REGIONAL MEDICAL RESEARCH CENTRE

(Indian Council of Medical Research)

Bhubaneswar

Annual Report 2007-08

RMRC

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2007-08

REGIONAL MEDICAL RESEARCH CENTRE

Bhubaneswar

Contents...

Preface

During the year the Centre has addressed research issues on vector borne diseases, diarrhoeal disorders, hepatitis and nutrition. Research was focused on applied as well as in operational areas. There are 21 projects, of which 5 are completed and most of which are extramural in nature.

During the period the Centre has addressed research issues on protective immunity in lymphatic filariasis to identify the candidate antigen for immunoprophylaxis. Multi-centric clinical trials are being initiated to address key issues for evaluating appropriate drug regimen for MDA. Host parasite interaction was addressed in both malaria and filariasis to generate knowledge in the area of cerebral malaria and host immune responses in filariasis. Multicomplex PCR assays were developed to facilitate diagnosis of vector borne infection. This Centre has reported a new strain of V.cholerae Eltor variant isolated from Kashipur block of Rayagada district of Orissa first time reported in the country during investigation of the epidemic of severe diarrhoeal disorders.

The Institute has established linkages with other ICMR Institutes in area of transfer of technology, training and scientific exchange of knowledge. The technology of molecular detection of HBV and genotyping was established here after the training obtained by our staff at NIV, Pune. Scientists received training on sequencing and AIDS diagnosis at NARI, TB culture technology at TRC, Chennai, reverse dot blot technique for detection of mutation in thalassemia at IIH, Mumbai, HPLC and haematological tests at NIN, Hyderabad, MAMA PCR at NICED, Kolkata and GCP & GLP at RMRI, Patna in the WHO workshop. New colaboration have been established with WHO & GATES foundation for sponsoring research projects at this Centre in the area of filariasis. Colaborations with DBT and DST and other non-ICMR institutes were also established during the year.

Colaboration with State Govt. and NVBDCP were augmented in national and state run programmes in area of malaria while keeping the research focus. For diarrhoeal disorders, the state government has referred to this Centre for diagnosis during epidemic and inter epidemic period. For lymphatic filariasis control programme, this Centre has provided training to medical officers of the State of Orissa and undertook survey of two districts for evaluation of the programme. On request of the State AIDS Control Society, diagnostic facility for CD4 count for referral cases of AIDS was undertaken.

This Centre has added new area of research during the year i.e. establishment of TB culture facility and Virus culture lab which are under process. These facilities will subsequently help in area of research besides complementing towards services in this region.

Human Resource Development facility was augmented by imparting training to the existing manpower in the advanced area of bio-medical research. Around 60 M.Sc students from various reputed universities had undertaken 6 months dissertation work. Summer training was imparted to 50 university-sponsored students during the year.

International workshop in the area of malaria was organized. Symposium on Emerging & Re-emerging infection was organized under the Chairmanship of Hon'ble Minister of Health & Family Welfare, Govt. of Orissa. Besides several scientific interactive meetings involving Professors of medical colleges and other reputed institutes locally were organised. Human Ethical Committee and Animal Ethical Committee meetings, invited lectures by the outside experts and regular weekly seminars, journal clubs were organised. Six monthly News Bulletin and Library News letter were published.

With the total sanctioned staff strength of 102, only 93 are in position, out of these only 13 scientists are currently in position. During the year, this Cente has already generated funds around one crore and twenty lacs through extramural projects and training. There are several other projects that have been taken up with foreign collaboration which are to be initiated this year and will add to the resource generation besides above.

This Centre published 12 research papers in 2007 and in the year 2008, 26 papers have been published or in press. All publications are published in index journals. Library subscribes 41 foreign and 42 Indian biomedical print journals for 2008. Online accesses of full text journals are made accessible to all computers of the centre. During this year, library has procured more than 1000 research books and monographs on biomedical and health science subjects. Library subscribes 5 online journals through ICMR e-jornal consortium. In addition to that, our library has started e-journal consortium between NML, ICMR & AIIMS, where 1505 medical journals will be accessed freely through NML-ERMED e-journal consortium of Directorate General of Health Services (DGHS), Ministry of Health & Family Welfare, Govt. of India. Library facility has been extensively used by outside institutions besides our scientists and staff.

The scientists and staff of this Centre have made continuous effort and contributed to significant output of the centre. I sincerely thank scientists and staff for their endeavour and contributions. I am also thankful to the State Health Department and other agencies and collaborating institutes for their assistance and co-operation. I extend my deep gratitude to Director General and the Council for continuous support, guidance and encouragement. With all round support, the Centre can continue its endeavour to achieve its goals.

S. K. Kar
DIRECTOR

Research Highlights

Malaria imposes great socio-economic burden in Orissa. In absence of a suitable vaccine, treatment of malaria largely depends on the available drugs. Our study on therapeutic efficacy had shown that chloroquine drug resistance was high in some parts of Orissa. Both infant mortality rates and maternal mortality rates in Orissa are high, largely attributed to malaria. High prevalence of anemia was reported by earlier studies undertaken among adolescent girls and pregnant mothers residing in malaria endemic regions. Hence, efficacy of chloroquine chemoprophylaxis amongst the pregnant mothers was evaluated prospectively in a cohort in 17 Gram Panchayats of Nayagarh district of Orissa. Around 85% of the pregnant mothers were found to have anaemia, of which 11% had severe form. Significant difference in incidence of malaria was recorded between chloroquine compliant and non-compliant mothers. However, the incidence of low birth weight (LBW) babies amongst the non-compliant mothers was 4 times more than that of compliant mothers.

Development of cerebral malaria is attributed to inflammatory Th1 types of responses. Such hyperactivity of T-cell responses are now known to be down-regulated by regulatory T lymphocytes. The levels of T regulatory cells in human falciparum malaria were studied. T-regulatory cells (CD4+ and CD25+) were found to be significantly low in complicated malaria in comparison to non-complicated malaria. The CD4+T cells with the highest levels of CD25+(FOXP3 cells) were also quantified in both complicated as well as uncomplicated malaria. The CD4+ and CD25+ high cell population were significantly low in complicated in comparison to uncomplicated malaria indicating their role in inflammation observed in complicated malaria.

The human glycoprotein CD36 is a major receptor for *P. falciparum*-infected erythrocytes and possibly contributed significantly to the patho-physiology of severe malaria. The study reveals the T188G stop mutation that reduces the synthesis of CD36 has shown protective effect (OR=3.71, 95% CI =1.69-2.22) against severe (cerebral) malaria.

Flowcytometric assay of micro particle produced by vesciculation of blood cells as well as endothelium during inflammation was found to be significantly high among the severe falciparum malaria cases indicating its role in the pathogenesis of cerebral malaria. Further, the (CCTTT) pentanucleotide microsatellite (MS) at the 2,5KB region of Nitric Acid synthase (NO) 2A was found to be highly polymorphic among the hospitalized cerebral malaria cases. The physiological significance of this polymorphism is being investigated.

Novel primers have been designed to identify the intra-specific differences between the sibling species of *An. annularis*, a secondary vector of malaria in Orissa, by SSCP analysis. To monitor the transmission of malaria in the community, it is important to detect the sporozoite in mosquito, mosquito blood meal and the species of the vector mosquitoes. The scientists have been able to develop a single step multiplex PCR assay for the first time which will detect sporozoite, source of blood meal and sibling species of *An. annularis* in one go. Based on the ecology, study on malariogenic stratification carried out in Angul district revealed *P. falciparum* sporozoite in An. subpictus indicating its role as a local vector. Data indicate that it contributes to the high SPR rate in the summer season in Angul district studied. HPTLC fingerprinting pattern has been developed to understand the chemical composition and consistency of mosquito larvicidal activity of highly active plant extracts. Three plant extracts tested for mosquitocidal efficacy in vectors transmitting malaria has shown promising results that being evaluated

Lymphatic filariasis has been considered as one of the major public health problems of this region affecting large population. Twenty out of 30 districts of Orissa are endemic for filariasis. The efficacy and tolerability of low dose of DEC (100mg) given in uniform single dose annually was compared with higher annual doses of DEC, i.e., 200 mg or 300 mg given uniformly in all age group population in 3 comparable ecological set up. The cohort followed periodically at 3, 6 and 12 months indicated comparative efficacy of all three dosage schedule while reduction of frequency and severity of side reaction was observed in the lowest dose (100mg) administered. The entomological indices assessed at 3 sites were also comparable. The continuing study results may help the annual Mass DEC programme strategy against filariasis.

The dlfferentlal Immune responses between two chronlc cllnlcal manlfestatlon of filariasis like hydrocele formation and elephantiasis have not been elucidated. Our study revealed that the ratio of TNFRI and TNFRII was significantly different in two forms of chronic filarial diseases. Therefore, we analyzed the genetic polymorphism of TNFR-II (M 196 R) genes in the clinical spectrum of human bancroftian filariaisis. Genetic polymorphism of TNFRII (M 196 R) is observed in significantly lower frequency among elephantiasis cases as compared to that in hydrocele cases indicating that this immunological marker may be useful in differentiating the two clinical forms of chronic diseases manifestation.

Glutathione binding protein with Glutathione S-Transferase (GST) activity was purified through Glutathione Agarose column from soluble extracts of cattle parasite *Setaria digitata.* The protective potential of GST has been studied in *S.digitata* implanted Mastomys. It was observed that microfilaria could persist in the circulation up to day 160 in control group of mastomys. Immunization of Mastomys with GST antigen before implantation of worms resulted in significant reduction in microfilaria (Mf) density and a complete clearance in about 95 days indicating its potential in protective immunity.

Lipids were extracted from the adult stages of cattle parasite *Setaria digitata.* The IgG antibody response to this lipid fraction was earlier demonstrated to be decreased in asymptomatic microfilaraemic individuals, but high in asymptomatic amicrofilaraemic (endemic normals) and chronic filariasis cases. It is of interest to note that higher antibody levels were observed in circulating filarial antigen (CFA) negative sera compared to lower levels in CFA positive individuals irrespective of microfilaraemia and clinical status. Thus asymptomatic microfilaraemic individuals exhibited low IgG levels similar to symptomatic (chronic filariasis with elephantiasis, hydrocele) or asymptomatic amicrofilaraemic individuals with antigenemia indicating protective role of lipid fraction.

To check the trans-placental transfer of filarial antigen, cord blood as well as maternal blood samples were analysed in an area endemic for *W. bancrofti* infection. About 45% of mothers were found to be CFA positive. Only 25% of CFA positive mothers were observed to transfer filarial antigen to their offsprings through placenta.

A flow cytometric assay developed by the centre indicates that apoptosis of intrauterine developing stages of *S. digitata* could be a possible contributing factor for the absence of microfilariae in amicrofilaraemic animal model.

Prevalence of hepatic viral infection (A,E,B &C) and associated risk factors were studied in five primitive tribes of Orissa indicated high prevalence of hepatitis C infection in 2 of these tribes, i.e., *Jaunga* (13%) and *Mankdia* (9%). Out of HBV infected subjects, Genotype D was observed in all the tribes. The risk factors for high transmission of above parental infection is studied to develop future strategy for prevention

The weekly surveillance for diarrhoeal disorders was undertaken in different health facilities of Puri and Bhubaneswar indicated *E coli* (60.9%) as the major diarrhoeogenic agent followed by *V. cholerae* (29.8%), *Aeromonas* spp (7.3%) and *Shigella* spp (2%). Amongst the *V. cholerae*, O1 Ogawa(19.2%) was the predominant serotype followed by Inaba(9.9%) and O139 (0.7%). Above observation was found associated with environmental data of prevalence of nonO1 nonO139 *V cholerae* which was found to be 35.2% in Planktons, 30.8% in water samples and 25.5% in roots of the floating vegetation. During the epidemiological investigation of diarrhoea in three districts of Orissa i.e Raygada (Kashipur, Kolnara), Koraput (Dasmantpur, Laxmipur), Kalahandi (Th Rampur) and Gajapati (Malanta) from July to September 2007, a new eltor variant strain of *V cholerae* (mixed character of classical and El tor biotype) has been identified both in diarrhoea as well as in environmental water samples (stream, river, *Nala* and *Chua*) and reported for the first time in this region. The centre has rendered timely assistance to the local government for control of the epidemic. During past epidemic period the environmental water samples were also monitored. This indicated the persistence of pathogen, hence effective method of disinfection of water sources was undertaken by Govt to prevent future epidemic.

Thalassemia is identified as common hemoglobinopathy disorder prevalent in the state of Orissa. Molecular characterization of β-thal. mutation in 210 subjects revealed IVS (I $\rightarrow$ 5) G $\rightarrow$ C as the major mutation. Two of the above samples have identified as (CD 15 G $\rightarrow$ A.) being reported for first time in this region.

Ongoing Studies

1

1.1 Immunochemical characterization of filarial Glutathione-S-Transferase and its protective potential in experimental filariasis.

Status : Extramural (Funding : DST)
Investigator :
Dr. M. K. Beuria (PI)
Co-Investigators :
Dr. M.K. Das, Dr. M.S. Bal,
Dr. N.N.Mandal
Starting date : March 2005
Closing date : Feb. 2008, Extended to Aug. 2008

Background:

Glutathione-S-Transferases (GST), a group of detoxifying enzymes, help in parasite survival against host-induced damage. These enzymes have been used as component of anti-parasitic vaccine in Schistosomiasis, Fascioliasis and in Chaga's disease. Glutathione binding protein with GST activity was purified from soluble extracts of adult cattle parasite *Setaria digitata* through Glutathione agarose column. On SDS-PAGE it revealed a broad band of 26-28 KDa. Antibody recognition pattern to this fraction was checked by both ELISA and Immunoblotting assay. More than 90% of infected subjects were positive for IgG antibody compared to 45% in endemic normal. Sera collected from individuals with other helminthes infection from non endemic area were negative for any isotype indicating the filarial specificity of GST. Sera collected from endemic subjects identified both the two bands of GST by immunoblotting. Sera from non-endemic normal (NEN) individuals could not recognize GST.

Objectives:

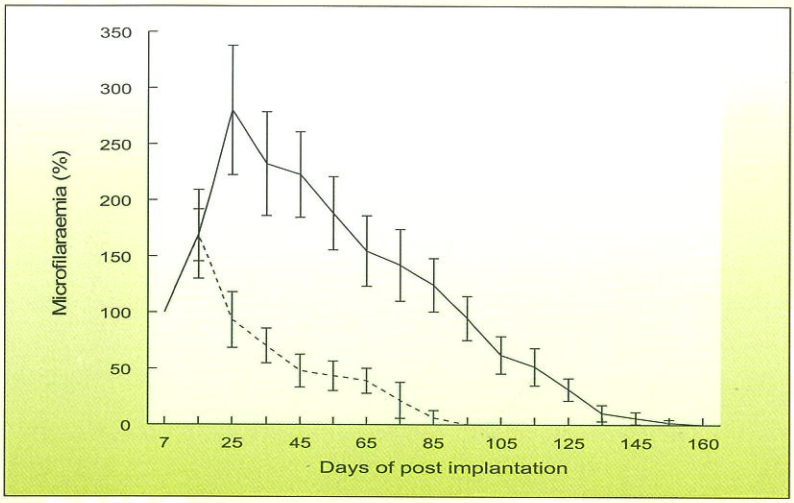
1. To determine recognition pattern of anti-Glutathione-S-Transferase (GST) antibodies (SDS-PAGE and immunoblotting) in filarial sera.
2. To determine the cytokine responses specific to GST in filariasis.
3. To evaluate the protective potential of GST to clear microfilariae in experimental infected animal.

Work Progress:

Intraperitoneal implantation of adult female worms of *Setaria digitata* in *Mastomys coucha* could induce microfilaraemia in circulation. The microfilaria could be detected in peripheral circulation as early as 4th day of post implantation. Microfilaraemia in animals lasted about 160 days with the peak Mf counts of about 36/20 µl of blood by 25 days (Figure 1). Immunization of mastomys with GST antigen, purified from the cattle parasite *Setaria digitata*, before implantation of worms resulted in significant reduction in microfilaria (Mf) density. Complete clearance of circulating microfilaria was achieved by about 90 days, indicating the protective efficacy of GST antigen in clearance of microfilaria in the infected mastomys. Reduction of Mf density (about 7%) was observed in immunized group of mastomys vs. 180% increase in control (non-immunized) animals on day 25. The mean microfilaria count on day 25 was observed to be 13.3 ± 2.6 and 36.1 ± 6.3 in immunized and control group of mastomys respectively. The circulating Mf in immunized group were found to get cleared very rapidly in comparison to control group animals, indicating that microfilaria clearance in vivo was significantly potentiated

by antibodies to GST. Decline in Mf density was observed about 52% on 45th day, 57% on 55th day and 78% on 75th day of post implantation in immunized animals. The microfilaria density was more than 100% till 85th day in non-immunized control animals. Immunization of GST after appearance of microfilaria in mastomys is being evaluated.

Fig-1



Effect of immunization with GST antigen on microfilaraemia in *Setaria-digitata*-implanted *Mastomys coucha*: animals were immunized with 2 doses of GST before implantation of worms (....., n=8); normal microfilaraemic controls (———, n=8). The results are expressed as mean percentage of microfilaraemia (mean±SD) for each group calculated with reference to Mf density for each of the animals. The Mf density on day 7 was taken as 100% for each of the animals.

T cell proliferative response of peripheral blood mononuclear cells of normal and infected subjects was determined stimulating with GST. About 75% of endemic normal and chronic patients were found positive. None of the asymptomatic microfilaraemic individuals showed T cell proliferative response to GST. Culture supernatants are being collected to determine cytokines from the cell culture experiments taking peripheral blood mononuclear cells from endemic subjects stimulating with GST.

1.2 Effect of maternal infection on neonatal immune responses in Bancroftian filariasis

Status : Extramural
(Immunology Task Force)
Investigators : Dr. M. K. Beuria
Dr. S.K.Kar, Dr. A. K. Satapathy,
Dr. M.S. Bal, Dr. N. N. Mandal
Starting date : August 2008
Duration : 3 years

Objectives :

1. To study the B cell response (antibody isotype) to filarial antigens in cord blood samples of offspring and in 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 neonates born to infected and uninfected mothers.

Background:

The study was approved by Immunology Task force of ICMR for funding but funds are awaited. The work has been initiated in June 2008 with intramural funding of the Centre.

Lymphatic filariasis caused by the nematode *Wuchereria bancrofti* is a major vector borne disease in many tropical countries including India. Maternal filarial infection represents a risk factor for infection in children and is associated with alteration in the parasite specific immune reactivity of their offspring. Children of infection free mothers have been shown to respond vigorously to filarial antigen with lymphocyte proliferation, production of IL-2 and IFN-γ. Cellular hyporesponsiveness has been observed in children born to microfilaraemic mothers.

These observations support the hypothesis that the prenatal exposure may affect development of subsequent immune responses. It has been postulated that placental transfer of filarial antigens may develop a state of tolerant in the offspring diminishing the anti filarial immune reactivities within them rendering increase in susceptibility to infection. The study aims to carryout immunological investigations such as anti- filarial antibodies, T cell proliferative and cytokine response and expression of T regulatory cells in cord samples of infants from infected and uninfected mothers. This study will help to understand the immunological consequence of offspring upon exposure to filarial antigens during gestation.

Results:

In order to check the transplacental transfer of filarial antigen, cord blood along with their maternal blood samples were collected from Khurda (district hospital), an area endemic for *W. bancrofti* infection. Presence of circulating filarial antigen (CFA) was also determined in both maternal and corresponding cord blood samples by Og4C3 enzyme linked immunosorbent assay. About 45% of mothers were found to be CFA positive. 25% CFA positive mothers were observed to transfer filarial antigen through placenta.

1.3 Human Bancroftian filariasis: Identification of immunological markers of morbidity in hydrocele and elephantiasis

Status : Intramural
Investigator :
Dr A.K.Satapathy (PI)
Co-Investigators :
Dr A.S. Kerketta, Dr P.K. Sahoo, Dr. B.Ravindran
Starting date : March 2006
Closing date : February 2009

Introduction

Human lymphatic filariaisis displays diverse forms of clinical manifestations. It has been assumed that repeated episodes of acute attack could eventually lead to development of chronic forms of the disease. Hydrocele and elephantiasis are two major clinical manifestations associated with chronic Bancroftian filariasis. However,

it is not clear why some individuals develop one form of pathology, and others develop another form of pathology. Although hydrocele and lymphedema / elephantiasis are two diverse forms of chronic manifestation, immunological features distinguishing patients with elephantiasis from that of hydrocele have not been very successful. Identifying immunological markers may be useful in differentiating the two forms of chronic disease manifestations as well as crucial for understanding the mechanism of pathogenesis of the disease. An attempt has been made to address these issues in this study.

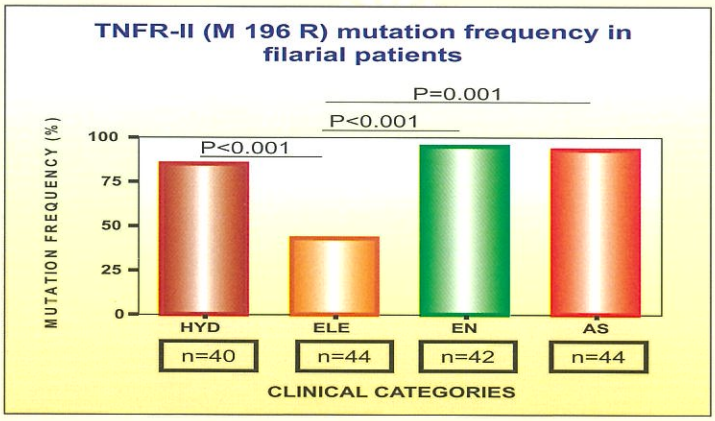
Objectives:

1. To evaluate filaria specific as well as mitogen induced T-cell proliferative responses in hydrocele and lymphoedema patients
2. To quantify inflammatory cytokines and chemokine levels in patients with hydrocele and lymphedema and correlate with severity of chronic manifestation.
3. To type genetic polymorphism of TNF receptors (TNFR-I & TNFR-II) in hydrocele and lymphedema patients.

Work Progress:

Genetic polymorphism of TNFR-II occurred at a lower frequency in elephantiasis cases and was significantly more in hydrocele cases. There was a significant difference in the mutation frequency between the two forms of chronic disease. This finding leads us to study the TNFR II polymorphism in other clinical manifestations of filarial disease. Fig-1 shows the frequency of TNFR II mutation in different clinical categories of filariaisis. TNFR-II mutation frequency was significantly more in endemic controls (95%), asymptomatic carriers (93%) and hydrocele cases (85%) in comparison to patients with elephantiasis (43%). The observed difference in elephantiasis does suggest that TNFR-II mutation might predispose patients for development of hydrocele.

Fig-1



Polymorphism of Endothelin-1:

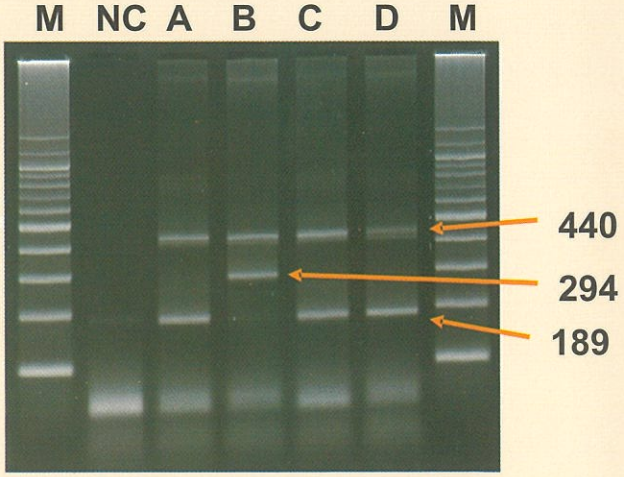
The endothelin system has a physiological vasoconstrcting role and sometimes a pathophysiological participation in systemic endothelial activation. Increased ET-1 levels have been associated with vascular hypertrophy and neighboring fibrosis. It has been presumed that progression of infection to chronic manifestations may be due to over expression of lymphangiogenic factors such as VEGF and endothelin.

The ET-1 (Ala 288 Ser) mutation is caused by substitution of the G/T at codon 288 in exon 5 position 862 of the ET1 gene. The polymerase chain reaction confronting two pair primers method (PCR-CTPP) was used to analyze the Ala 288 Ser polymorphism in ET-1 gene. we design the 4 primers for PCR-CTPP were as follows: the forward primer was 5'-CCT CGC TCC CAT TCT AAG CAT AAG G-3', the reverse primer was 5'-CCT TTG CCA GTC AGG AAC CA-3', the G allele-specific forward primer was 5'-GAT CCC AAG CTG AAA GGC AAG-3' and the T allele specific reverse primer was 5'-TCA CAT AAC GCT CTC TGG AGG GA-3'. The total volume of PCR reaction solution was 20 µl for each sample containing 20 pmol of forward and reverse primer each and 10 pmol of G specific and T specific primer each, 2 µl of 10mM dNTP mix and 1 U of Taq DNA polymerase. The DNA was denatured at 95°C for 10 min, followed by 35 cycles of 95°C for 30s, 65°C for 30 s, and 72°C for 60s. The final extension step was prolonged to 7 min. PCR products were visualized by electrophoresis on a 3.5% agarose gel and staining with ethidium bromide for genotype assessment, as shown in Fig. 2.

To check the possibility of endothelin–1 polymorphism playing a role in the outcome of different clinical manifestations, the prevalence of ET-1 (Ala 288 Ser) genotypes was assessed in about 80 subjects of filariaisis with different clinical manifestations. The genotype frequencies of these polymorphisms are described in Table 1. The prevalence of wild genotype (Ala/Ala) was found to be more significant in hydrocele case in comparison to elephantiasis (41% vs 16%; P=0.0028; odds ratio=0.2738; 95% CI=0.1239 to 0.6266). A significant difference was found in the distribution of heterozygous (Ala/Ser) individuals among the chronic categories. In elephantasis the Ala/Ser genotype was more prevalent in comparison to hydrocele case (65% vs 43%; P=0.0213; odds ratio=2.386; 95% CI=1.177 to 4.839).

Fig-2

Genotyping of Endothelin 1 (288) by Polymerase chain reaction confronting two pairs primers method (PCR-CTPP)



A, C, D - Wild Type, B – Homozygous mutant

Wild type (GG) - 189bp+440bp, Heterozygous (GT) – 189bp+294bp+440bp Homozygous mutant (TT) – 294bp+440

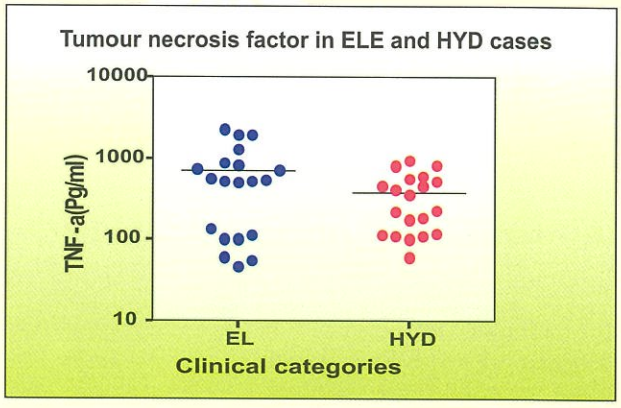
Table1

Genotype and allele distribution of Endothelin-1 (Ala288Ser) polymorphism among chronic filarial patients.

Genotype	Elephantasis (n=73)	Hydrocele (n=58)	P value	Odds ratio (95% CI)
Ala/Ala	12 (16%)	24 (41%)	**0.0028**	0.2787 (0.1239 to 0.6266)
Ala/Ser	47 (65%)	25 (43%)	**0.0213**	2.386 (1.177 to 4.839)
Ser/Ser	14 (19%)	9 (16%)		
Allele	2n=146	2n=116		
Ala	71 (49%)	73 (63%)		
Ser	75 (51%)	59 (37%)	0.2813	1.307 (0.8149 to 2.096)

Quantification of plasma levels of pro inflammatory molecules in patients with hydrocele and lymphedema is another approach expected to offer markers of morbidity. Extensive studies have resulted in growing evidence in support of involvement of various inflammatory cytokines in parasite infection, where a central role for TNF-α has been attributed. TNF-α has been known as an important inflammatory mediator capable of producing symptoms such as chills, fever and myalgia. Since, hydrocele and elephantiasis are two diverse forms of chronic manifestation, quantification of plasma levels of TNF could be useful for the differentiation of both forms of chronic manifestations. There is no significant difference in the plasma levels of TNF between hydrocele and elephantiasis (Fig-3).

Fig-3



1.4 Role of CD5⁺ B-lymphocytes in human lymphatic filariasis

Status:
Extramural (Applied for DST)
Principal Investigator:
Dr A. K. Satapathy
Co Investigators:
Dr P. K. Sahoo, Dr B. Dwibedi & Dr S. K. Kar
Starting date: August 2008
Closing date: July 2011

Introduction:

The importance of T cells in host protection against filarial infection is well documented. In contrast, the role of B cells in host protection against filariasis remains unclear. Several studies have shown that B1 subset of B cells play an important role in the outcome of infection in Schistosomiasis, S. pneumonie and

experimental filariasis. However, no information regarding status of B-1 cells in clinical manifestation of human bancroftian filariasis exists. Although all the published data indicated a role of B1 cells in the outcome of experimental filarial infection and a variety of diseases, the biological role played by B1 lymphocytes to provide host protection against filarial infection is largely unknown. Attempt is being made to study the profile of B1 cell populations and its association with poly reactive antibodies in filarial infected human population. The role of B1 cells in cytokine responses by filarial proteins and carbohydrates - antigens in filarial infected human cells will be analyzed.

Background:

The proposal is under consideration of DST. Preliminary work was initiated in August 2008 with intramural funding from the Centre.

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 protein and carbohydrate antigens in filarial infected human cells.

Work Progress:

Efforts are being made to collect samples from the following categories of filarial patients (1) endemic normals (2) asymptomatic microfilariaemic carriers and (3) chronic patients. About 50 blood samples of endemic normals, asymptomatic microfilariaemic carriers and chronic patients were collected from a filarial endemic village. Plasma were separated and preserved which will be used subsequently for quantification of antibodies reacting to various antigens. CD 5^+ B cells expressing CD 19 are B-1 cells. Attempts are being made to quantify B-1 cells in the clinical spectrum of human filariaisis. B-1 Cells ($CD5^+CD19^+$) were found to be low in microfilariaemic carriers in comparison to chronic patients and endemic normals - individuals.

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

Status : Extramural
(ICMR Task Force)
Investigator : Dr. N. Mahapatra (PI)
Co-Investigators :
Dr. R. K. Hazra, Dr. S. K. Parida
Collaborator :
ORSAC,Govt. Of Orissa
Starting date : March 2007
Closing date : February, 2010

Introduction:

Geographic Information System(GIS) and Remote Sensing (RS) has now been used as a tool to develop the epidemiological maps of vector borne diseases. The derivation of these maps are vital for the effective design and implementation of successful programme for control of

parasite and their vectors. The data on filariasis of Orissa is inadequate which does not reflect the real prevalence of the disease and also vector population. Thus using GIS and RS as a tool, filariasis risk map can be developed by studying and mapping the breeding habitat of the vector.

Objectives :

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

2. Development of filariasis risk map.

Work Progress:

To map the breeding habitats of vector (*Cx quinquefasciatus)*, the two endemic block Jatni (Khurda district) and Satyabadi (Puri district) were taken as study areas and one non endemic block ie Angul (Angul district) was selected for comparision. The above three districts are covered in two scenes of LISS-III (Multi spectral sensor) and five scenes of Pan chromatic sensor of IRS-IC/ID Satellite.

Initially the interpretation of data of the districts were taken for selection of Geo-environmental Zonation of the Block. Two index villages were selected from each environmental zone. Adult mosquitoes and larval collection were done from these villages. Vector breeding sites survey were done through GPS.

A total of 1267, 927 *Cx quinquefasciatus* mosquitoes were collected from Jatni and Satyabadi blocks respectively. Infectivity rates were 10 % and 5.4 % in the above blocks. *Ma.annulifera* & *Ma.uniformis* were also found *in* Satyabadi blocks. Infectivity rates were 12 % and 6.8 % in the above two blocks.

From the nonendemic Angul block only 123 *Cx.quinquefasciatus* were collected and dissection did not show presence of any stages of *W. bancrofti* larvae. Vector density and larval density were very low. The soil type was found to be **ustochrepts**, (which does not facilitate stagnation of water). Therefore breeding of *Cx.quinquefasciatus* was very low due to non availability of polluted water.

Water analysis of the breeding sites of the above three blocks were done for the following parameters like temperature, pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids

and total dissolve solids. It was seen that COD and total dissolved solid were more in the endemic blocks (where the larval density were higher) in comparison to the nonendemic blocks.

Nonspatial database on soil and water have been generated. Climatological information related to the project site has been compiled. Linking of terrain parameters derived from RS with entomological, epidemiological & climatological parameters through GIS software has been done for Angul block. The thematic layers like block, village boundry, water bodies, canal and river network of all the three blocks were developed. Further study on integrating other geoenvironmental parameters with vector proliferation is in progress.

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

Status :
Intramural
Investigator :
Dr. B. Dwibedi (PI)
Co investigator :
Dr. S. K. Kar, Dr. N. Mahapatra
Starting date : March 2006
Closing date :
March 2007(extended to April 2009)

Objectives :

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

Back ground:

Mass drug administration (DEC) is being undertaken in the country targeting for elimination of lymphatic filariasis by 2015. The programme is based on mass administration of single annual dose of DEC in the recommended dosage of 6mg/kg body weight averaged for three age groups of populations i.e. 2-5, 5-14 & >14 years. But the population compliance of the programme is limited largely because of the fear of side reactions of the drug and also by confusion of the drug dosage distributed by volunteers in various age groups. The present study aims at generating evidence for low dose DEC given in uniform doses for all age groups which may be equally efficacious and well tolerated. This results may help the national programme for elimination of filariasis in increasing the population compliance of the drug.

Work progress:

Three endemic villages from 2 districts (Cuttack & Khurda) with population around 2000 were selected after screening several endemic villages based on mf status (>6%) and clinical disease. After baseline examination for microfilarimia and antigenimia two successive annual rounds of mass DEC administration either 100, 200 or 300 mg was undertaken in uniform dose to all age groups in the study villages for successive annual rounds in year 2007 and 2008. The study population were subjected to reassessment of filarial infection status periodically during the year following the first annual round of DEC. It is planned to continue the assessment of effectiveness of the three dosages for three successive annual rounds of MDA while observing side reactions and compliances.

Predrug Assessment:

Assessment includes morbidity status of population under study and evidence of filarial infection like night blood mf count and ICT test for adult worm antigen. Entomological studies for filarial transmission of infection were assessed periodically to evaluate the effect of M.D.A. in 3 sites.

Table 1: Baseline Investigation & Clinical Parameters

Parameters	Site A 100 mg	Site B 200 mg	Site C 300 mg
Total population(dejure)	1808	2124	1907
Eligible population(excluded < 2 yrs, pregnant, very ill subjects)	1493	1710	1613
2-5 yrs	213(14.6)	250(14.6)	224(13.8)
6-14 yrs	299(20)	342(20)	275(17.04)
> 14 yrs	981(65.7)	1118(65.3)	1114(69)
Population covered	1126(75.4)	1191(70)	1225(76)
ICT Positives (%)	(23.4)	(27.3)	(17.1)
Mf +Positives	86(9.8)	98(8.7)	82(6.6)
Lymphoedema cases	42(2.4)	89(4.3)	84(4.5)
Hydrocele cases	93(5.3)	101(4.8)	96(5.2)

*Nos in parentheses are in percentage

Entomological Indices			
	Site A (100mg)	Site B (200mg)	Site C (300mg)
C. quinquefasciatus Density(PMHD)	11.8	11.0	10.1
Infection eate(%)	0.5	1.5	0.5
Infectivity rate (%)	0.5	1.5	0.5

The study population was comparable and covered all ages and both sexes

Drug distribution, supervised consumption & follow-up for side reactions:

During first year (2007), after obtaining baseline information the regimens (100mg/200mg/300mg) were allocated randomly to the three study sites for uniform single dose mass administration. Children below 2 years, pregnant women & those who are critically ill were excluded from DEC consumption. Supervised DEC consumption was ensured by physician. Side effects if any were observed daily for 7 consecutive days by the physicians, recorded and managed by providing treatment at the doorstep. The second annual round was instituted in the study sites in similar fashion at 12th month in the year 2008. Side reactions were recorded and infection status will be monitored at 18 and 24 months.

DEC compliance and side reactions:

DEC compliance was 86%, 85% and 80% in the first annual round and 79%,73% and 75% in the second annual round of MDA in 100mg,200mg and 300mg regimen sites respectively (Fig 1). Side reaction frequency was noted to be higher in the 300mg dose and the frequency was reduced in the second round of the MDA(Fig 2). Majority of the side reactions were mild and no severe reaction observed.

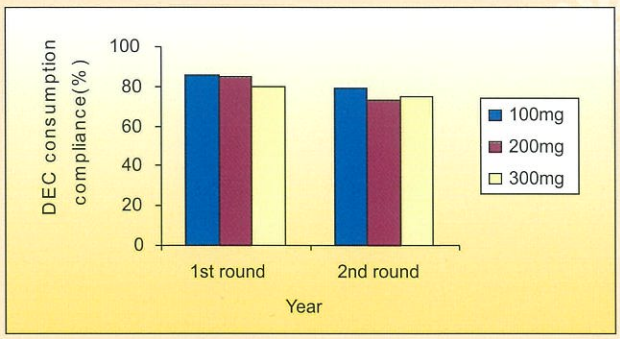


Figure 1 : Population coverage in 3 regimen sites in 2 annual rounds

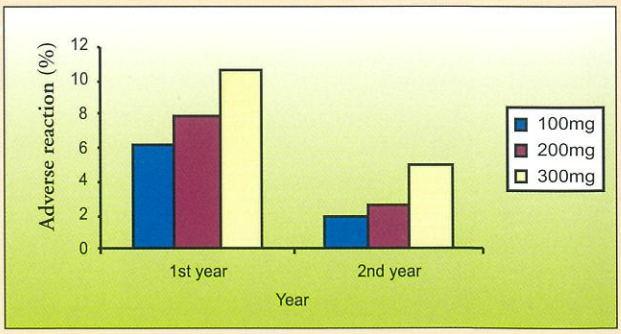


Figure 2: Frequency of side reaction to DEC in three regimen groups in 2 successive annual rounds

Effect of first annual dose of DEC on microfilarimia in three regimens:

Microfilaria clearance and reduction in mf count was assessed in all the three regimen sites periodically at 0, 4th, 12th month for one year following first round (2007) of MDA. Post MDA follow-up in all three study sites for Mf status with mean duration at 4th month has shown 48.7% , 44% & 64% Mf clearance in the 100mg, 200mg & 300mg dosages respectively & that at 12 months follow-up was 54%, 52% & 62% respectively (Fig 3), which was indicative of DEC efficacy in clearance of microfilaria. Mf clearance was also comparable in all the three regimens in the population of age group above 14 years (Fig 4). The difference was statistically not significant. Effect of the three doses on community mf load (Geometric mean of mf dencity) was identical (Fig.8).

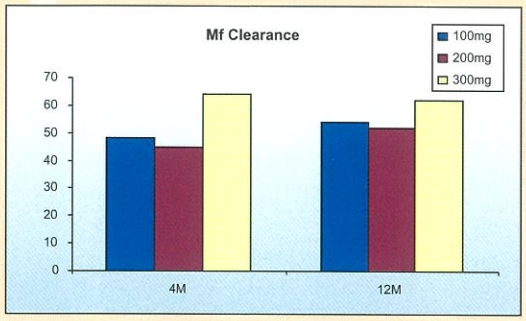


Fig 3 Mf clearance in the three regimens

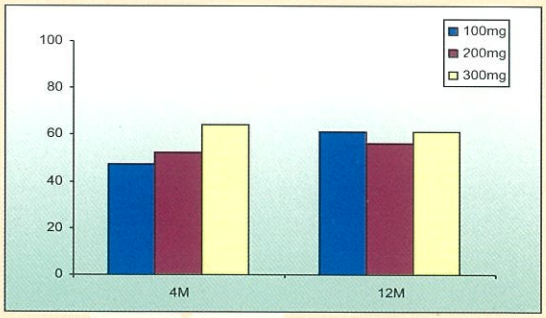


Fig 4 Mf clearance in>14 years age group

Besides, partial microfilaria clearance among the residual microfilarimics was observed in 35.8%, 39% and 20% cases at 4th month and 28%, 27% and 24% cases at 12th month following MDA in 100, 200 and 300 mg regimens respectively. Decline in Mf rate and mean microfilaria density(fig 8) among the residual microfilarimics was also noted in all the three regimens.

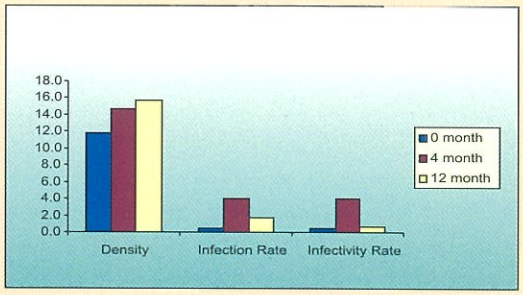


Fig 5 Vector indices (**100mg site**)

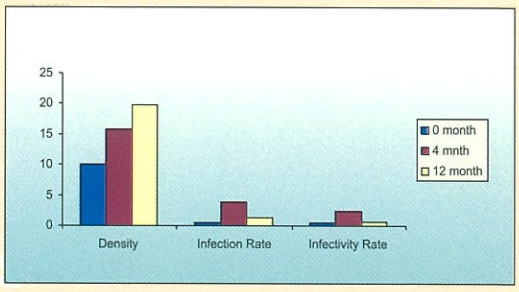


Fig 6 Vector indices (**200mg site**)

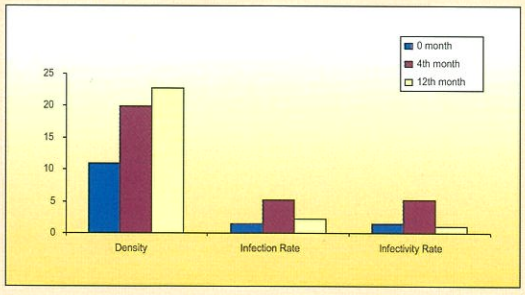


Fig 7 Vector indices (**300mg site**)

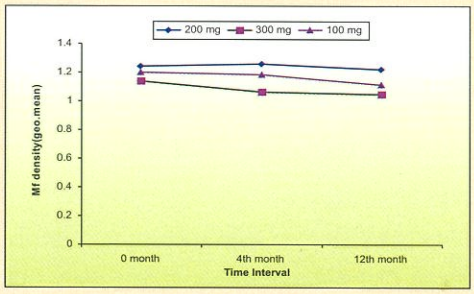


Fig 8 Effect on community mf load (MF density in geometric mean)

Entomological indices in Three regimen sites in 1st Year:

Entomological survey was carried out in the three sites before and after the first annual round of MDA undertaken in April. The parameters included mosquito (*Culex quinquefasciatus*) density (per men hour,PMHD), infection rate and infectivity rate. In site A(100mg) (Fig 5) vector density varied from 11.8 to 15.7 and infection rate from 0.5 to 1.4% and infectivity rate from 0.5 to 0.7% in different seasons of the year. The vector density in 200mg sites (fig 6)ranged from 11-28 PMHD and infection rate ranged from 1.5 to 2.2 % while infectivity rate was between 1.5-1.0%. In 300mg regimen site(Fig 7) vector density was 10.1 to 19.7 % with infection and infectivity rate of 0.5 to 1.2% and 0.5 to 0.6% respectively. In the subsequent years these parameters will be studied and be compared with this information for interpreting on comparative efficacy of the three regimens on transmission indices.

Subsequent plan of activity

The study population will be subjected to night blood examination for microfilarimia & CFA at 18 and 24th months following second annual round of MDA; following which the third round of annual single dose DEC will be administered. The drug administration will be supervised with close follow-up of the population for side reactions if any, with management of such cases at the door step. The effect of third round of MDA will be assessed for both the above parameters to check sustainability of the drug efficacy at three dosage levels .

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

Status :
Extramural (ICMR Task Force)
Investigators :
Dr. A. K. Satapathy (Co-PI),
Dr. B. Ravindran (PI), ILS
Starting date :
January 2006
Closing date :
December 2008

Introduction:

One of the severe pathological manifestations observed in *P.falciparum* infections is cerebral malaria. However, only a subset of *P.falciparum* infected patients suffer from such clinical symptoms. The factors responsible for development of cerebral malaria amongst *P.falciparum* patients are not yet clearly identified. It is envisaged that cerebral malaria is caused due to genetic and immune responses of man towards *Plasmodium falciparum*. In the current study an attempt has been made to study the host gene polymorphism involved in several arms of the immune response have been investigated for correlation with the clinical manifestation of *P.falciparum* malaria. Innate immune recognition of Plasmodium and subsequent release of cytokines and inflammatory mediators are important for parasite clearance but may also contribute to disease

severity. The family of Toll-Like Receptors (TLRs) has been identified as key host molecules in the induction of innate immune responses to microbial ligands. Recently, P. falciparum GPI was reported to induce signaling via both TLR-2 and 4. GPIs have been identified as an important parasite factor that activates the host innate immune system. These molecules initiate the production of excess levels of the cytokines, tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), leading to a systemic inflammatory cascade, renal failure, multiorgan inflammation, hypoglycemia and death. Purified *P. falciparum* malarial GPIs also increase expression of cell adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and nitric oxide production in human vascular endothelial cells through cytokine-independent pathways. Thus, GPI molecules of the parasites are proposed as the key malaria pathogenicity factors. Studies using human monocytes and anti-TLR2 and anti-TLR4 antibodies have confirmed that GPIs are recognized mainly by TLR2 and to a lesser extent by TLR4. Since, *P. falciparum* GPI was reported to induce signaling via both TLR-2 and 4, we hypothesized that modified recognition or signaling via variants of TLR-2 and TLR-4 could influence susceptibility to and manifestation of malaria.

Objectives:

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

Work Progress:

Patients reporting at the out–patient department and/or admitted to the Department of Medicine, SCB medical college, Cuttack with short history of fever associated with unarousable coma were assessed clinically. Based on the status of clinical manifestations the patients were divided in to complicated and uncomplicated malaria group. Blood samples (2 ml venous blood in heparin) have been collected from both complicated and uncomplicated malaria cases. DNA were purified from the leukocytes and preserved for typing genetic polymorphism.

In our earlier study we had reported that TLR-4 (Asp 299 Gly) mutation was significantly high in complicated malaria cases in comparison to non-complicated cases suggesting a genetic predisposition of subjects

with this mutation to clinical complications associated with human malaria (P=0.0309). Since GPIs are recognized mainly by TLR2, we also analyzed the possibility of TLR-2 mutation playing a role in the clinical outcome of human *P.falciparum* infection.

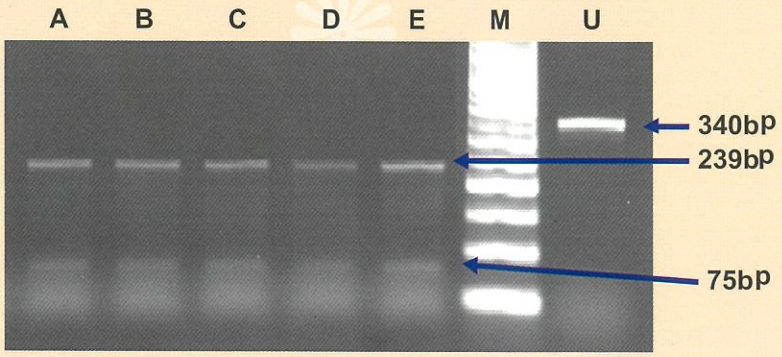
TLR-2 Polymorphism:

Two common polymorphism Arg753Gln and Arg677Trp which quantitatively decreased the inflammatory molecule productions, were found to be associated with susceptibility to many infectious diseases. A total of 52 samples were genotyped for TLR2 (Arg753Gln, Arg677Trp) polymorphisms by PCR-RFLP method as described below. Primers were designed For TLR2 which spans a region of 340 bp including both polymorphisms (Arg753Gln, Arg677Trp). The two primers sequences were as follows forward; 52 - GCCTACTGGGTGGAGAACCT-32 Reverse; 52 -GGCCACTCCAGGTAGGTCTT-32 . Reactions were set up in a total reaction volume of 20 µl, which contained 2µl of 10X PCR buffer, 10 pmol of each primer, 0.1 ng genomic DNA, 1 U Taq DNA polymerase, 0.5µl of 10mM dNTP mixture, and 1.5 mM magnesium chloride.

PCR assay was performed as follows: initial denaturation of 94^{0}C for 5min followed by 30 cycle of 94^{0}C for 30s 58^{0}C for 30s, 72^{0}C for 30s. The final extension step was for 7 minutes at 72^{0}C. QIAquick Gel Extaraction Kit (Qiagen, Hilden, Germany) was used for extracting and purifying amplicons after PCR. 5 µl of the resulting products were used for an overnight digest with the appropriate restriction enzyme (A*ci* I) in a total volume of 20 µl at 37^{0}C, and digests were run out on a 3% low melting agarose gel and visualized under UV light.

For the TLR2 wild type, there are two restriction sites by AciI. From these restriction sites, three bands appear on 38 bp, 75 bp, and 227 bp in wild type. If TLR2 polymor-phisms (Arg753Gln or Arg677Trp) are present, there is only one restriction site and therefore, only two bands (75 bp, 265 bp in Arg753Gln and 38 bp, 302 bp in Arg677 Trp) should be appeared. In our results, however two bands (239 and 75bp) appeared (38bp band is so small that we were unable to trace it in agarose gel) in all the patients and healthy volunteers which represented for wild type as shown in Fig-1.

Fig-1



A,B,C,D,E- Wild type M– 50bp marker U– undigested

The association between TLR-2 polymorphism and clinical manifestations of malaria has been analyzed. All subjects were found to be wild type for TLR2 Arg753Gln and Arg677Trp polymorphism (Table-1). The prevalence of wild type alleles was 100 percent. To verify these findings we performed sequencing analysis of about 40 samples randomly and we confirmed that there were no TLR-2 polymorphisms in this studied subjects. Hence these polymorphisms may not have any role in the clinical outcome of human *P.falciparum* malaria.

Table-1

Frequencies of TLR2 gene polymorphism in clinical manifestation of P.falciparum malaria

TLR2 gene polymorphism	Genotype	Frequency (%)
Arg753Gln	Wt / wt Wt / mut Mut / mut	52/52 (100%) 0/52 0/52
Arg677Trp	Wt / wt Wt / mut Mut / mut	52/52 (100%) 0/52 0/52

1.8 Role of *PfEMP1* subtypes in clinical manifestations of severe *falciparum* malaria

Status :
Extramural (applied to DBT)
Principal Investigator :
Dr M R Ranjit
Co-Investigator(S) :
Dr A K Satpathy, Dr S K Kar
Starting date : April 2008
Closing date : March 2011

Introduction

The pathogenecity and virulence of *P falciparum* has been linked to its expression of variant surface antigens (VSAs) that subvert acquisition of protective immunity and mediate infected RBC sequestration. Several studies have shown that parasites causing severe malaria in young children with little protective immunity tend to express VSA_{SM} which are serologically distinct from VSA_{UM} expressed by most parasites causing uncomplicated malaria and sub-clinical infection in older, and more immune, individuals. The PfEMP1expressed in the membrane of the late-stage-infected erythrocytes is a family of VSAs which binds to various receptors –such as CD36 or ICAM-1 on the vascular endothelium, CSA in the placenta, and CR on the red blood cells-that leads to microvascular obstructions in various organs. The PfEMP1 proteins are structured in to several semi conserved domains-namely an N terminal segment(NTS), various

Duffy binding-like(DBL) domains, a cystein rich inter domain region (CIDR), in some instances a constant 2(C2) region, a trans membrane domain, and a conserved, C terminal acidic terminal segment(ATS), which represents the intra cellular part of PfEMP1 that anchors the protein to the cytoskeleton. In the *P falciparum* line3D7, PfEMP1 is encoded by approximately 59 *var* genes per haploid chromosome. Most var genes can be assigned to 1 of 3 types (var groups A, B and C) mainly according to their conserved 5' upstream sequences. In 3D7, the majority of *var* genes belong to the subtelomerically located *var* group B, whereas 13 *var* group C genes are arranged in chromosome internal clusters. Ten mostly larger, subtelomerically located *var* genes with a distinct domain structure belong to *var* group A. *Var* genes are exclusively expressed but undergo switching within parasite lineages. Recent molecular phenotypic studies conducted in Southern Tanzania and Papua New Guinea have found differential expression of *var* genes in severe (cerebral) and uncomplicated malaria cases. Since no studies has been done in this regard in India, the present project proposal has been aimed to find out the association *var* gene sub groups with clinical manifestation of severe falciparum malaria expressed by the *P falciparum* isolates prevalent in Orissa.

Objectives

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

Work Progress

This project proposal has been submitted to DBT for extramural funding. But funding is awaited. Therefore the project has been initiated with intramural fund since April 2008.During this period about 23 mild cases and 12 severe cases (cerebral malaria) has been selected for the study. About 2ml of venous blood was collected in EDTA and total RNA was isolated using TRI-Reagent as recommended by the manufacturer (INVITROGEN).The RNA was purified and dissolved in DEPC water. The cDNA was synthesized using first strand cDNA synthesis kit (Fermentas) and random hexamer primers in a 20ul reaction mixture as recommended by the manufacturer. The quantitative estimation of the PfEMP1variant specific cDNA was done by Real Time PCR using QuantiTect SYBR Green PCR kit. Rox was taken as the internal reference dye and expression level was studied by relative quantification. The RT-PCR results indicate that the var group A of transcripts are more abundant in mild group of patients while var group B of transcripts are more abundant in severe group of patients in Orissa.

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

Status :
Extramural (NVBDCP, Govt of India)
Principal Investigator :
Dr M R Ranjit
Co-Investigator :
Dr A S Acharya
Starting date : March 2006
Cosing date : August 2009

Introduction

The EDPT is the most practical approach for control of malaria. According to national drug policy CQ is the first line of drug and SP is the second line of drug for treatment of uncomplicated malaria. But widespread resistance to the first line of drug might be the cause of high incidence of malaria attributed deaths in the state of Orissa, which needs continuous monitoring /surveillance to assess its efficacy and develop a strategy to prevent its spread. The WHO has out lined three ways of measuring drug efficacy (i) in vivo clinical response (ii) in vitro drug sensitivity assay and (iii) by using molecular marker. Though the first two methods are specific and quiet 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 falciaprum* mutidrug resistant gene analog (*Pfmdr*1) has been observed to be associated with the development of resistance to chloroquine in different studies including our own study conducted during 2002-03. However, the frequency and distribution of CQ and S-P drug resistance markers has not been known. The proposed study aims at (i) generating a baseline data on the frequency and distribution of CQ and S-P resistance markers in different physiological regions of the state and (ii) predicting the origin and spread of these genotypes through *P falciparum* populations in this particular region of the country. This information will serve as a public health tool to develop a rational drug policy and combat spread of drug resistance.

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.

Progress

During this period we have examined the polymorphism of 8 microsatellite markers (4 upstream and 4 down stream of the 76 codon) in about 160 *P falciparum* isolates having *Pfcrt* K76T mutations to investigate the

probable origin of this mutation in this geographical region of the country .All markers except one seem to be highly polymorphic with two or more alleles (Fig 1). Comparison of the frequency distribution and type of polymorphism prevalent in Orissa with the other parts of the world (Africa and Papua New Guinea of Asia) indicates independent origin of this mutation, by positive Darwinian selection at the pfcrt locus. (Fig 2). The study is in progress to answer this question more precisely.

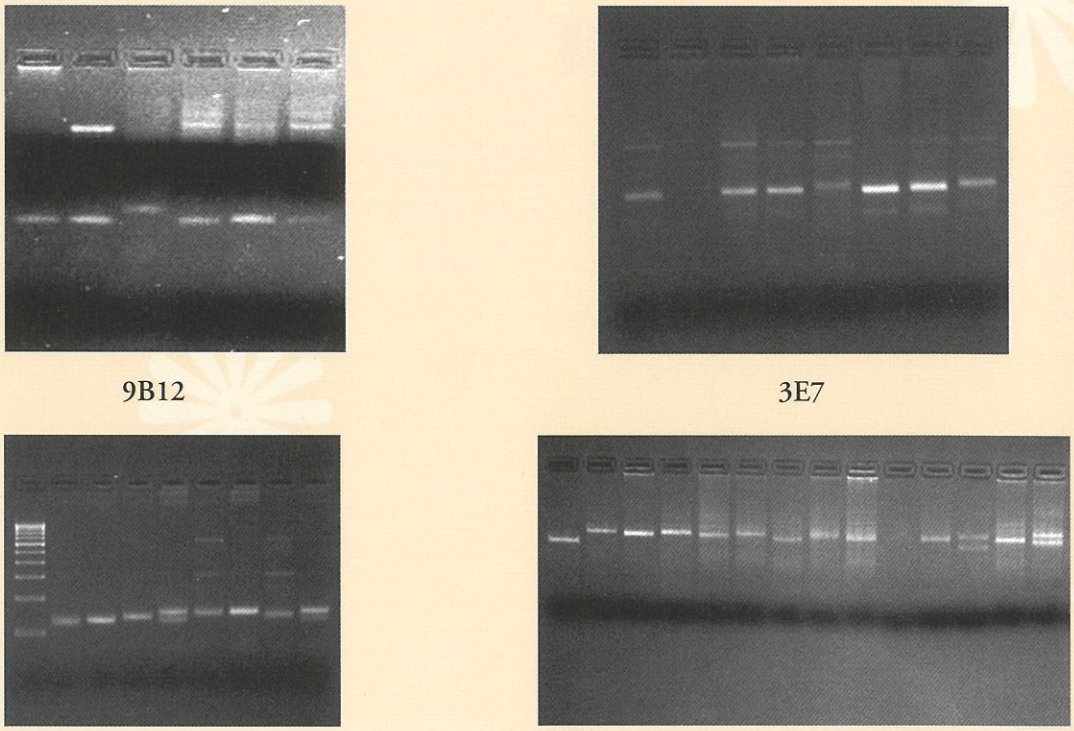


Plate1: Gel photo of some microsatellite showing size polymorphisms

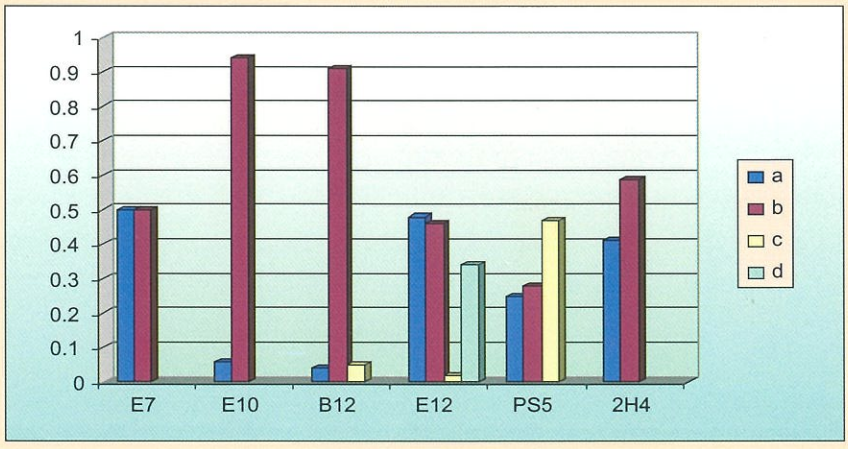


Fig 1: Genetic diversity on microsatellites

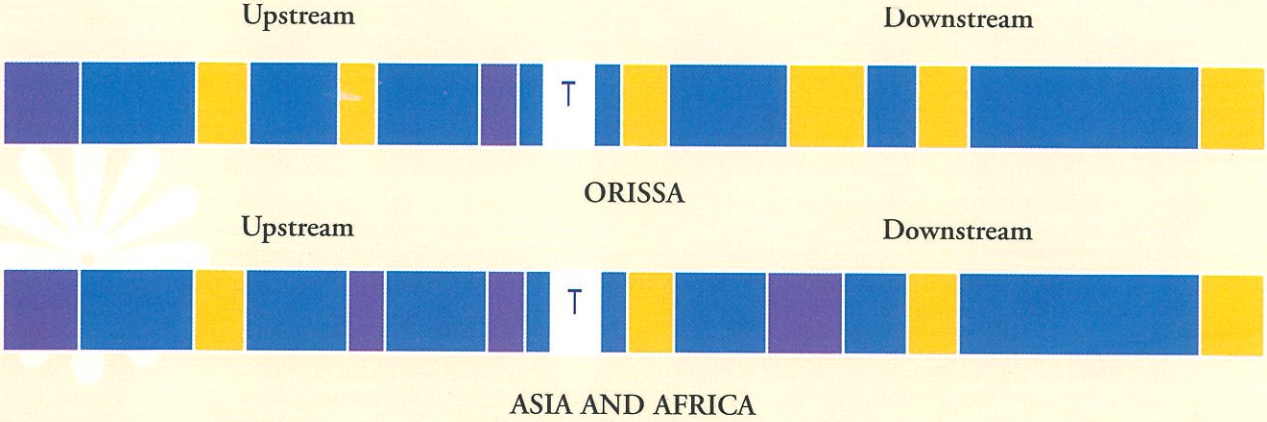


Fig 2: Genetic Polymorphism in Microsatellites flanking the *Pfcrt*

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

Status : Extramural (Funding : CSIR)
Investigator :
Dr. R.K.Hazra (PI)
Co-Investigators :
Dr. N.Mahapatra, Ms. Sunita Swain,
Starting date : March 2006
Closing date : Sept. 2008
(Extended to December 2010)

Introduction :

The project was initiated with intramural fund since March-2006. Funds received from CSIR for two year since December-2007 hence period was extended up to 2010.

The present study aims to distinguish the members of the *An. annularis* group. Vectorial and behavioral variations found among these species groups or complexes constitute the major reason for the need of accurate and precise identification. Realizing the difficulties of morphological identification and the need to elucidate the role of individual species in malaria transmission, molecular methods have been introduced for distinguishing the members of the *An. annularis* group.

Objectives:

1. To compare cytotaxonomic technique with new molecular technique like PCR, PCR-RFLP and SSCP specify to establish the accurate identification of the sibling species.
2. To develop microsatellite markers for *Anopheles annularis* species for population genetics analysis.

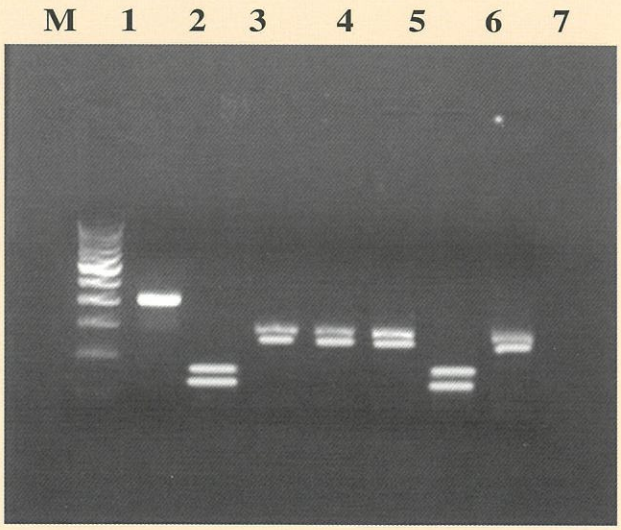
3. To develop multiplex PCR technique to achieve simultaneous detection of sporozoite identification, blood meal analysis and sibling species identification from single mosquito.

Work Progress:

The PCR-RFLP method has been shown to be more appropriate for dealing with a greater number of species and particularly in the case when the local fauna is not thoroughly studied. The assay developed for differentiating three members of the *An. annularis* group of species by digesting the domain-3 (D3) of 28S rDNA with the endonuclease *Msp*I, *Hae*III. Digestion of D3 with these two enzymes proved to be useful in the identification of the members of the *An. annularis* group of species, which are prevalent in Orissa. The D3 fragments were PCR-amplified individually using the above-mentioned methods and digested with *Msp I* and *Hae III* enzymes, which shows clear and distinct banding pattern differentiating the species of the *An. annularis* group. For *Msp*I the *An. annularis* have two cutting site where as *An. philippinensis* has one cutting site. Digestion of the D3 locus with *Hae*III differentiated these two members i.e. *An. annularis* and *An. philippinensis*, which were characterized by two and three cutting sites respectively.

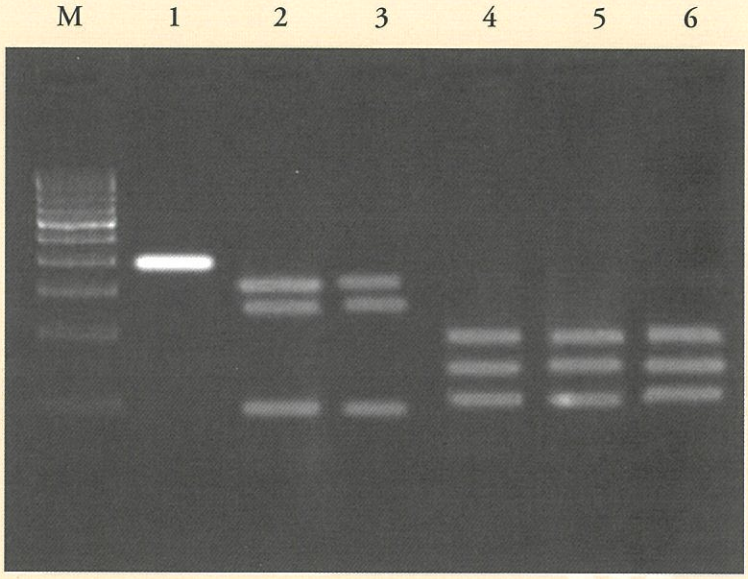
We developed a single step multiplex PCR assay for an easy and reliable way of identification of *An. annularis* group. The multiplex PCR assay will help to determine the two essential factor of vectorial capacity i.e. human host preference and sporozoite presence along with the species-specific diagnosis of *An. annularis* group in a single PCR assay. For discriminating closely related members we designed novel universal primers that bind to the D3 region of 28S rDNA 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 rDNA of the *P. falciparum* locus. For feeding preference analysis the human specific primers were used.

Figure 1



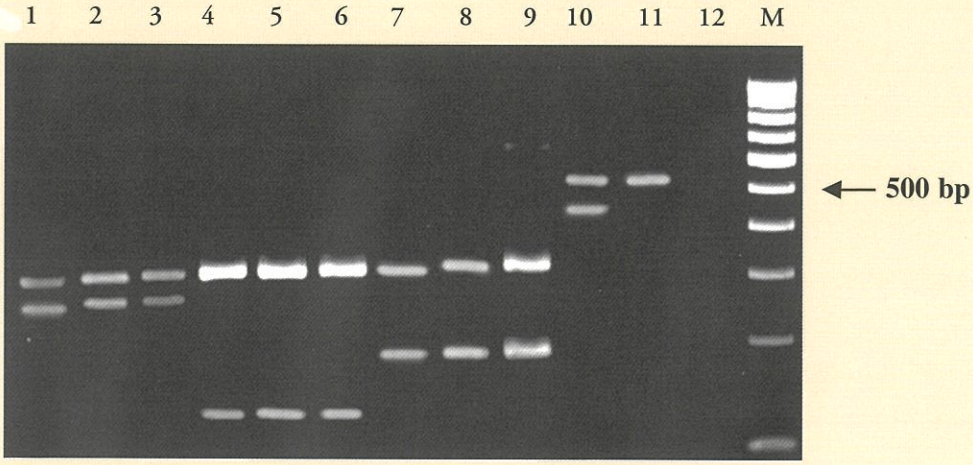
Gel shows digestion by MspI enzyme for *An. annularis* group of species. Lane M-Marker, Lane1-Undigested samples, Lane 2,6- *An.philippinensis*, Lane 3-5 & 7-*An. annularis* samples

Figure 2



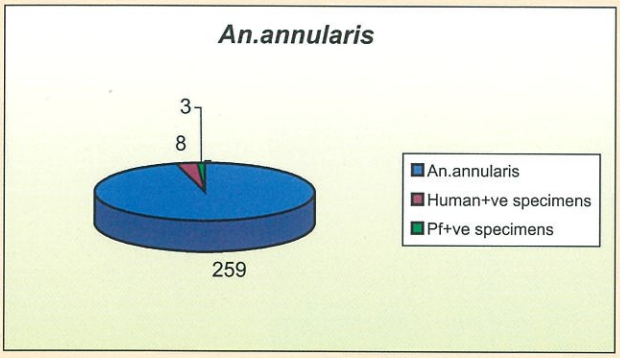
Gel shows digestion by Hae III enzyme for *An. annularis* groups of species. Lane M-Marker, Lane1-Undigested samples, Lane 2,3-*An. annularis*, Lane 4-6-*An.philippinensis* samples

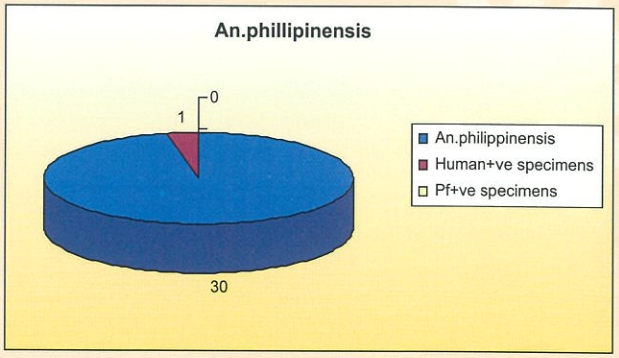
Figure 3

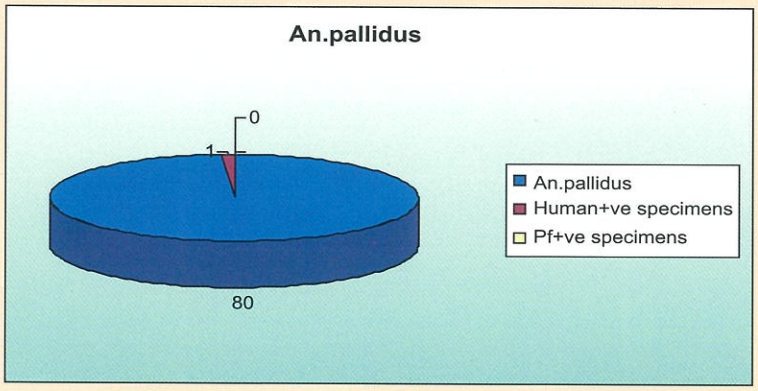


Lane 1, 2, 3, *An. annularis species* (285bp); Lane 4, 5, 6, *An. philippinensis* species (138bp); Lane 7,8,9, *An. pallidus* species (194bp); Lane 10, Presence of *P. falciparum* (429bp) as well as human (519bp) specific amplicons from the DNA isolated from blood of *P. falciparum*-infected persons;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

Multiplex PCR assay showing results of misidentified *An.annularis* group species along with their vectorial attributes







Major Achievement

A novel technique was developed for mosquito processing. Multiplex PCR method was developed for sporozoite identification, blood meal analysis and sibling species identification from single mosquito of *An. fluviatilis*. Single step multiplex PCR method was developed for simultaneous detection of *Anopheles annularis* group, human host preference and *Plasmodium falciparum* sporozoite presence.

Future work

Primer designing and characterization of microsatellite will be done for all the samples collected. Collection of *An.annularis B* from Uttar Pradesh. Cytotaxonomic study already started and will be compared with *An.annularis B* population genetics and evolutionary study of *An.annularis* from microsatellite data.

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

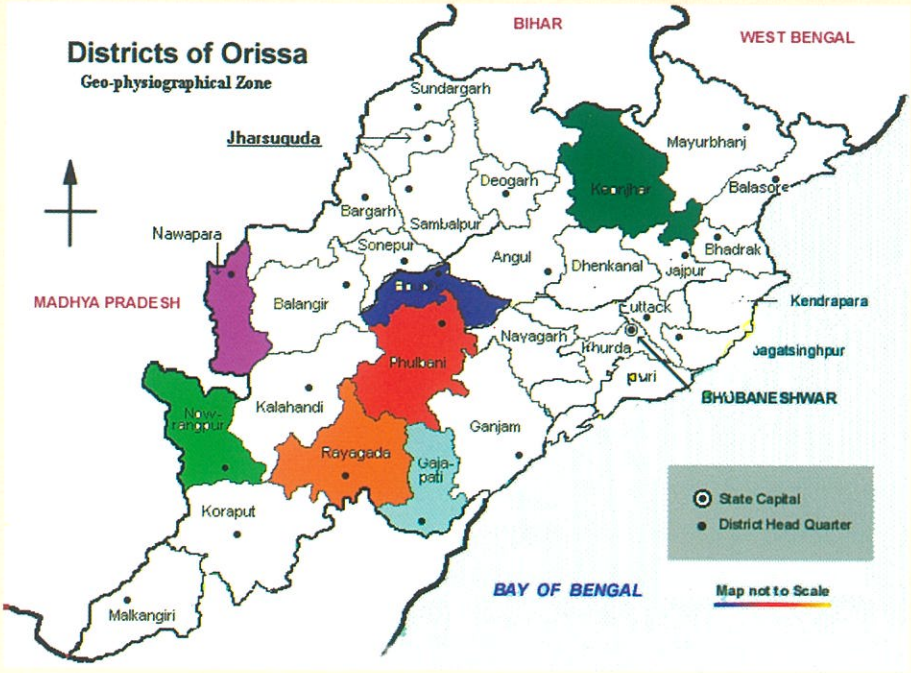
Status :
Extramural (NVBDCP)
Investigator :
Dr. R. K.Hazra
Co-Investigators :
Dr.N.Mahapatra, Mr.H.K.Tripathy
Starting date : March 2008
Closing date : Feb. 2009

Introduction

Mosquito fauna that transmits malaria and its insecticidal susceptibility status in Orissa has not been precisely studied, although few reports exist from patchy regions of state. In view of high morbidity reported in Orissa due to malaria it is essential to identify the vector responsible for transmissions of malaria in different region and its susceptibility to different insecticides so as to plan for appropriate insecticide spray and to develop IEC to curtail the spread of the diseases. As requested by State health department, seven high-risk districts are being studied.

Objectives

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



Work Progress:

Out of seven districts (i.e, Baudh, Gajapati, Kandhamal, Keonjhar, Nawarangpur, Nawapara and Rayagada) only five were surveyed. Mosquito collections were done in Baudh, Gajapati, Kandhamal, Keonjhar and Rayagada district. This was repeated in all seasons.

District survey done:

BOUDH DISTRICT
PHC-Adenigarh No. of villages visited - 19
GAJAPATI DISTRICT
PHC-Guma, No. of villages visited - 5
PHC-Mohona, No. of villages visited – 13
KANDHAMAL DISTRICT
PHC-Phiringia No. of villages visited - 5
PHC- Nuagaon
KEONJHAR DISTRICT
PHC – Ghatagaon, No. of villages visited – 3
PHC – Jhumpura, No. of villages visited – 1
PHC – Padmapur, No. of villages visited – 3
RAYAGADA DISTRICT
PHC – Muniguda, No. of villages visited – 7
PHC- Bisama cuttack, No. of villages visited – 3

1. Vector survey of Keonjhar district

The study was undertaken in three PHCs of Keonjhar district, they are PHC – Ghatagaon, Jhumpura, Padmapur. Twenty-one species belonging to three genera of mosquitoes were collected from nine villages. From the total mosquito collected, Anopheles were 81.9%, Culex 17.8% and Mansonia 0.27% (Fig 1). Total 1101 mosquitoes were collected among which 902 were Anophelines. Among fifteen species of anophelines collected, five were identified as vectors of malaria they are *An.annularis, An.culicifacies, An.fluviatilis, An.varuna* and *An.philippinensis* (Fig 2). The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. The main malaria vectors were collected from both cattle shed and human dwelling.

The susceptibility status of *An.culicifacies* and *An.annularis* revealed that *An.culicifacies* and *An.annularis* were resistance to DDT (Table 1). All the species were observed susceptible to synthetic pyrethroid.

Figure 1

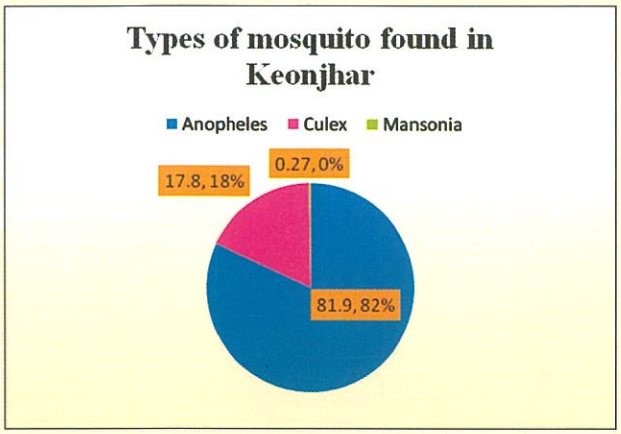
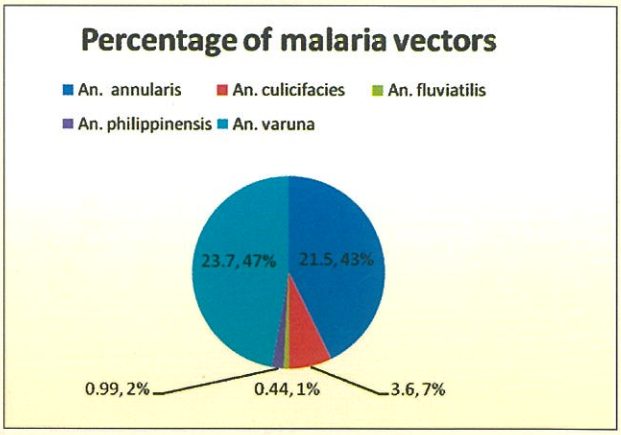


Figure 2



Susceptibility status to insecticides

Table 1

Vectors	DDT		Synthetic Pyrethroid	
	Susceptible	Resistance	Susceptible	Resistance
An.annularis		+	+	
An.culicifacies		+	+	

2. Vector survey of Gajapati district

Gajapati district comprises seven PHC including district hospital, they are Kashinagar, Gumma, Rayagada, B.K.Pada, R.Udayagiri, Mohana, Gurandi and Parlakhemundi. Mohana PHC was selected, as the area is maximum and highest no. of malaria cases were recorded. Fourteen species belonging to three genera of mosquito were collected from nine villages, were surveyed for vector collection. From the total mosquito collected Anopheles are 36%, Culex 62% and Mansonia 2% (Fig 3). Among nine species of anophelines collected, three were identified vectors of malaria they are *An.annularis, An.culicifacies and An.fluviatilis* (Fig 4). The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. *An.culicfacies and An.annularis* were collected from both cattle shed and human dwelling.

Figure 3

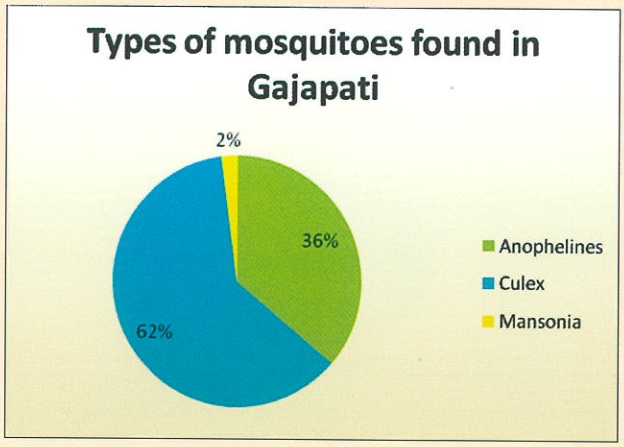
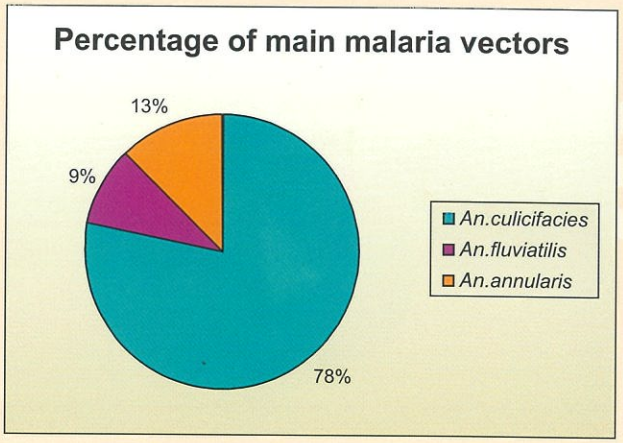


Figure 4



3. Vector survey of Rayagada district

The study was undertaken in two PHC of Rayagada districts, they are PHC –Muniguda, Bhisamacuttack. Nineteen species belonging to five genera of mosquito were collected from five villages. From the total mosquito collected Anopheles are 61.46%, Culex 35.88%, Mansonia 1.32%, Aedes 0.99% and Armigeries 0.33%. Twelve species were from genus Anopheles, the rest were of other genera. Among twelve species of anophelines collected two were identified as vectors of malaria they are *An.annularis, An.culicifacies*. The abdomen of major species showed half gravid (HG), full fed (FF), and unfed. The main malaria vectors were collected from both cattle shed and human dwelling. The following work has been done in all five districts.

Observation on biting habits

We have studied the biting habit of mosquitoes in indoor, outdoor and cattle shed in evening hours. *An.culicifacies, An.annularis* though bites animal and human in indoor habitats mainly, also found as a out door biter.

Observation on biting habits of *An.annularis*

The biting rhythm of *An.annularis* was crepuscular most of the biting was between dusk and 20.30hrs. Some reports said that *An.annularis* bites throughout the night .

An.annularis were found both in out door and indoor in resting time.

Observation on biting habits of *An.culicifacies*

Rao, 1951 found in warm eastern region, most of the feeding took place in the open. There is enough evidence that a good proportion of *An.culicifacies* adults rest both outdoors and indoors during day times. We observe *An.culicifacies* adults biting in the out door bait and also rest out side in Gajapati and Phulbani.

Conclusion

The prevalence of vectors and their susceptibility status to different insecticide which will ultimately help for planning of appropriate intervention measure to tackle the problem by the state health department.

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

Funding : Intramural
Investigators :
Dr. A. S. Kerketta (PI),
Dr. S. K. Kar
Collaborator : Dr. B. V. Babu (Social Scientist), ICMR
Starting date : August 2007
Closing date : July 2009

Background of the Project:

Malaria is a major public health problem of concern in the state of Orissa. In one hand there is substantial generation of knowledge on diagnostic tools and treatment and on the other hand beautifully designed malaria control programme still the malaria morbidity and mortality is persistent or in increasing trend. Indicating the non-accessibility or low utilization of the knowledge as well as the programme. Some important factors must be responsible from consumer as well as provider side. Therefore the present study has been developed to identify the factors responsible for the low utilization of the programme and to cover maximum population under Early Diagnosis and Complete Treatment (EDCT), by developing an innovative community tuned strategy for optimizing the utilization of the services provided by the grass root service providers through involving community participation and augmenting provider related bottlenecks.

Objectives:

1. Assessment of the community, the health providers and other related personnel and factors associated with Early Diagnosis and Complete Treatment (EDCT) process through a formative research.

2. Development, intervention and evaluation of an innovative strategy for access and utilization of EDCT the key strategy of malaria control programme.

Methodology:

Study design: The study involves two phases–
Phase – 1: Formative research – 4 months
Phase – 2: Strategy development & Intervention and evaluation of the strategy – 18 months (Consolidation – 2 months)

Formative research:

1. To understand the people's knowledge and perception on fever.
2. To assess the knowledge of the people to identify fever as malaria and triggering agent for treatment seeking.
3. To understand the treatment seeking behaviour of the community.
4. To assess the perception of the community regarding the existing health infrastructure and their functioning.
5. To examine the existing linkage between the health system and community

Rapid Malaria Test in the Field in Nayagarh

Survey method: Qualitative methods like questionnaire survey, illness narratives and in-depth interviews, focus group discussion.

Study area: One community development block Narasinghpur, of the Cuttack district. The selection of the block is based on the following criterion: poor accessibility, poor economy, lesser development, poor health infrastructure, low literacy and malaria endemicity.

Sampling: Stratified random sampling of the villages were undertaken. 2 PHC villages, 4 Health sub-centre villages, and 8 villages with no health facility (4 with anganwadi centre and 4 without them) were chosen.

Work progress:

The preliminary visit was made to the block to collect the base line socio demographic information. The block covers a total population of 135490 in 33 gram panchayat and 251 villages. The total area of the block is 635.5 square km of which forest land is 5256 hectors, cultivation land 2256 hectors and irrigated land is 2974 hectors. The villages are located in different geographical location like top hill, foothill, riverbank and plane areas. The population belongs to different ethnic groups. The Schedule Caste comprises 30.25% and the Schedule Tribe comprises 6.07 % of the total population.

The available health infrastructure in the block is one area hospital, one government hospital, three PHCs (new) and one CHC. There are 9 Medical officers at different health facilities. Of which 5 medical officers and 9 health supervisors, 44 health workers and one technician are involved in the malaria control programme. There are 138 Anganwadi worker and 137 ASHAs who act as the grass root health provides named as drug distribution center (DDC) holders. In the state the DDCs have been converted to as fever treatment depot (FTDs), after imparting training on blood slide collection and distribution of full course of antimalarial drug like chloroquine. Till date 125-grass root provides has been converted to FTDs in the block. The average malaria surveillance data of last three years shows ABER 6.6%, SPR 3.56% Pf % 82.5 and API 2.78% indicating the area as high malaria transmission area.

Two villages were surveyed for fever cases during the visit. One at top hill and other at riverbank. The total populations covered in the villages are 363. Of which female comprises 172 (47.4%) and male 191(55.6%). A total of 159 fever cases reported on the day of visit in the villages. The information on the treatment seeking behaviour was collected. That revealed a merely 27.0% reported to the government health facility for the treatment of fever. Rest 52.8% reported to the private practitioners and 20.1% received treatment from other providers like traditional healer or alternative medicine practitioners.

The preliminary information shows that the use of government health facility is very poor in the area. Most of the people opted to visit the private practitioners. The study will help to establish the reason for low utilization of health facility.

1.13 Mapping of P.falciparum susceptibility to Chloroquine in seven high malaria endemic districts of Orissa.

Status :
Extramural (NVBDCP, Govt of India)
Principal Investigator :
Dr. Anna S. Kerketta
Starting date :
November 2006
Closing date :
November 2008
(Extended up to 2009)

Background:

The fund from NVBDCP, New Delhi has been received in the month of March 2008 with sanction of study period of 18 months. The proposed study districts namely Keonjhar, Kandhamala, Gajapati, Ganjam, Rayagada, Kalahandi and Nabarangpur has been visited and the baseline information has been collected. However with the financial support from NVBDCP, Orissa and on request of state health department the susceptibility to chloroquine by P falciparum malaria parasite has been studied in Nayagarh district of Orissa during July 2008.

Objective:

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

Materials and Method:

Test system:

WHO guideline (2001) for assessment of therapeutic efficacy of antimalarials.

Test type: 28 days extended test.

Sample size:

WHO guideline for estimating the population proportion (2001) was applied for calculation of sample size.

Patient examination during door to door malaria survey

Study area:

The Madhyakhanda PHC area of Nayagarh district, Orissa. The table-1 depicts the malaria situation in the PHC area.

Table 1-. Malaria situation in PHC area as per last three years surveillance data

Year	Population	ABER	SPR	Pf%	API
2005	99445	6.49	3.99	85.32	2.59
2006	98994	6.93	5.99	83.70	4.15
2007	100568	6.39	2.43	89.10	1.55
Average	99002	6.6	4.1	86.04	2.76

Study procedure:

The study was conducted in Madhayakhanda PHC area of Dasapalla block, Nayagarh district of Orissa in collaboration with the state health department. The team comprised of two physicians, two technical assistants, and three technicians (one from state health department and two project staff). The health workers of the particular sub-centres accompanied the team to the study villages. Initially to select the eligible case a rapid fever survey was done by door-to-door visit in six villages namely Jamusahi, Jagannathprasad, Takra, Tangiapalli, Sankurdengi and Pankua. In a central place of the village the camp was organized and all the fever cases were

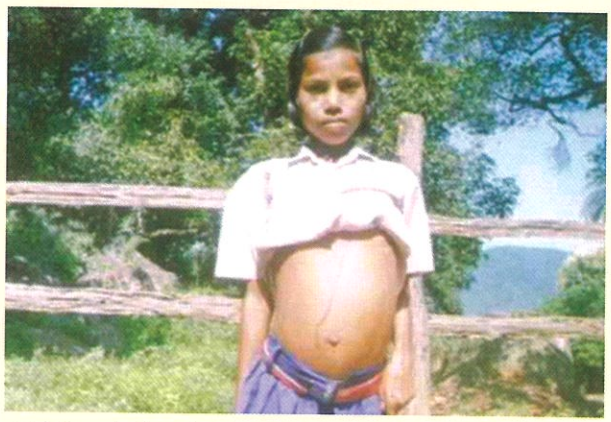
asked to report there. A total of 97 fever cases were screened after taking a careful and precise registration of address. Prior to the enrolment the patient and his/her attendant were briefed in details about the aim, procedure and the benefits of the study. There after informed and written consent was obtained from each subject. Each patient underwent physical and clinical examination with a careful history of the duration of fever and on treatment sought. The axillary temperature was recorded with the help of an electronic thermometer and the body weight was measured using a calibrated scale-weighing machine. A finger prick blood was collected from each patient for a thick and a thin smear in duplicate and also for rapid diagnostic kit test (RDK). Simultaneously the thick and thin smears were prepared and were air dried rapidly. The thin smear was fixed with anhydrous methanol. The thick smears were dehaemoglobinised and were stained with Giemsa stain 3% and at pH 7.2 and examined on same day. The parasite count was done against 200 WBC in thick smear. Thin smears were used to confirm the parasite species. Taking consideration of inclusion and exclusion criteria, 57 eligible cases were selected for the study. (The detail is given in Table-2).

Table- 2 Enrollment characteristics of the study population

		Mean	Range
Age (in years)		14.4	4-65
Gender	*M=32	52.8	6-65
	*F=25	47.2	6-40
Axillary temperature		37.7	37.5-39.3
Asexual parasite		6190	1000-48000

*M=Male, * F=Female

All 57 cases were administered with Chloroquine 150 mg base (Brand name Lariago, from Ipca Pharma Pvt Ltd) on the spot and under supervision of the medical team, after noting down the Batch No, MFD and EXP date. The dose schedule followed the WHO recommendation (10mg/Kg Body weight on days 0 and 1 and 5mg /kg on day 2) after or with food. The study subjects were monitored daily by the medical doctor for initial 3 consecutive days.



Girl from high malaria endemic area with huge splenomegaly

Follow-up:

The study subjects were followed up on post treatment days 1, 2, 3, 7, 14, 21 and 28, with detail clinical examination and blood smear for parasite count. Besides, on each day the danger signs like not able to drink or feed, repeated vomiting, convulsions during present illness, lethargic or unconscious and unable to sit or stand (as per WHO guideline) noted. The case found to have treatment failure with CQ were treated with second line of drug Sulfadoxine pyrimethamine and Artesunate according to the standard NVBDCP guideline. In all cases the health of the study subject was given priority over the test.

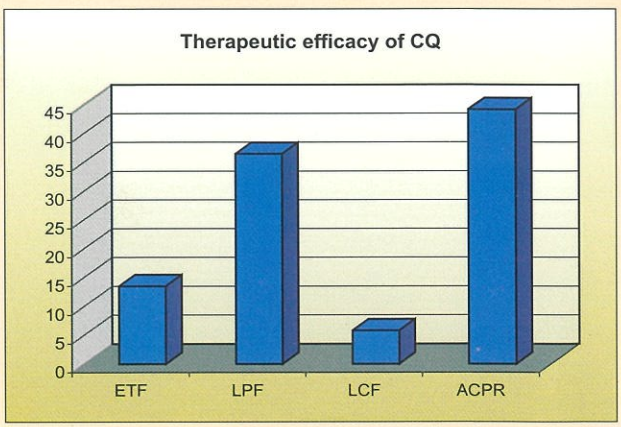
Dropouts:

Out of total study population 1(1.7%) dropped out from the study, as he could not be traced since he moved to his relative's house, which is out of our reach of active follow up. One girl (1.7%) was given treatment from out side. Three (5.3%) were detected having *Plasmodium vivax* trophozoites on day 7 and 14. Thus a total of 52 (91.2%) subjects could be followed up till 28 days study.

Results:

Out of the total cases studied, 52 cases followed up till the completion of study period of 28 days. The study revealed, Early treatment failure (ETF) in 7 (13.5%) cases, of which 2 (28.6%) had very high fever i.e axillary temperature more than 39.5^{0}C, one case (14.2%) developed danger signs and had severe vomiting with high fever, two (28.6%) cases had parasitaemia on day 2 was more than day 0 and 2 cases (28.2%) had parasitaemia on day 3 found to be more than 25% of day 0.

The Late treatment failure (LTF) was marked in 22(42.3 %) cases. Of which 19 (36.5%) had late parasitological (LPF) i.e. 10 (52.6%) cases found to have parasitaemia on day 7, 5 (26.3%) on day 14, 2 (10.5 %) on day 21 and 2 cases (10.5 %) on day 28. The late clinical failure (LCF) was marked in 3 (5.8%) cases. Of which one case (33.3 %) developed severe malaria as convulsion on day 7 and 2 (66.6%) had history of fever with presence of parasitaemia. Thus the total treatment failure with CQ is found to be 29(55.8%). Adequate clinical and parasitological response (ACPR) was marked in 23 (44.2%) cases.



Inference:

The present study reported a high prevalence of Chloroquine resistance in Madhyakhanda PHC area of Dasapalla block, Nayagarh district of Orissa. Since the CQ resistance is more than the cut off limit, it indicates urgent need of switching over to second line of antimalarial drug in the area.

1.14 Effectiveness of combined regimens with iron-folic acid, vitamin B12 supplementation, deworming and nutrition education in control of anemia in tribal adolescent girls in Gajapati district of Orissa.

Status :
Extramural (ICMR Tribal Task Force)
Investigator : Dr.G.Bulliyya, (P.I)
Co-Investigators :
Dr. B. Dwibedi, Dr. A. Maharana, Mrs. G. Mallick
Starting date : November 2006
Closing date :
October 2008 (Extended to 2010)

Objective

1. To carry out an intervention study on effectiveness of combined regimens with iron-folic acid, vitamin B12 supplementation, deworming and nutrition education in control of anemia in tribal adolescent girls in Gajapati district.

Background

The study was initiated with intramural fund and ICMR tribal task force has approved it in 2008 for funding for two years and funds awaited. The study has been done so far with intramural fund.

Anemia 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 anemia is the major cause. Prevalence of anemia among pregnant women and adolescent girls reported 81% and 96% respectively. Despite the national anemia control program, anemia 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 pre-existing iron deficiency anemia in women at the time of conception. Adolescence, as a period of growth and development, is considered the best time to intervene in order to assist in physical and mental development, and to prevent later maternal anemia, thereby determine the well-being of the next generation. To achieve the goal of controlling anemia in adolescent

girls, a 5-arm regimen approach is being adopted to compare the efficacy of iron and folic acid administration when combined with deworming, vitamin B_{12} and nutrition education through routine monitoring by the existing ICDS network.

Methodology for Baseline survey

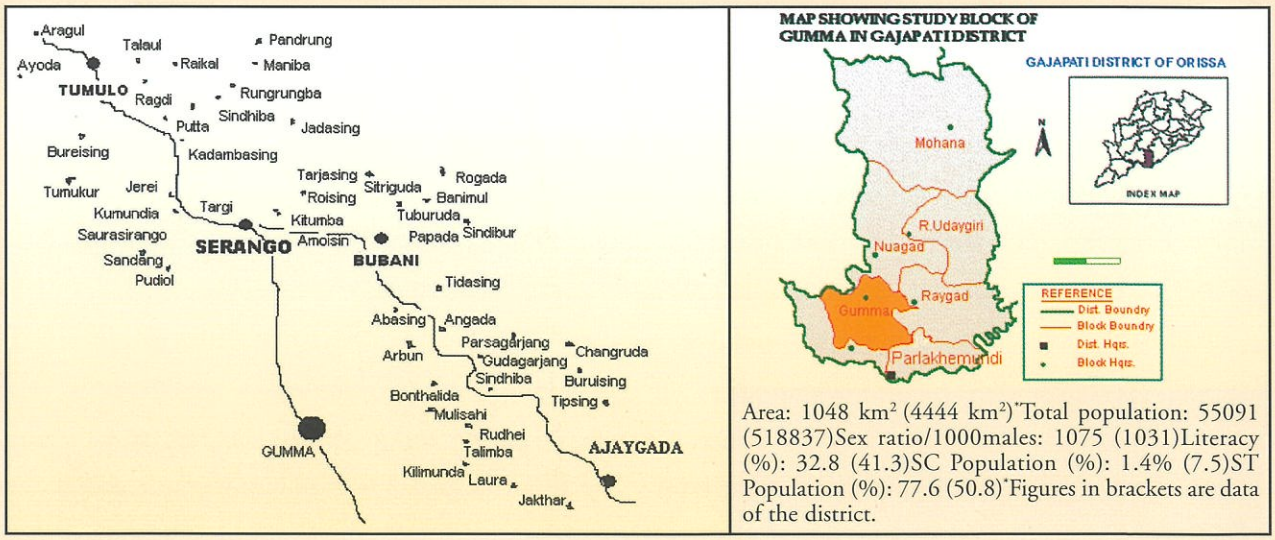
Serango sector in Gumma blocks of Gajapati district is selected for the study because it is pre-dominated by tribal people and primitive tribal groups (Figure-1).

Calculation of sample size is based on the anticipated increase in mean haemoglobin levels by 1.0 g (taking Hb= 10.7 g/dl and standard deviation [SD]=1.46 g/dl at 95% confidence and 80% power), the required sample number is 60, which is multiplied by 2 for allowing analysis to examine between age groups (12-14y and 15-18y) separately, considering 20% dropout rate to follow-up, and excluding 5% severe anemia grade for referral treatment, thus, a total of 750 adolescent girls included.

A pre-tested questionnaire was used to collect the household data, socioeconomic information of parents, adolescent girls, age, sexual maturity, personal hygienic practices, morbidity, clinical examination, KAP on anemia and its control measures and food consumption patterns. Anthropometric measurements were taken using standard equipment and procedures. Blood samples were collected for haemoglobin and blood smear (thin and thick) for detection of malaria. Serum samples were collected after centrifuging blood samples for ferritin and transferrin receptor levels using ELISA techniques. Anemia was considered to be present if the Hb value was below 12 g/dL for adolescent girls. Levels of anemia 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 <8.3ug/ml were considered to indicate depleted body iron stores and iron deficiency respectively. Stool samples were collected and examined for intestinal worm infestations.

Results

Figure 1. Map of Serango Sector showing study villages Gajapati district (Orissa)



Area: 1048 km² (4444 km²)*Total population: 55091 (518837)Sex ratio/1000males: 1075 (1031)Literacy (%): 32.8 (41.3)SC Population (%): 1.4% (7.5)ST Population (%): 77.6 (50.8)*Figures in brackets are data of the district.

A total of 301 (altogether 8/5 with 5/4 girls in last year) adolescent girls aged 12-18 years were studied from four Gram Panchayats in Serango area of Gajapati district. Majority of study population belonged to ST, while 6% SC community. Two thirds of the households had kutcha house. About 31% of the adolescent girls were literate. Electricity was available in 17% and piped water was available in 4% of households. Sanitary latrine available in less than 5% of the households.

Clinical examination by physician was carried out and illness history recorded to find out the common illness, which might contribute towards the anemia and to exclude other diseases in adolescent girls. History of fever due to malaria was reported by 29-53% of girls. While features of recurrent malaria and hepatosplenomegally were not of high magnitude (2%), intestinal helmenthesis as visible worm passage in stool was reported in 15% of individuals. Diarrhea and upper respiratory tract infections were the other common disorders, the prevalence of which was 5.1% and 13% respectively. Three-fourth of adolescent girls had clinical sign of pallor. The study from the four GPs were almost similar in terms of clinical illness and of common disorders. Worm infestation was the most common associated feature. Recurrent illness leading to anemia was not predominant in the study population as per clinical impression.

Table 1. Clinical examination among adolescent girls in Serango sector in Gajapati district

Clinical sign	Total (253)	Serango (50)	Bubani (79)	Tumulo (49)	Ajaygad (75)
History of fever	62.8 (144)	62.0 (31)	59.5 (47)	73.5 (36)	40.0 (30)
Malaria	37.5 (95)	36.0 (18)	29.1 (23)	53.1 (26)	37.3 (280
Diarrheal disorders	5.1 (13)	4.0 (2)	5.1 (4)	8.2 (4)	4.0 (3)
R.T.I	13.0 (33)	14.0 (7)	15.2 (12)	22.4 (11)	4.0 (3)
Common cold	18.5 (47)	36.0 (19)	25.3 (20)	14.3 (7)	1.3 (1)
Joint pains	4.0 (10)	8.0 (4)	22.2 (2)	0.0 (0)	5.3 (4)
Worm passage	15.0 (38)	14.0 (7)	25.3 (20)	12.2 (6)	6.6 (5)
Pallor	66.0 (167)	62.0 (31)	68.2 (46)	67.3 (33)	76.0 (57)
Lymphadenopathies	7.1 (18)	8.0 (4)	10.1 (8)	10.2 (5)	1.3 (1)
Liver enlargement	0.4 (1)	0.0 (0)	1.3 (1)	0.0 (0)	0.0 (0)
Spleen enlargement	1.6 (4)	2.0 (1)	22.2 (2)	2.0 (1)	0.0 (0)

Figures in parenthesis are sample numbers.

Iron deficiency anemia

The mean haemoglobin concentration of adolescent girls was 10.3 g/dl, ranged between 6.5 g/dl and 15.5 g/dl. Overall, 81% of adolescent girls had anemia, of which 34%, 33% and 4% had mild, moderate and severe grades of anemia respectively. The proportion girls having moderate and severe anemia was relatively higher in younger (12-14 y) than in older age group (15-18 y).

The mean serum ferritin concentration was 34 ± 21.5 ug/L in a sample of 136 adolescent girls. The proportion of girls with inadequate iron stores (< 15 ug/L) was 36%, while a considerable proportion (46%) had ferritin levels below 50 ug/l, which is considered the cut-off value for defining hypoferritinaemia. The serum soluble transferrin receptor concentrations revealed that 69% of 136 tested samples had iron deficiency.

Malaria slide examination

Microscopic diagnosis of malaria in blood smears revealed that out of 216 slides examined, about 11.3% were found to be slide positive for malaria parasites. Of total positive for malaria, 5.7% were identified as having *P. vivax* and 4.7% as *P. falciparum* infections, while 0.9% of slides were interpreted as having mixed *P. vivax* and *P. falciparum* infections.

Field team conducting assessment of nutrition knowledge of Lajia Saura primitive tribal community in Serango sector, Gajapati district

Haemoglobinpathies

The blood samples were analyzed in BioRad-Variant analyzer for haemoglobinopathies. Out of 301 cases, 3 (1%) were identified as homogygous sickle cell disease and 37 (12.3%) sickle cell trait. β-thalassemia minor was seen in 11 (3.7%) cases, and none were β-thalassemia major.

Peripheral blood smear collected and examined by a pathologist from 181 slides. These sliders were stained and examined for type of anemia. Microscopic examination of blood smear revealed that majority had normocytic hypochromic (46%), followed by normocytic normochromic (27%), microcytic hypochomic (20%) and dimorphic (7%) anemia.

Intestinal worm infestations

Out of 18 stool samples examined, the prevalence of intestinal parasites was 27.7% and mixed infestation was seen in 8% of samples. Vitamin A in serum samples is under process of study. After coverage of 1000 samples, the intervention regimens (5-arms) will be instituted.

1.15 Epidemiology of viral hepatitis in tribal populations of Orissa, Madhya Pradesh /Chhattisgarh and Jharkhand, India. – Multicentric study

Status : Extramural Funding ICMR Tribal Task Force
Investigator : Dr. S. K. Kar(PI)
Co-Investigator: Dr.B.Dwibedi, Dr. B. V. Babu, Dr. A. Mohapatra, Dr. A. S. Acharya,
Collaborator: Dr. (Prof) S.P.Singh, (HOD, Gastroenterology), SCB Medical College, Cuttack
SRF : Dr. LalMohan Ho, Dr. Jyotsnamayee Sabat
Starting date : March 2006
Closing date : March 2009

Aims & Objectives:

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

1. To determine the prevalence of hepatitis A, B, C, D & E viruses along with the circulating genotypes of HBV & HCV.
2. To assess the risk factors of transmission of hepatitis virusesPrevalence of pre-core and basal core promoter mutants of HBV

Rationale of the Project:

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

Summary of progress:

Study has been initiated to assess the prevalence of hepatitis virus infections and associated risk factors for transmission in five primitive tribes (eg. Lodha, Saora, Khadia, Mankidia and Juanga) in Mayurbhanj and adjacent areas of Orissa. (Annual Report 2006-07) . Minimum sample size of 0.99% of the total population from each of

the tribes was considered adequate, taking HBsAg prevalence of 5%. Representative samples covering all ages and both sexes were drawn from different clusters/inhabitations of the above tribes for inclusion in the study. A total of 1426 individuals were covered during the study from the five tribes (Table 1) the distribution is given below.

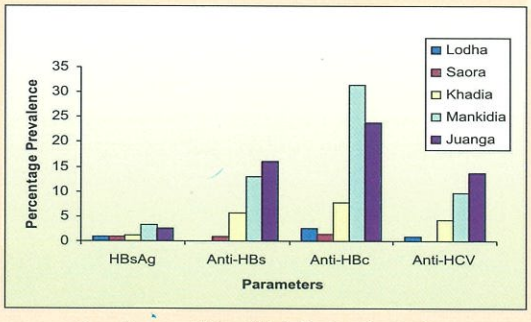
Table-1: Distribution pattern of five primitive tribes

Name of the tribe	Census Population	Population covered(%)	Block covered	District
Lodha	2405	242(10)	Morada,Suliapada,Udala	Mayurbhanj
Saora	3740	212(5.6)	Morada, Suliapada	
Khadia	15405	450(3.0)	Karanjia, Jasipur, Udala	
Mankidia	713	262(36.7)		
Juanga	15719	260(1.6)	Gonasika	Keonjhar
Total	37982	1426 (3.7)		

Clinical examination findings and history of illness were recorded. Predesigned risk factor questionare was filled in while discussing with the individuals or guardians. Blood samples were collected aseptically and transported to RMRC laboratory in freezed condition. Serological screening test were done on all the samples and molecular diagnostic tests performed on HBV and HCV seropositives.

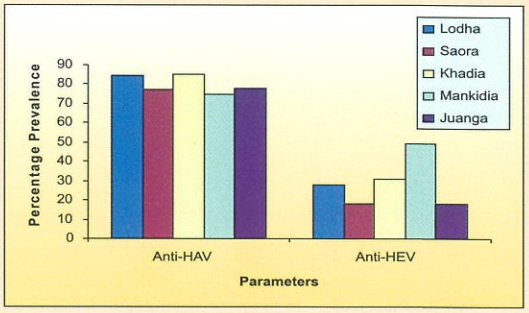
Serological tests were conducted on all the samples collected HBsAg positivity was noted in 1%, 0.9%, 1.1%, 3.2% and 2.7% cases in Lodha, Saora, Khadia, Mankidia and Juanga tribes respectively where as Anti HBc IgG positivity was 2.5%, 1.4%, 7.8%, 31.5% and 24% in those tribes(fig 1). HBV DNA was detected by PCR in 36% of HBsAg positive samples and the HBV viral load ranged from< 250 to 2.62 x 10^8 copies/ml.Occult HBV DNA was detected in 28% of the samples tested. All HBV DNA positive samples were subjected to genotyping and all were found to be Genotype D and all were wild type for pre-core DNA.

Fig 1



Prevalence of Hepatitis infection in 5 tribes

Fig 2



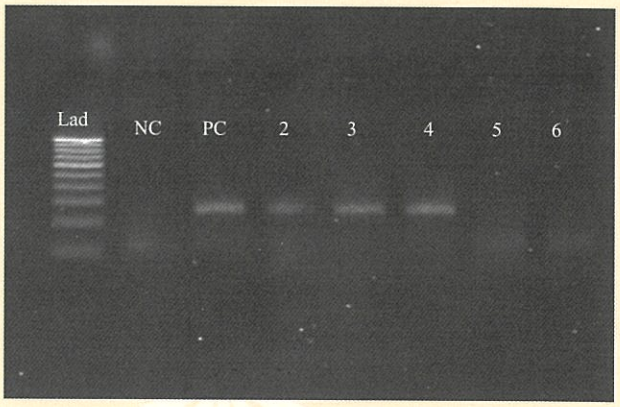
Prevalence of Hepatitis infection in 5 tribes

Antibody to HCV was detected in 1%, 0%, 4%, 9.8% and 13.8% in Lodha, Saora, Khadia, Mankidia and Juanga tribes respectively. Evidence of exposure to Hepatitis A virus infection(i.e HAV IgG) was 75-85% and to that of HEV was 18-49% in the above tribes. HCV RNA was positive in 3 out of 6 anti HCV positive sera tested by RTPCR Fig. 3.

Factors relating to both enteral & parenteral routes for transmission of hepatitis viruses were looked in. Unsafe drinking water, unhygienic preservation of cooked food (Rice & Kanji), was observed as a common practice in all the tribes. The observed risk factors for parenteral transmission were; tattooing (9-31%), Sharing of razor(3-18%), Body piercing(21-42%), Blood letting(2-24%), H/O Multiple injection (26-58%),Shaving in barber shop/by village barber (18-33%) and scarification(2-25%) (Table 2).

Association/contribution of these risk factors towards transmission of the infection will be analysed in-depth subsequently.

Fig.3



Lane 2, 3, 4 are positive. Lane 5, 6 are negative samples
Amplification of 5'NCR Region of HCV RNA

Table 2: Prevalent Risk Factors for Parenteral Transmission

Tribe	Lodha	Saora	Khadia	Juanga	Mankidia
Tattooing	18 (9.04%)	29 (13.69%)	50 (13.92%)	18 (7%)	29 (31.52%)
Sharing of Razor	7 (3.51%)	54 (25.47%)	103 (28.70%)	58 (22.3%)	17 (18.47%)
Body Piercing	42 (21.10%)	66 (31.13%)	135 (37.60%)	92 (35.38%)	39 (42.39%)
Blood Letting	4 (2.01%)	52 (24.52%)	40 (11.14%)	2 (0.76%)	9 (9.78%)
H/O Multiple Injection	52 (26.13%)	97 (45.75%)	211 (58.77%)	125 (48.07%)	49 (53.26%)
Shaving in Barber shop	66 (33.16%)	61 (28.77%)	104 (30%)	58 (22.30%)	17 (18.47%)
Scarification	50 (25.12%)	17 (8.01%)	21 (5.84%)	6 (2.30%)	5 (5.43%)

Quality control and technology transfer:

All positive and 5 % of negatives were tested at NIV Pune and external quality control assured . Transfer of moleculat technology could be undertaken through man power training and successful implementation of the molecular diagnostic test related to HBV and HCV at the centre.

Subsequent plan of activity:

The above observations has shown that two tribes (Mankidia and Juanga) are having high prevalence of Hepatitis C virus infection hence the risk factor for transmission needs to be studied in depth. Questionare for addressing each of the risk factors has been developed for population groups, key informants etc which will be pretested and modified for utilisation during in-depth interview and focus group discussion. The data generated will be validated with the already collected the individual information.

Because the present study population was derived from Mayurbhanj and adjacent areas it was suggested by the review committee that, to make the data representative for the state of Orissa, for the respective tribes, information on risk factors for hepatitis transmission need to be obtained on similar proportion of samples from geographically separated inhabitations of these tribes if any, which will be helpful for formulating prevention strategy for the tribes with high risk in the state. So the distribution pattern of the studied tribes has been ascertained in other parts of the state and are now included in the study. As per recommendation, proportionate samples will be covered for the two tribes with high HCV infection from geographically distinct areas. Hence, the distribution of

Table 3: Distribution of Mankidia and Juanga tribes.

	Total population in the district					
Tribe	Baleswar	Deogarh	Jajpur	Anugul	Cuttack	Dhenkanal
Mankidia	108	134	36			
Juanga			707	926	480	16104

Mankidia and Juanga tribes outside the studied area in the state of Orissa (2001 census) was ascertained and given below.
After serological investigation the data will be compared . Risk factor analysis will also be done after generating data both through individual and group questioniare to identify specific contributory factors if any.

Because of inclusion of above activities which are pertinent to obtain risk of Hepatitis C infection in 2 vulnerable tribes and data to be representative of Orissa region, the work need more period of time, hence period of study needs to be extended for 12 more months than the schedule.

1.16 Preventive strategy of severe diarrhoeal disorders in tribal dominated Kashipur, Dasamantpur and Th. Rampur blocks of Orissa

Funding : Govt. of Orissa
Principal Investigator : Dr. B. B. Pal
Co-investigators :
Dr. H. K. Khuntia, Mr. S. K. Samal
Mr. B. K. swain
Collaborator : Dr. Bikash Pattanaik, IDSP,
Govt. of Orissa
Period : Three months, 2008

Background:

As per the request of the DHS, Govt. of Orissa, the study was initiated in the three tribal blocks to look for different bacterial enteropathogens including the *V. cholerae* and the recent emergence of new variant of El Tor Variant of *V. cholerae* O1 Ogawa with *ctx*B gene of classical strains.

Objectives:

1. To isolate and characterize any bacterial enteropathogens including *V. cholerae* isolated from diarrhoea patients and different environmental water samples.
2. To study the behavioral practices of the villagers with relation to use of safe drinking water, defecation practices, hand washing and food habits.
3. Health seeking behavior, migration habits among the tribals for spreading the disease in their localities.

Progress:

By the request of the Directorate of Health Services Govt. of Orissa with financial support, the study has been initiated in the tribal dominated Kashipur block of Rayagarh district, Dasamantapur block of Koraput

Table 1. Evaluation of water samples collected from tribal areas (April, 08– May, 08).

Month and Year	Place	Water	
		Total No.	No. positive for *V. cholerae* non O1 non O139 (NAG)
March, 2008	Kashipur	28	3 –NAG** One positive for *ctxA* gene
April, 2008	Kashipur	41	1-NAG
May, 2008	Dasmantpur	43	0
May, 2008	Th. Rampur	35	0
Total		147	4-NAG* 1* - Positive for ctxA gene

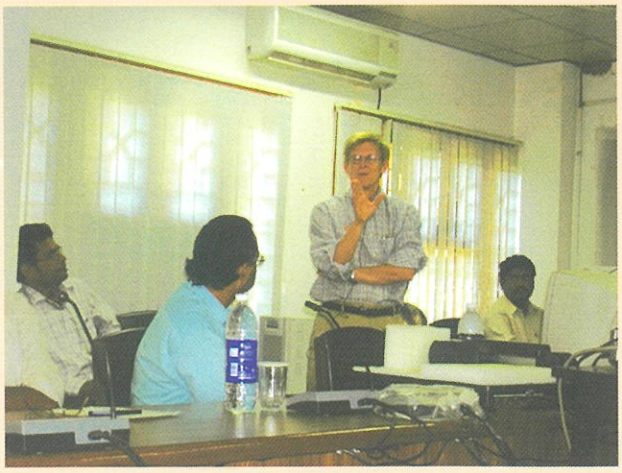
district and Th. Rampur block of Kalahandi district to look for the presence of *V. cholerae* including the El Tor variants of *ctxB* gene of classical strains from the diarrhoea patients and its existence in different environmental water bodies. The results are as follows:

The rectal swabs collected from severe diarrhoea patients of Hinjili block and Bhanjanagar blocks of Ganjam districts were positive for *V.cholerae* O1 Ogawa, biotype El Tor. The above report indicates that chances of localized outbreaks of diarrhoeal disorders may occur due to *V.cholerae* in other adjacent blocks of Ganjam district. So, adequate control measures should be implemented specifically in this district to check further spreading.

Table 2. Bacteriological analysis of rectal swabs received from severe diarrhoea patients from different districts (14.07.08- 4.08.08).

Sl No.	Place	Total samples	Organism
1	Rourkela	3	3- Non significant
2	Hinjli Block, Ganjam	2	1- *V. cholerae* O1 Ogawa; 1- Non significant
3	Kalahandi	7	7- Non significant
4	Jagatsinghpur	3	3- Non significant
5	Bhanjanagar, Ganjam	3	2- *V. cholerae* O1 Ogawa; 1- Non significant
Total		18	15- Non significant3- *V. cholerae* O1 Ogawa

Training programme for Bhubaneswar Municipal Corporation (BMC) anti-larval supervisors for vector control



Guest lecture by Dr. Eric Ottesan

Completed Studies

2

2.1 Malariogenic stratification of Angul district of Orissa using sibling species prevalence of malaria vectors.

Status : Intramural
Investigators :
Dr.N.Mahapatra
Dr.R.K. Hazra
Dr.S.K.Parida;
Mr. N.S .Marai
Mr.H.K.Triparthy.
Starting date : October 2003;
Closing date : September2006.

Introduction :

Our study from three different geophysiographical regions of the state reveals the presence of *An.culicifacies* B and C and *An.fluviatilis* T (RMRC annual report, 2001). The study was fragmentary and point survey, therefore an intensive in-depth study was undertaken in Angul district of Orissa, which has reported high malaria death during last three years. Hence a study was carried out for the stratification of the district based on the prevalence sibling species of malaria vector(s) in order to develop an innovative, sustainable, situation specific and cost effective control measure

Objectives :

1. To study the prevalence of different sibling species complex of malaria vectors and their susceptibility status to insecticides in Angul district of Orissa.
2. To study the bionomics of the complex like resting, feeding and biting behaviour, anthropophilic indices, gonotrophic cycle, and preferential breeding habit.
3. Malariogenic stratification of the district basing on the above parameters

Results:

Angul district has eight PHCs having a total population of 11,39,341(Census 2001) . It has forest, riverine and plain ecotype and it has also developmental dam project areas as well as mining areas. Out of eight PHCs , three PHCs viz Bantala, Godibandh and Kaniha, each of which represent separate ecotype, were selected for entomological studies. From each PHC, 6 representative villages were selected based on different ecotypes (Hilly forest 2 Nos, Plain 2 Nos, Riverine 2 Nos). Each PHC was visited in two different seasons winter and summer from November to June . Each village has 100 houses on an average. Mosquitoes were collected from 10%(10 nos.) of the households(HD) and Cattlesheds (CS) from each village. The sampling for all the entomological studies were done as per the WHO procedure (WHO, 1975). After collection the mosquitoes were identified .Blood meals were collected on Whatmans filter paper for processing by gel diffusion technique. The ovaries were dissected from semigravid females and were placed in modified Cornoy's fixative. Ovaries were processed in 50% propionic acid and stained in 2% lacto- aceto-orcein according to the method of Green and

Hunt (1980) for making polytene chromosome preparation. The chromosomal preparation were studied under phase contrast microscope.

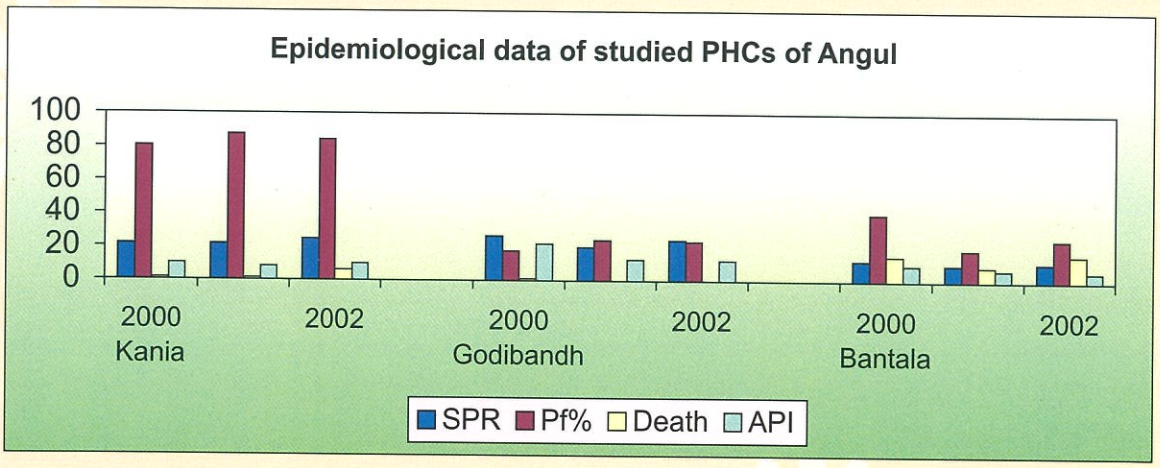


Fig-1 Epidemiological data of studied PHCs of Angul district of Orissa.

The epidemiological data of malaria like SPR, API, Pf% and death due to malaria of the selected PHCs were collected from Directorate of Health Services. The epidemiological situation of the three PHC is depicted in Fig- 1.The data depicts that among the three studied PHCs SPR and Pf% is low in Bantala PHC but death is high compared to other two PHCs.

The entomological study reveals the presence of 15 species of mosquitoes *belonging to four genera ie Anopheles, Culex, Aedes and Armigeres. Overall, 7299 female Anopheles* representing the following 11 species were captured. The species were *An.aconitus(9), An.annularis(218), An.culicifacies(475), An.fluviatilis(11), An.hyrcanus(54), An. maculates, An pallidus, An pseudojamsei, An jeyporiensis(8), An karwari(4), An subpictus(3432), An.splendidus(11), An tesselatus, An vagus(3064) and An varuna(13)* in the district. The numberof mosquitoes collected and the species diversity were relatively high between September to January.

Seasonal studies showed,the three known malaria vectors *An. culicifacies and An. annularis* were prevalent in all the three seasons in all the three PHCs except *An. fluviatilis* which was not collected in summer in Godibandh. The density of *An. culicifacies* was highest during rainy followed by winter and summer while *An. fluviatilis* was collected more in winter in all the PHCs. *An. annularis* was predominant during winter followed by rainy season. *An. culicifacies* was found to be the predominant vector. In different ecotype the distribution of species were different. In foot hill villages sixspecies of *Anophelines* including *An. culicifacies, An. fluviatilis* and *An. annularis* were prevalent. In riverine and plain villages both *An culicifacies An. annularis* were foundalong with other six species of *Anophelines*.

An culicifacies B, and C were found in all the ecotypes and *An. fluviatils* S was found in foothill villages. The composition of species complex is presented in table1 The prevalence of the three main vectors in different PHCs during winter ,summer and rainy are depicted in Fig- 10. The density of *An culicifacies* was highest during rainy followed by winter and summer while *An. fluviatilis* was collected more in winter in all the PHCs. *An. annularis* was predominant duri ng winter followed by rainy season. *An. culicifacies* was found to be the predominant vector in Godibandh and Kania PHCs while *An annularis* was dominant in Bantala PHC.

Ditection of sporozoite by molecular method:

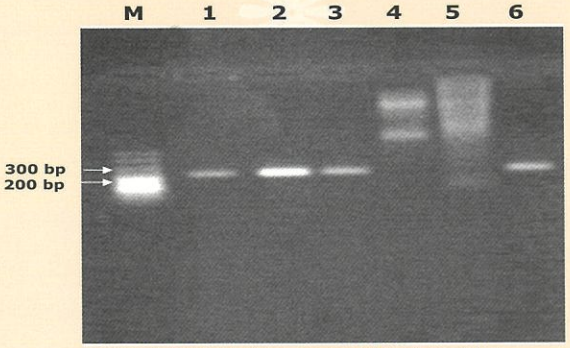
Anopheline mosquitoes (1946) are being processed for detection of sporozoite by using PCR (Fig-2). Six specimen of *An annularis*, one specimenof each *An varuna*, and *An aconitus* and six species of *An subpictus* was found positive for *P. falciparum* sporozoite by PCR method along with 8 species of *An culicifacis* and 2 species of *An fluviatilis*. The detail result is given in Table 1.

Table-1 Sporazoites detection

SL. No.	Anopheline Species	No Tested	PCR detection No.Positive
1	*An.culicifacies*	860	8
2	*An.annularis*	805	6
3	*An.fluviatilis*	240	2
4	*An.varuna*	23	1
5	*An aconitus*	18	1
6	*An subpictus*	1158	6

Fig-2

Detection of sporozoite by PCR method in Anopheline

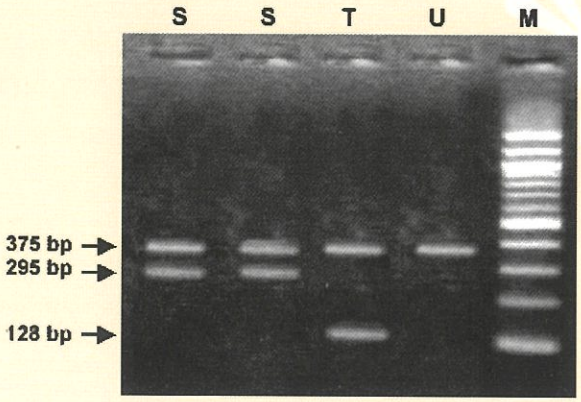


Lane M: 100 bp ladder, Lane 1&2 test samples *An. culicifacies* and 3 to 5 test samples of *An.annularis* Lane 6 : Positive Pf sample

An culicifacies, An fluviatilis and *An annualris* are found to be the main vcteors in Angul district. By molecular identification out of five sibling species of *An.culicifacies (A,B,C,D,&E),* species A, B, Cand D and from three sibling species of *An. fluviatilis (S,T &U)* only S and T were identified from the district. *An. culicifacies, An.fluviatilis* and *An. annualris* are found to be the main vectors in Angul district. Out of five sibling species of *An.culicifacies (A,B,C,D,&E),* species B and C are found in plain area and from three sibling species of *An. fluviatilis (S,T &U)* only S and T were collected from the foothill areas. The percentage of *An. culicifacies* C were 78% and B comprises 22 % of the collection.

Molecular identification of *An.fluviatilis* was also done .In the molecular analysis the D3 region of the ribosomal DNA were analyzed using primers developed by MRC (Singh et al 2004) (Fig 3). The result of the molecular study revealed the composition of *An. fluviatilis* S were 89%and 11% is 'I' (fig- 2). One specimen of *An. fluviatilis* U was collected. The compositionon of *An. fluviatilis* S were 78 %, 88%and 50% in Bantala PHC and 75 %, 98% and 66% in Kaniha PHC during rainy, winter and summer season respectively. In Godibandh PHC all the *An. fluviatilis* collected were found to be only *An. fluviatilis S.*

Fig-3 Molecular analysis of *An.fluviatilis*



The percentage of *An. culicifacies* C were 80 %, 55% and 70% in Bantala, 85 %, 68%, and 75% in Godibandh and 78 %, 65% and 72% in Kaniha PHC during rainy, winter and summer season respectively.(fig 4-5)

Fig-4

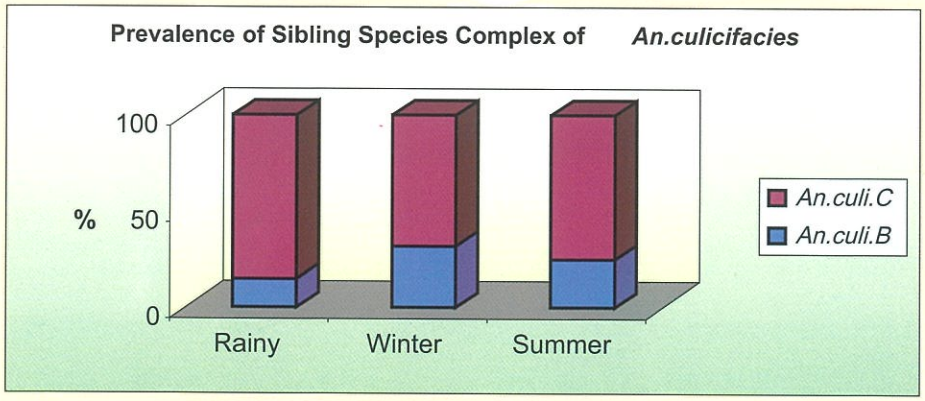
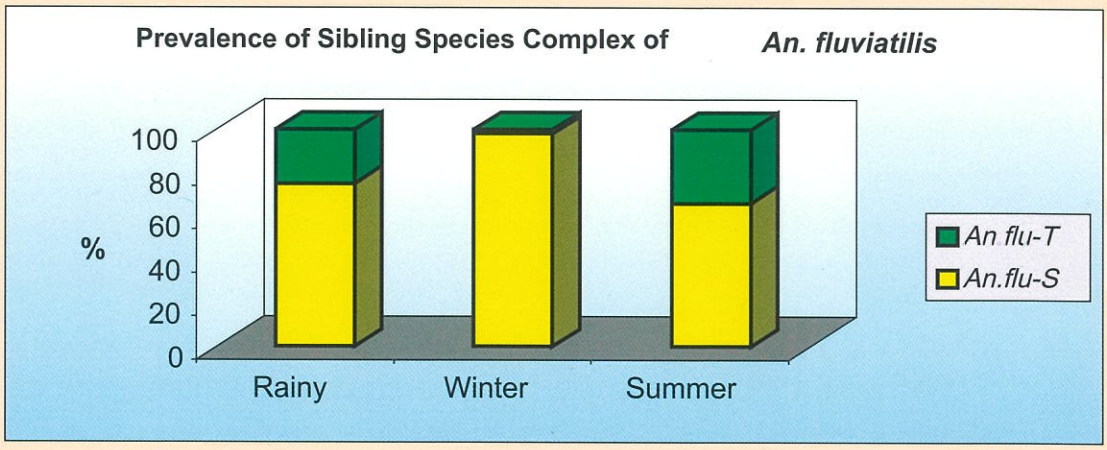


Fig-5



Anthropophilic index :

Precipitin test was conducted for the identification of source of blood meals of anopheline vectors by using gel-diffusion technique. *An. fluviatilis* was found to be highly anthropophilic (.>75%) where as *An. culicifacies(>97%)* and *An. annularis(>96%)* were highly zoophilic.(Fig 6,7 & 8) There was no significance variation in the feeding pattern from last year.

Resting Habitat:

An. culicifiacies adults prefer to rest in cattle sheds and houses during daytime, also shelter in straw, mud cakes, dense vegetations, bushes, cattle sheds.

Biting habitat:

The biting activity of *An. culicifacies* species A, B and C was found all through the night. The peak biting activity of species A and B was in the second quarter of the night, between 10,00 pm to 11.00 pm. The peak biting activity of species C and B was in the night between 6.00 pm to 9.00 pm. The peak activity of species D was between 9.00 pm and 10.00 pm.

Larval ecology:

An. clucifacies breeds in streams, river bedpools, wells, tanks, rice fields, water irrigation channel, agricultural wells, field channels, rice fields, ditches, seepage and spring pools, rain water pools.

Gonotrophic cycle:

An. culicifacies is generally regarded to have a gonotrophic cycle of 72 hours after the first blood meal and 48 hours in the subsequent feeds.

Anthropophilic Indices of *Anophelines* of Angul district

Fig-6

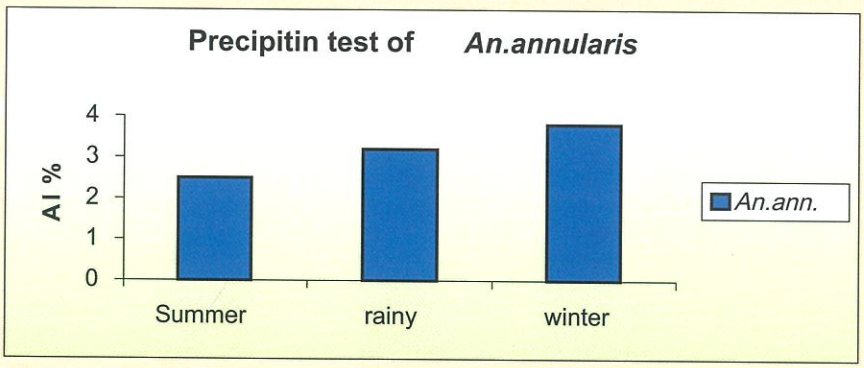


Fig-7

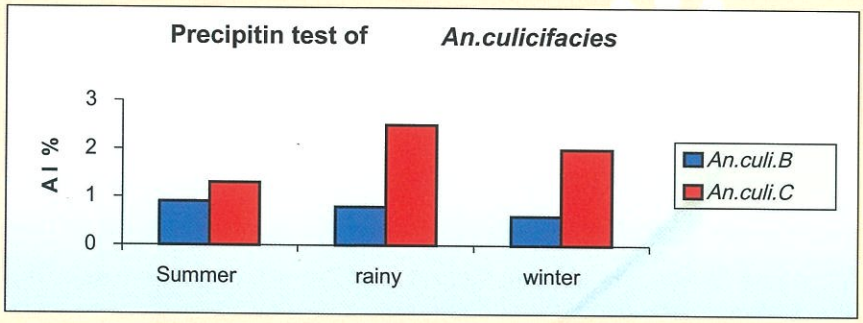
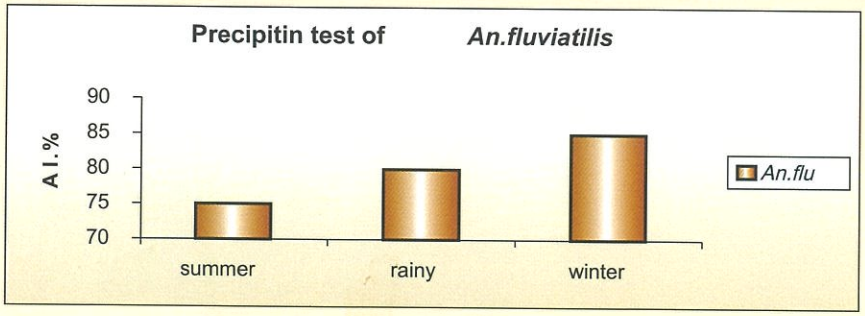


Fig-8



Susceptibility Test

The susceptibility status of *An.culicifacies* B and C was done and it was observed that B and C both are resistant to DDT but they are susceptible to Deltamethrine 0.5%. *An culicifacies* species A remains more susceptible to DDT than species B. Species C is resistant to malathion. *An fluviatilis* was found to be suspeptible to DDT.

Though two sibling species reported in *Anopheles annualris*, the presence of species complex in Orissa is unknown. We have started the detection and identification of sibling species of *An. annualris*, which also plays an important role in malaria transmission. Molecular methods for identification of sibling species work initiated with standardization of D3 and ITS2 region.

Stratification

The remote sensing data, land use and land cover of Angul district were collected and analyzed for forest coverage and elevation from the sea level. Three strategies of forest coverage (0-10%,11-29%, more than 30%) were done and it was found that Angul district is stratified into three ecozones and also three stratified zone tally with the altitude from sea level(Fig11). In each ecozones all the three vectors were present and also seasonal variation of theses vectors are also observed (fig-10) .The sporozoites are also detected in all the three species with different sporozoites rates (Fig-13). *An.culicifacies* A and D was reported first time in Orissa and also *An.annularis* plays an important role as secondary vector. Sporozoites were also found in *An subpictus*, *An varuna* and *An aconitus* .The epidemiological data showed no difference in SPR value in three seasons in each of the blocks(Fig-12). In three ecozones distribution of sibling species were different. In the first ecozone, Zone-1(>30% forest coverage A, B,Cand D are prevalent. In the second ecozone, Zone-2 (>10%forest coverage) A, B, C and in Zone-3 (<10% forest cover)only An culifacies A, B, C, D were found. *An. fluviatilis* S and T were prevalent in Zone-1 and Zone-2.This shows three types of Malariogenic stratification can be done in Angul and the control measure also can be made zone specific.

Fig-9

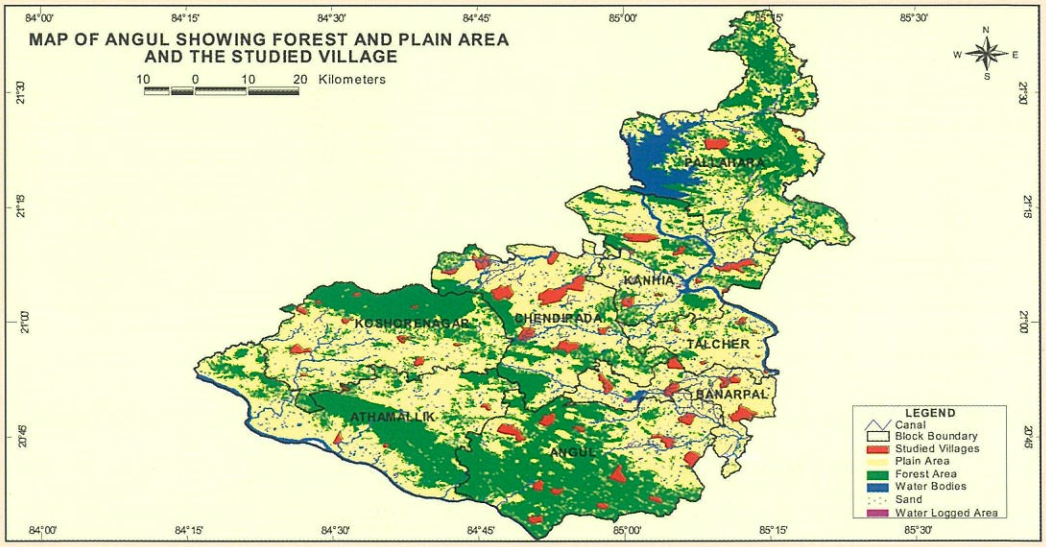


Fig-10

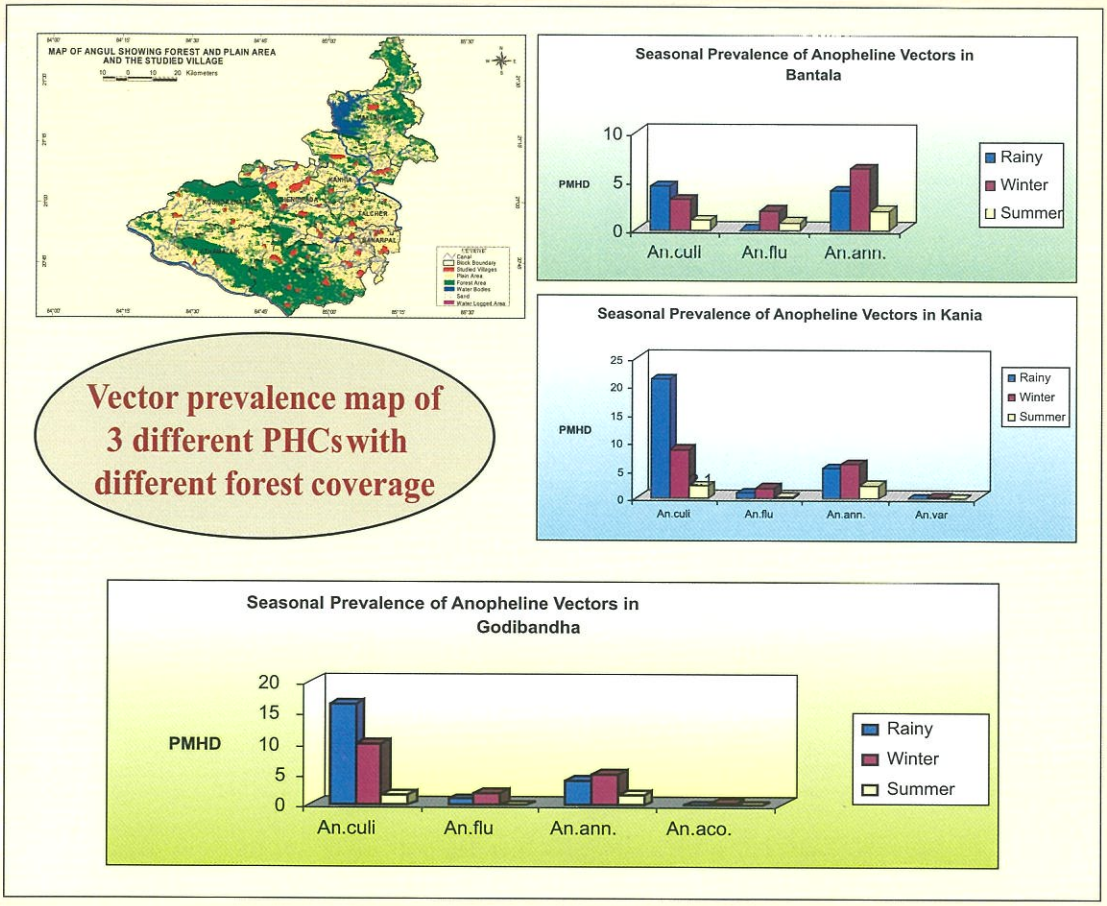

MAP OF ANGUL SHOWING FOREST AND PLAIN AREA AND THE STUDIED VILLAGE
Seasonal Prevalence of Anopheline Vectors in Bantala
PMHD
An.culi
An.flu
An.ann.
Rainy
Winter
Summer
Seasonal Prevalence of Anopheline Vectors in Kania
An.var
Vector prevalence map of 3 different PHCs with different forest coverage
Seasonal Prevalence of Anopheline Vectors in Godibandha
An.aco.

Fig-11

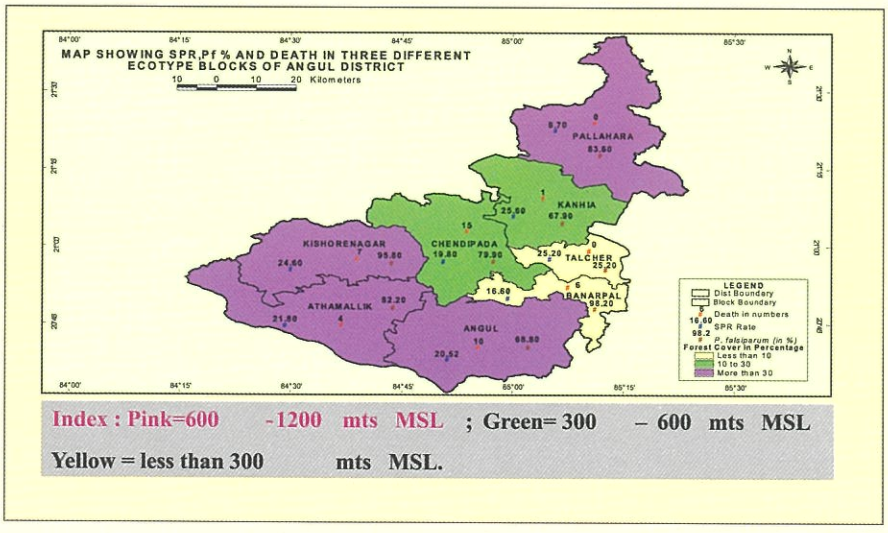

MAP SHOWING SPR,Pf % AND DEATH IN THREE DIFFERENT ECOTYPE BLOCKS OF ANGUL DISTRICT
PALLAHARA
KANHIA
KISHORENAGAR
CHENDIPADA
TALCHER
RANARPAL
ATHAMALLIK
ANGUL
LEGEND
Index : Pink=600 -1200 mts MSL ; Green= 300 – 600 mts MSL
Yellow = less than 300 mts MSL.

Fig 12

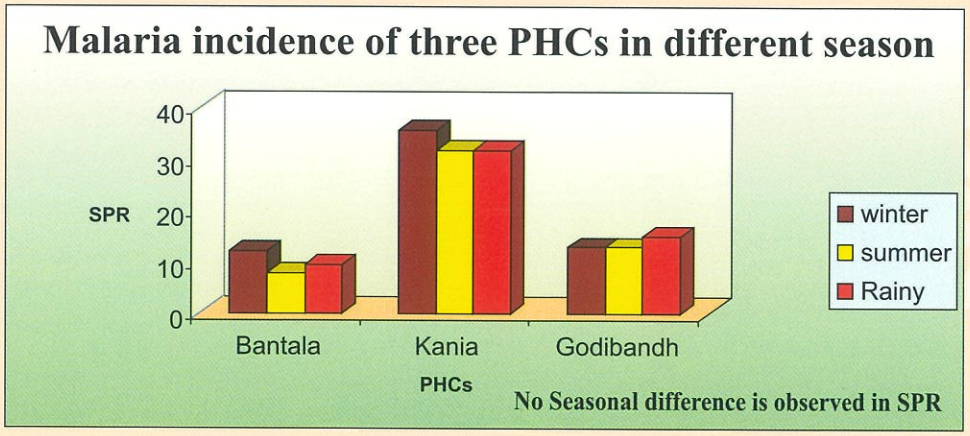
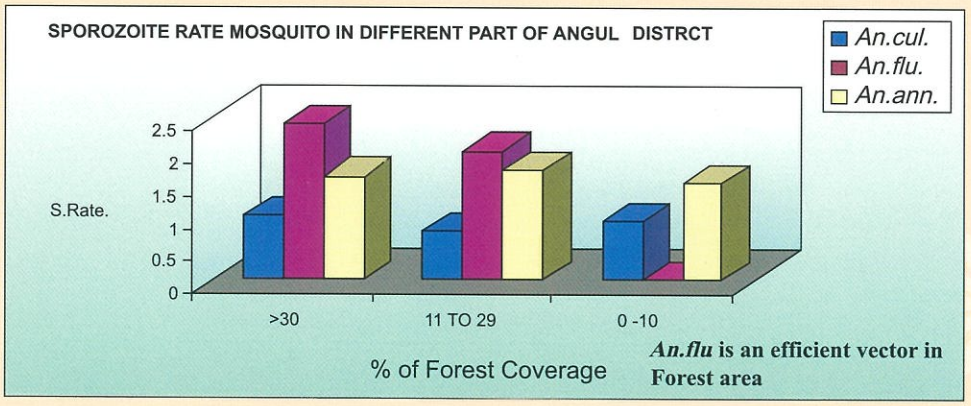


Fig-13



Conclusion:

The Malariogenic stratification taking into account the following factors that is malaria endemicity, vector sibling species prevalence of *An.culicifacies* (A,C,D) and *An.fluviatilis* (S), sporozoite rate, GIS data like forest coverage, altitude, land use, and land cover showed that the district can be devided into three ecozones ie Zone-1, Zone-2 and Zone-3 . Zone-1 contains four blocks (Angul, Athamallik, Kishorenagar, Pallahara). Zone-2 consist of two blocks (Chendipada and Kania). Talcher and Banarapal belongs to Zone-3. Zone-1, highly malarious having prevalence of A, C and D of *An.culicifacies* (65.3%) and *An.fluviatilis* S (81.4%). In Zone-2 species A and C constitute 52.4% and *An.fluviatilis* S (68.2%). In Zone-3 *An.fluviatilis* was not found only *An.culicifacies* A,C,D (71.7%). So it is recommended that in Zone-1 and Zone-2 needs continuous surveillance and insecticide spray with DDT/pyrethrum and impregnated bednet distribution as a control major. Where as for Zone-3 (where only *An.culicifacies* vector are present) the control major should be targeted towards only *An.culicifacies.*

2.2 Development of potent mosquitocidal agents from natural sources

(A Collaborative Project between Regional Research Laboratory (RRL), Bhubaneswar and RMRC, Bhubaneswar)

Status : Extramural (ICMR Vectors Taskforce)
Investigators :
Dr. Namita Mahapatra
Dr. U. V. Mallavadhani, IMMT, BBSR
Dr. R. K. Hazra, Dr. S. K. Parida
Date of commencement : 1. 8. 2005
Date of completion : 31. 7. 2007

INTRODUCTION

The major diseases transmitted by mosquitoes are malaria, filarial, dengue, Japanese encephalitis and yellow fever. WHO is emphasizing vector control is the best strategy to tackle vector borne diseases. Chemical insecticides proved to be highly toxic and causing environmental hazards. It has now been realized that natural insecticides have to be developed on priority basis as these are safe, efficient and cost effective. In view of this, a study has now been taken up to develop potent natural mosquitocides.

Objectives

i. Identification, collection & extraction of potent natural sources (Terrestrial plants, mushrooms, high altitude taxa like lichens, orchids & ferns)

ii. Generation of abundantly available natural products and analogues.

iii. Mosquitocidal screening of the natural sources and natural products/ analogues against mosquito vectors, *Anopheles stephensi* (malaria), *Culex quinquefasciatus* (filariasis), *Aedes aegypti* (Dengue)

iv. Development of potent natural mosquitocides.

Work has been carried out in two parts by the two collaborating institutes as specified below:

Part A: Extraction and sample generation (Institute of Minerals and Materials Technology (Formerly RRL), Bhubaneswar.

Part B: Mosqutocidal screening (Regional Medical research Centre, Bhubaneswar).Details of work done under each component are given below:

PART-A: Extraction and sample generatioin

(*Component of: Institute of Minerals and Materials Technology, Bhubaneswar*)

The 10 natural sources were collected from authenticated areas and their identity was confirmed by taxonomists. Voucher specimens have been deposited in the Herbal Drugs and Bio – Remedies Cell of IMMT, Bhubaneswar.

The sources have been processed by removing the foreign matter followed by washing with fresh water and shade drying. The dried plant materials were powdered in a pulveriser. As plants contain a wide range of secondary metabolites with varying polarities, they have been extracted with various polar solvents as per the protocols given. However, in case of *Cinnamomum zeylanicum* the volatile oil extract has also been generated by hydrodistillation method using Clevenger apparatus, as it contains considerable amount of monoterpenes and sesquiterpenes. Further, in case of *D.sylvatica* and *L.specicosa*, the solvent systems dichloromethane – methanol (1:1) and methanol found to be highly efficient in extracting their marker compounds, these two plant materials have been extracted accordingly. In total 45 extracts have been prepared from 14 plant parts of 10 plant sources. The details of various extracts along with their yields and codes are presented in Table. 40 plant extracts (except RRL - 031a, RRL - 051 – to RRL – 054) and 7 pure isolated single molecules have been submitted to RMRC, Bhubaneswar for mosquitocidal screening.

PART B: MOSQUITOCIDAL SCREENING

(*Component of: Regional Medical Research Centre, Bhubaneswar*)

The natural source extracts and pure compounds received from IMMT have been subjected to detailed mosquiticidal screening against thee following 3 vectors:

1. *Anopheles stepheni* (Malarial vector)
2. *Culex quinquefasciatus* (Filarial Vector)
3. *Aedes aegypti* (Dengue Vector)

Students working in the Lab.

MATERIALS AND METHODS

BIOASSAY

For evaluating the larvicidal activity of a compound, mosquito larvae (late 3rd or early 4th instars larvae) should be exposed to a range of test concentration in 250ml of chlorine free water. The bioassay test of the plant extracts were carried out in the laboratory condition against the three species of mosquitoes. The plant extracts were tested at concentration ranging from 1 to 100ppm. After addition of test materials to water, the water was stirred vigorously and left for 30 minutes to evaporate the solvent. Twenty/ Twenty five (20-25) early third instars larvae of each species were introduced into 500ml glass beakers containing 250ml test solution. Each test concentration was replicated five times. Two replicate of control were also maintained. Mortality of larvae was recorded at 24 hours interval. After 24 hours, percent mortality is determined scoring the dead and moribund larvae in test

replicates. Larvae, which have pupated during the test were discarded and counted for calculation of mortality. The experiments were repeated if more than 10% larvae pupated or when more then 20% larval mortality occurs in the controls. Percentage mortality was calculated using "Abbot's formula" where the larval mortality in control ranged from 5-20%. So as to remove the error, if any, on account of the mortality due to factors other than the toxic effects of the extract 25, 26. Observation was continued till all the larvae in the control beaker emerged to adult27. The LD50 and LD90 values were also determined for some of the highly active extracts. In total 32 plant extracts and 7 pure compounds have been screened against the 3 mosquito vectors. Out of 62 experiments carried out, 17 against *Anopheles*, 34against *Culex* and 11 against *Aedes*. The % mortality in each case after 24, 48, 72 and 96 hrs and thereby cumulative larval death was calculated. It is highly significant to note that activity was found in 34 screenings and the remaining are inactive. The data of the active experiments was given in Tables. From data it is seen that out of 34 active screenings, 13 are against *Anopheles*, *16 Culex* and 5 *Aedes* vectors. Among the active samples, the highly potent ones against each vector are given in Tables. .From these results it is interesting to note that the plant extracts RRL-030, RRL–031 and RRL- 036 are active against all the three vectors. Whereas, three plants extracts RRL-010, RRL-028 and RRL-035 and one pure compound RRL-033 are active against two vectors each. The remaining samples are active against one vector only. Details of the highly active extracts are given below:

i) **RRL – 030:** This is the n-hexane extract of the plant species and showed Activity against all the three vectors screened.

a) *Anopheles* - It showed 100% mortality at 100 ppm concentration in 72 hrs.The activity is dose dependent and at lower concentrations, the mortality rate is not only decreasing but also time period is increasing.

b) *Culex* - It showed 100% activity at exceptionally low concentration of 10 ppm in 48 hrs.

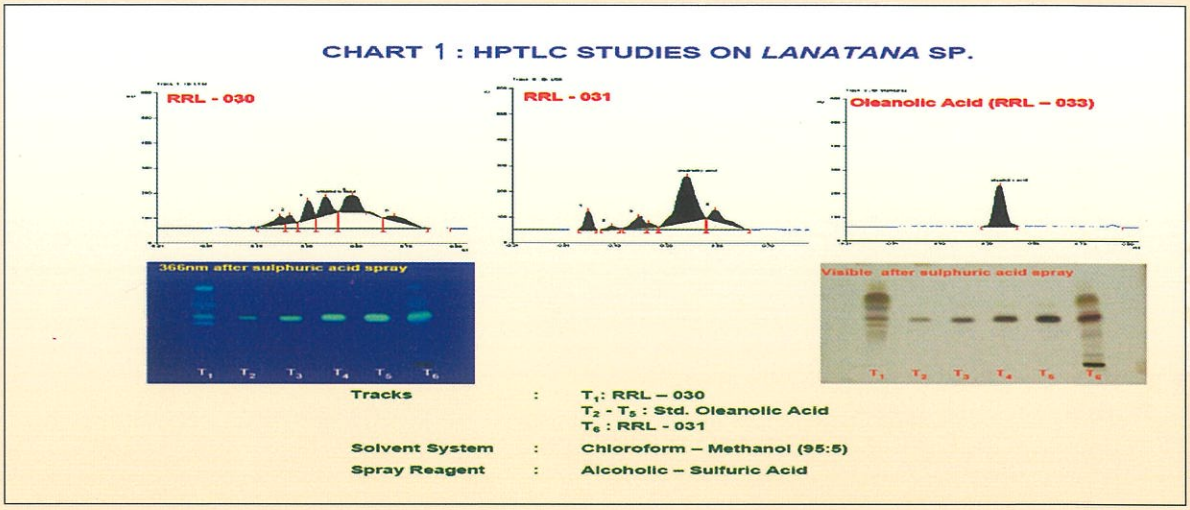
c) *Aedes* - The 100% mortality was shown at 50 ppm concentration in 48 hrs.

ii) **RRL - 031:** This is the ethyl acetate extract of the plant species and showed activity against all the three mosquito vectors.

a) *Anopheles* – This extract is more potent than the n-hexane extract. It showed 100% mortality at 10 ppm in 48 hrs. With the increase of concentration to 100 ppm, the time period has come down to 24 hrs.

b) *Culex* – Against this vector also it showed 100 % mortality at 25 ppm in 48 hrs.

c) *Aedes* - In case of this vector, though the 100 % mortality was achieved, the concentration as well as the time period found more with 100 ppm and 96 hrs. respectively. Interestingly the above two extracts belong to same plant species. In order to see their chemical complexity and for comparison and reference purposes, their HPTLC fingerprint have been developed along with their major compound. (Chart 1)



iii) **RRL – 036:** This is the ethyl acetate extract of the plant species and showed 98% activity against all the three vectors screened at 100 ppm concentration in 96 hrs. In all the three cases it is found that activity is dose dependent. Its HPTLC finger print has been developed.

iv) **RRL – 010:** This is an hydrodistilled oil of a plant species. It showed activity against two vectors as per the details given below:

a) *Anopheles* - It showed 100% mortality at 15 ppm concentration in 48 hrs. By decreasing the concentration by 5 ppm, the activity has gone down to 49%.

b) *Culex* - It showed 100% activity at concentration of 50 ppm in 72 hrs. Interestingly at 25 ppm and 10 ppm, the mortality rates are 92 and 14 respectively indicating that a minimum of 25 ppm concentration is effective. It is to mention here that out of the other extracts of this plant screened, the ethyl acetate (RRL - 012) and acetone (RRL – 022) extracts are active. RRL – 012 showed 80% mortality at 50 ppm concentration in 96 hrs against the *Culex* vector. Whereas, RRL – 022 showed activity against both Anopheles (12%, 1 ppm, 96 hrs.) and *Culex* (11%, 1 ppm, 96 hrs).

In order to see the chemical complexicity, the HPTLC fingerprinting studies have been carried out on all the above mentioned three extracts along with the major metabolite of the plant.

v) **RRL – 028:** This is the methanol: dichloromethanol extract of the plant species. It showed highly potent mosquitocidal activity against the two vectors, Anopheles and Aedes.

a) *Anopheles* – It showed 100% mortality at 10 ppm concentration in 48 hrs.Even with enhanced concentrations the time period remained same at 48 hrs.

b) *Aedes* - A maximum of 92% mortality was observed with 10 ppm concentration in 96 hrs. Surprisingly activity profile is disturbed by increasing the concentration of the sample. The HPTLC finger prints of this extract along with its related extract (RRL-029) and their major compound (RRL – 034).

RRL – 035: This is the n hexane extract of the leaf part of the plant and showed activity against *Culex* and *Aedes*.

a) ***Culex*** : It showed 100% mortality at 100 ppm concentration in 96 hrs. The activity is dose dependent. With reducing concentration, the activity is decreasing.

b) ***Aedes*** : A maximum of 66% mortality is shown at 100 ppm concentration in 96 hrs.It is highly significant to note that , the larvae underwent significant colour changes from he original tea brown to yellow – green – reddish. The play of colours suggests that some mutations might have been taken place during the screening. Although, the mortality rate is less at lower concentrations and time period and lesser time periods, the larvae will be inactive due to the mutations as reported earlier29.HPTLC fingerprinting studies have also been done for this extract for reference purpose

vii) **RRL – 033:** This is a pentacyclic triterpenic acid, having functionalities at C–3, C12-13 and C17 positions. It showed potent activity against two mosquito vectors viz. *Anopheles* and *Culex*.

a) ***Anopheles:*** It showed 100% mortality at 100 ppm concentration in 96 hrs.Interestingly reduction in concentration to 25 ppm showed marginal decrease in mortality rate (94%).

b) ***Culex:*** It showed 100% mortality at 100 ppm concentration in 96 hrs. In this case also reduction in concentration to 50 ppm has marginal effect on the mortality rate. Incidentally, this is the major chemical constituent of the highly active extracts RRL-030 and RRL – 031.

The following samples showed potent activities against one vector only:

viii) **RRL- 027:** This is the dichloromethane – methanol extract of the heartwood part of a plant species. It showed 100% mortality at 50 ppm concentration in n72 hrs against the ***Culex*** vector. The HPTLC finger printing patterns of this extract along with other polar extract of the plant are given in Chart – 7.

ix) **RRL- 029:** This is the methanol extract of leaf part of a plant species. It showed 100% activity at 50 ppm concentration in 72 hrs. against ***Anopheles*** vector.

x) **RRL – 032:** This is a single isolated compound belonging to ursane class of pentacyclic triterpenic acid. It exhibited a maximum of 96%mortality at 100 ppm concentration against ***Anopheles*** vector.

xi) **RRL – 034:** This compound also belongs to ursane class of pentacyclic triterpene with additional hydroxyl functionality. It showed 100% mortality at 50 ppm concentration in 72 hrs. against *Culex* vector. It is to be mentioned here that this is the major compound of the active extract RRL – 029. **LD50 and LD90** values for the above highly active extracts were determined by employing the Logit Analysis software. The values are given in Table – 41.The samples taken up for this analysis are: RRL-010, RRL-012, RRL-030, RRL-031, RRL-033, and RRL-036, which exhibited almost 100% mortality against the mosquito vectors, *Anopheles, Culex* and *Aedes*. The extract RRL-010 was found active against both *Anopheles* and *Culex*. Interestingly LD50 values for both the species are almost equal, but LD90 value of Culex is double than the *Anopheles*

Lower the value higher in the potentially of the product as insecticide. Hence, RRL-010 is highly potent against *Anopheles* than *Culex*. The LD50 value of other samples for *Anopheles* ranges from 0.3315 to 1.5093ppm. So it is seen that RRL-033 is more toxic to *Anopheles* vector. In case of *Culex* the LD50 value for the samples RRL-012, RRL-030, RRL-031, RRL-033, RRL-036 were found to be 0.9870, 0.3081, 0.4351, 0.4365, and 1.4031 ppm respectively. So out of all the above, RRL-030 is more toxic to *Culex*. For *Aedes* the LD50 values of the three samples, RRL-030 RRL-031 and RRL-036 were found to be 0.4139, 1.3901 and 1.5234 ppm suggesting that RRL-030 is highly toxic to *Aedes* vector.

CONCLUSIONS

Some of the notable contributions of this project are

- 45 natural source extracts and 7 single compounds have been generated.
- 40 plant extracts and 7 pure isolated single molecules have been submitted for mosquitocidal screening.
- 32 natural source extracts and 7 pure compounds have been screened against the 3 mosquito vectors as per WHO protocols.
- In total 62 screenings have been done, 17 are against *Anopheles*, 34 against *Culex* and 11 against *Aedes*.
- The % mortality in each case after 24, 48, 72 and 96 hrs and thereby cumulative larval death was calculated.
- It is highly significant to note that activity was found in 34 screenings.
- Interestingly, as many as 16 samples showed activity against *Culex* vector, followed by 13 against *Anopheles* and 5 against *Aedes* respectively.
- Out of the 34 active screenings 30 are of natural source extracts and 4 are single compounds.
- Of the 30 extracts, 11 showed activity against *Anopheles*, 14 *Culex* and 5 *Aedes* vectors.
- Among the single compounds two samples each showed activity against *Anopheles* and *Culex* vectors.
- Three extracts (RRL - 030, RRL - 031 & RRL - 036) showed 100% mortality against all the three vectors (*Anopheles*, *Culex* & *Aedes*).
- Three extracts (RRL - 010, RRL - 028 & RRL - 035) and one pure compound (RRL - 033) showed activity against two vectors (*Anopheles* & *Culex*).

In conclusion extensive work has been done under this project and achieved very significant results. A good number of natural source extracts and pure compounds were identified with exceptionally potent activities against the three mosquito vectors. The identified samples have excellent scope to become highly potent mosquitocidal as they are natural, eco friendly and cost effective.

2.3 A randomized clinical trial with chloroquine and alternate drug regimens to study the comparative efficacy, in treatment of uncomplicated P.falciparum malaria in two endemic districts of Orissa".

Status :
Intramural
Investigators :
Dr. A S Kerketta,
Dr. M. R. Ranjit, Mr. P K Jangid
Staring date :
March 2006
Closing :
February 2008

Objective of the study:

1. To study the comparative efficacy of four antimalarial drug regimens in treatment of uncomplicated P. falciparum malaria.
2. To differentiate the recrudescence and re-infection by using molecular marker.

Work progress

The study was developed on the basis of high malarial death and first spreading of chloroquine (CQ) resistance in the state. At the time of development of the study, CQ was used as the first line of drug in treatment of uncomplicated malaria all over the state. Since the extramural fund could not be availed, the study was initiated intra muraly. It was undertaken at the Nuagaon PHC, Kandhamal district of Orissa. A total of 62 eligible cases were included in the study and allocated four different treatment regimens randomly. Of which 13 cases were in Chloroquine (Regimen- I), 15 cases in Sufadoxine pyrimethamine (Regimen-II), 12 cases in SP+ Artesunate (Regimen- III) and 15 cases in Co-artem ((Regimen- IV) could be followed up till Day 28. GCP was followed strictly during the study. The cases were closely monitored daily for first three days and thereafter on day 7,14,21 &28 days. Out of the total cases studied two cases, each from the regimen II and IV developed severe malaria on day two and shifted to the hospital on same day. The result of the small population sampled from the area studied revealed that, the study regimen III i.e SP and Artesunate is most efficacious drugs with adequate clinical response (ACR) of 91.7% in treatment of uncomplicated malaria followed by Co-Artem which is found to have ACR of 86.7%. However the study could not be continued, since the CQ resistance has became so rampant in the state and the treatment failure with CQ has been reported from more than 15 PHCs. The adjacent areas of these PHCs are also designated as CQ resistant area by the state health department. Secondly the ethical point of view it is unethical to use the drug, which has shown resistance to such an high extent. The state Government has already implemented the second line of antimalarial i.e SP+ Artesunate, in the World Bank funded blocks. In this present context it is not feasible to conduct the clinical trial with the drugs one which is not in use and other which is most efficacious and already been implemented as the second line of treatment in the state. In near future the study will be redesigned after reconsidering the trial drugs in treatment of uncomplicated malaria.

2.4 Comprehensive analysis of diarrhoeal and environmental isolates of *Vibrio cholerae* from a cholera endemic area of Orissa.

Status :
Intramural
Investigator :
Dr B. B. Pal
Co-Investigators :
Dr H. K. Khuntia, Mr S. K. Samal, Dr A. S. Acharya
Starting date : Oct. 2006
Closing date : March 2008

BACKGROUND:

The comprehensive analysis of *V. cholerae*, which were isolated both from indoor diarrhoea patients of different hospitals of Puri district and Capital hospital of Bhubaneswar was initiated. The *V. cholerae* were isolated from the water, planktons and roots of the floating vegetations.

OBJECTIVES:

1. Phenotypic characterization of *Vibrio cholerae* strain isolated from diarrhoea patients and environmental samples (water, sediment, plankton and roots of the floating vegetation) from selected areas of Puri district.
2. To find out the correlation between environmental and clinical isolates of *V. cholerae* by different molecular techniques such as PCR assay, RAPD, PFGE and ribotyping for the detection of virulence and regulatory genes.
3. To find out the critical environmental factors like pH of the sediment and water, salinity and temperature of different water bodies.

METHODOLOGY:

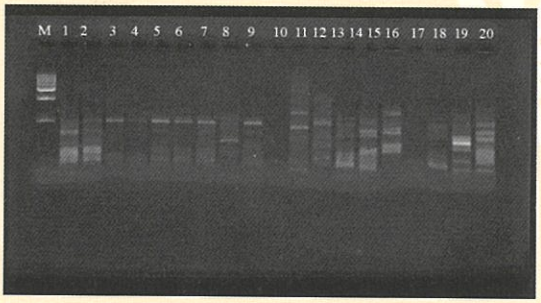
The proposed project was continued in Capital Hospital, Bhubaneswar, Pipli CHC, Mangalpur CHC, Sakhigopal Hospital and ID Hospital, Puri. The stool samples were collected in CBT medium and transported to RMRC, laboratory for bacteriological analysis (WHO Manual, 1987). The stool and environmental samples were collected weekly from the study area.

RESULTS AND DISCUSSION:

During this period (Sept, 07-Apr, 08), 398 stool samples were processed, out of which *E.coli* were 107 (56.3%), *V.cholerae* were 54, out of which O1 were 53 and only one O139 serogroup was isolated showing trend of extinction. The *V.cholerae* O1 Ogawa (n=37, 19.5%) serotype were showing dominance over Inaba serotypes

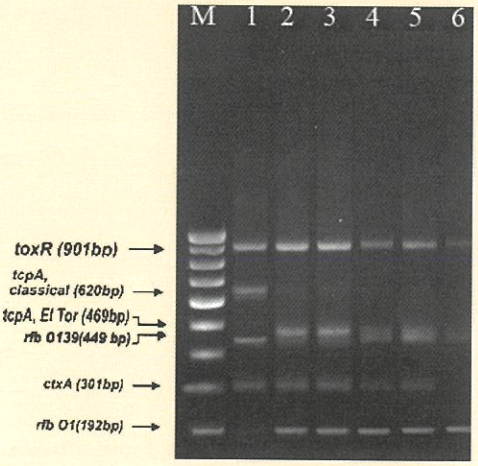
(n=16, 8.4%), Shigella spp. 4 (2.1%), Aeromonas spp. 25 (13.2%). *V.cholerae* non O1 non O139 strains were isolated altogether from environmental samples. The isolation of *V.cholerae* from plankton and water were more compared to roots of floating vegetation. The isolation rate of *V.cholerae* from environmental samples was very high during summer months in comparison to other months. The clinical isolates of *V.cholerae* O1 were resistant to Co, T, E, Pb, Na, Fr, A, S and sensitive to Nx, Cf, N, G, Az and C; whereas environmental isolates were sensitive to Co, Nx, Cf, T, C, G, Az and resistant to Fr, Na, S, E, N, A and Pb respectively. The clinical isolates of *V.cholerae* were positive for *ctx*A and *tcp*A genes showing biotype El Tor and positive for *tox*R gene. In contrast environmental isolates of *V.cholerae* were positive for *tox*R gene, whereas environmental isolates of one of the non O1 non O139 *V. cholerae* from Kashipur area was positive for *ctx*A gene. It was observed that the alkaline pH (7.2-7.9), the alkaline pH of water and the higher temperature of the environment favoured the growth of *Vibrio cholerae* in the water environment.

RAPD (PCR) ASSAY BETWEEN CLINICAL AND ENVIRONMENTAL ISOLATES OF V.cholerae with



1281 primer:

Lane M- 100bp ladder, Lane 1- O395 Classical strain, Lane 2- VC_{20} El Tor strain, Lane 3-9- clinical isolates of V.cholerae, lane 10-20- Environmental isolates of V.cholerae



QUADRUPLEX (PCR) ASSAY OF CLINICAL ISOLATES OF V.cholerae

Lane M- 100bp ladder, Lane 1- 6- clinical isolates of V.cholerae

Conclusion:-

The present study indicated that there was a good correlation between the clinical and environmental isolates of *Vibrio cholerae* observed in the Puri areas. The rate of isolation of the clinical and environmental isolates of *Vibrio cholerae* were higher in pre-monsoon, monsoon and post monsoon seasons as observed from the different parts of Puri district. High percentage of tetracycline resistance was observed for the first time from the state, which should be monitored regularly. Sensitive to polymixin B indicating the spreading of El Tor variants of *V.cholerae* O1 Ogawa from the tribal areas to the coastal districts which should be confirmed by MAMA PCR assay and to look for other cholera toxin genes. So, the continuous surveillance on diarrhoeal disorders including the environmental samples should be monitored together for better management, treatment and control of future outbreaks/epidemics of diarrhoeal disorders in the state of Orissa.

2.5 The effect of choloroquine chemoprophylaxis during pregnancy among the compliant and non - compliant cohort mothers of Orissa.

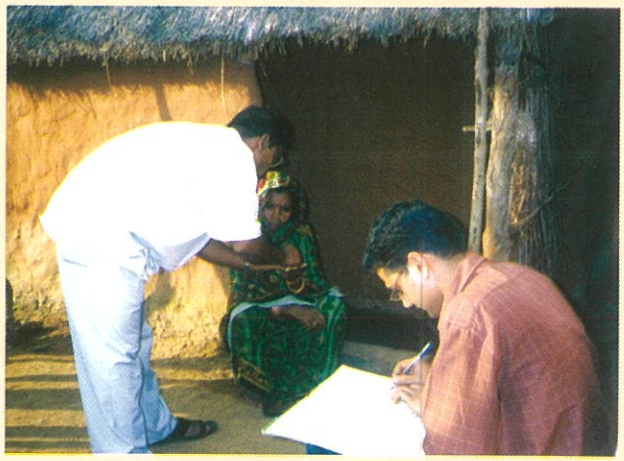
Investigators :
Dr. Amarendra Mahapatra,
Mr. D.P. Hansdah, Mr. H.S. Naik,
Mr. B. Purohit
Starting Date :
March 2006
Closing date :
March 2008

Background:

Pregnant women and infants are very much susceptible to malaria attack due to their weak immunity and special physiological condition. Repeated attacks of malaria leads to high anemia among the mothers, which results to low birth weight babies leading to high IMR and MMR. To overcome this problem, Government of Orissa, is distributing choloroquine tablets 600mg (2 tablets) weekly, among the pregnant women, under the IMR mission. The Choloroquine (CQ) chemoprophylaxis programme has not been assessed by its community acceptance and evaluation of the birth outcome as indicator. Hence the compliance & utilization has not been quantified and the socio-cultural reasons there off are not studied. So these are the new challenges for the policy makers and social scientists to think about the new alternatives and solutions for this. The present study will try to find out solutions and answers to these problems, identifying the salient feature with respect to behavioral changes among the community. This study will also elucidate the non-acceptance of malaria chemoprophylaxis, in a socio-cultural perspective.

The study has been initiated in the Nayagarh district of Orissa has been selected. From this district 189 villages of 3 PHCs namely Gania, Daspalla and Madhyakhanda were evaluated prospectively. For this purpose,

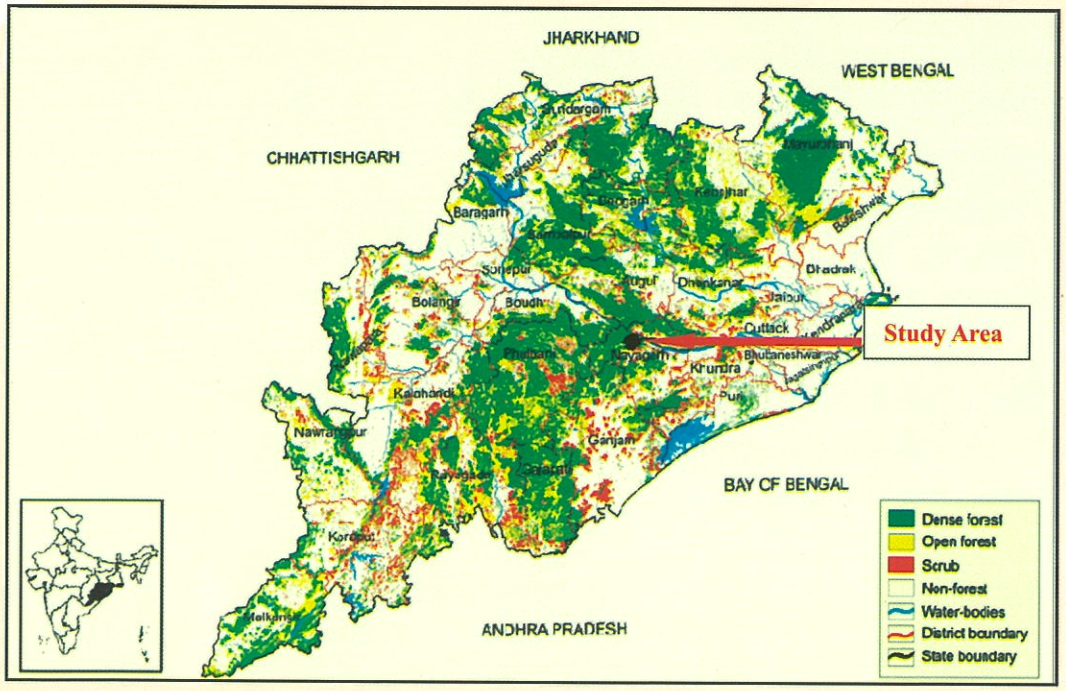
cohorts of 549 pregnant women were registered so far during last two years. These women were followed-up in each trimester (three monthly) and after delivery to record the birth outcome. During each follow-up the malaria fever, other morbidity history and ANC details were recorded. These ladies were subjected to both slide and ICT-KIT test for malarial conformation and 20 micro liter blood also been collected for Hb% estimation among the pregnant women during each visit.



Malaria data collection from pregnant woman in Nayagarh

During the follow-up data on Knowledge Attitude Believe and Practices (KABP) on malaria & the chemoprophylaxis programme was collected through interview schedule. In addition to this data on ANC and Iron tablet consumption were also recorded. In due course, all the pregnant women were divided in to two groups for analysis i.e. those who were taking CQ-Chemoprophylaxis and who were not taking CQ.

Fig. 1



GIS Map of the study area indicating the forest fringe & Mahanadi river basin in the location

Aim :

The aim of the present study is to assess the malaria chemoprophylaxis on the pregnant Cohorts of Nayagarh district Orissa, India.

Method :

Area: The Nayagarh district of Orissa State is selected as the study area due to its endemicity to malaria and operational feasibility of the cohort study. Thus Gania, Daspalla and Madhyakhanda PHCs were the sample showing high API. All Pregnant women in the villages are being selected and followed-up till 45 days post delivery. Registration of pregnant women for the study, from the Anganwadi Centers are being taken as base and besides this house-to-house rapid assessment of pregnant women registration are being carried out in each village. Then, these women are being followed from their 1st trimester every 3rd mont till postpartum 45days. After delivery their birth out comes are collected for epidemiological comparison of the CQ-compliant and Non-compliant cohort.

RESULT:

Out of 549 cohort samples, 501 samples were only analysed, rest were rejected due to loss to follow-up or incomplete data set etc. From this samples of cohorts 151 (30.1%) had suffered from malaria during pregnancy and a mean haemoglobin (Hb%) of 9%. Out of 501 cases delivered, 199 (40%) were compliant and 302 (60%) were non-compliant (Tab-1). Non-compliant women had more malaria incidence during pregnancy than the compliant counterparts; 23% and 10.6% respectively (p=0.005). The percentage of LBW babies born to non compliant cohort was significantly high (42.7%) than the LBW babies born to compliant cohort (13.6%), which is statistically very much significant (**P<0.0001**), the percentage of LBW babies in case of non compliance cohort was 3 fold more than the compliant samples. Among the studied pregnant women the mean Hb% was 9.15 among the compliant and 8.87 in the non complaints (Tab-1). According to WHO standards of haemoglobin cutoff levels, 85% of these cohorts were anemic, and 11% were severely anemic among the studied samples, only 15% had normal Hb% among them.

Correct Knowledge on Choloroquine (Cq) chemoprophylaxis was marked only in 40% of the cohort mothers and 27% had no idea at all of taking CQ-chemoprophylaxis, this was the out lined reasons of very poor CQ compliance among the study population i.e 40%.. However, it may be noted that, Knowledge regarding the

disease Malaria and about its vector mosquito is known to almost 90% of these pregnant women; The major reason of non-compliance was the fear of abortive fetus due to side effect & obstructive delivery fear, in 38.5% cases. Another 40.4% did not like the taste & frequency of Choloroquine weekly dose. The perceived benefit was not there in 14.6% of cases, among them 6.5% didn't receive the tablet due to service failure etc. CQ compliance rate was comparatively more in lower educational group. Malaria incidence percentage was 34.5% (**P= 0.0066**) and LBW was 46% (**P<0.0001**) more in case of iron tablet Non-compliant than the compliant cohort, even the mean Hb% was less in Non-compliant (8.8%) than the compliant (9.3%) cohort. Indicating Cq & IFA supplementation improves birth out comes and maternal health (Tab-2). Birth outcomes of institutional and home deliveries were also accessed and it was found that institutional deliveries were 62% and the results are encouraging.

Use of bed net is 50.3% in this studied cohort, the incidence of malaria is 20.2% among the users and 30.9% in case of non users which is statistically significant (P=0.0021; OR=1.92). Similarly the Low Birth Weight Babies born to the users is 29.36% and that of the non users is 40.56 (P=0.0089; OR= 1.64). This observation indicates a positive trend or improved birth out come and maternal health in the bed net users.

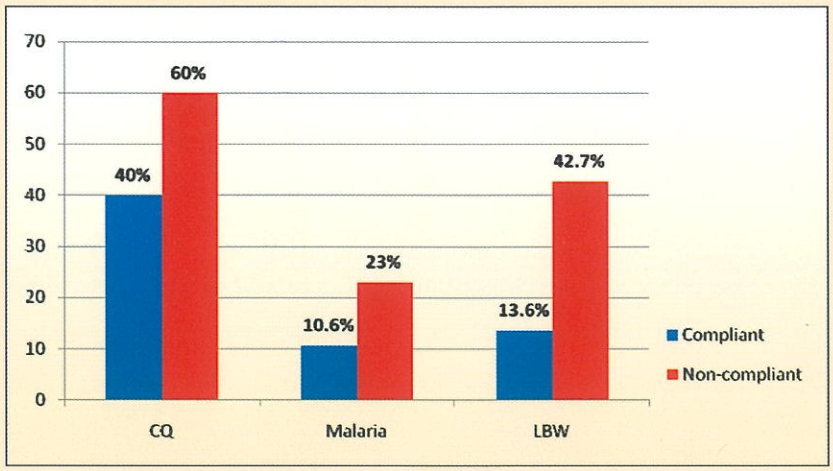
A total of 123 (51 compliant and 72 Non-compliant) cord and placental blood samples were assessed for PCR assay and 20 (5 i.e.10% compliant and 15 i.e.14% Non-compliant) samples were found to be Pf positive. However, Pfcrt estimation reveals a 46.0% resistance to Cq indicative of Choloroquine failure in these tested samples.

Table 1. Comparison of Malaria incidence, LBW & Hb% among the CQ Compliant and Non-compliant Pregnant Women, N=501

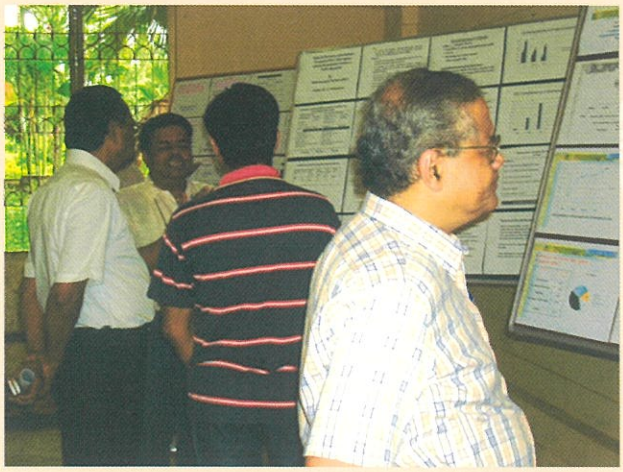
Parameters	Compliant, N=199 (40%)	Non-compliant, N=302 (60%)
Malaria Incidence	21 (10.5%)	69 (23%) **P=0.0005 OR=2.51**
LBW	27 (13.6%)	129 (42.7%) **P < 0.0001 OR=4.75**
Mean Birth Wt of LBW	2.7	2.4kg P=0.000, t= 24.139
Mean Hb%	9.15%	8.8% P=0.026 t = 4.95
Mean Birth weight	2.62kg	2.49 kg P=<0.001 t = 10.80

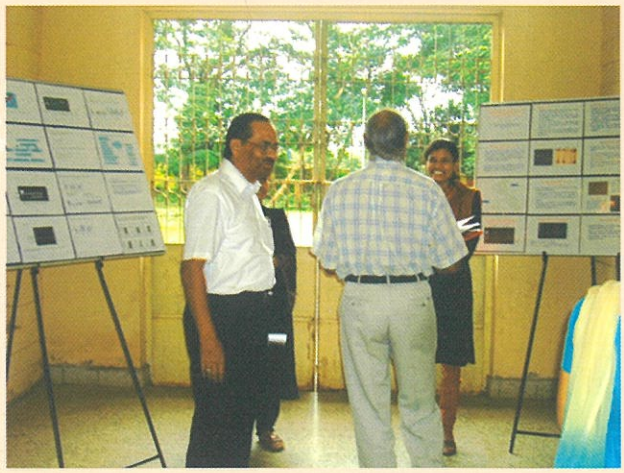
Table -2. Iron tablet (IFA) Consumption of Pregnant Women and the Outcomes, N=501

Parameters	Compliant, N= 188 (37.5%)	Non-compliant,N=313 (62.5%)
Malaria Incidence	43 (23%)	108 (34.5%), **P=0.0066 OR= 1.77**
LBW	31 (16.520%)	144(46%), **P< 0.0001 OR=4.31**
Mean birth wt of LBW	2.7	2.4 P< 0.000 t = 24.13
Mean Hb%	9.33%	8.8% P< 0.000 t = 18.71



Cq Compliance, Malaria incidence and the proportion LBW in the Compliance & non Compliance Cohort





Poster presentation by research scholars during SAC meeting

Other Scientific Studies

3

3.1 Evaluation of the programme for the Insecticide Treated Bed Net and entomological studies for malaria control in Orissa

Status :
Extramural
NVBDCP, Orissa

Investigators :
Dr. R. K. Hazra (P. I)
Dr. N. Mahapatra
Dr. A. Mahapatra

Objective :

a) Impact assessment

b) Vector assessments

c) To identify malaria vectors and its species complexes, bionomics, feeding and susceptibility status in three districts of Orissa.

Background:

This study is sponsored by Govt. of Orissa, EMCP programme, as a concurrent evaluation and document the operational aspect of the treatment of the mosquito nets. It is desirable that the methodology followed and the lessons learnt are recorded for future guidance since the initiative for the treatment of owned mosquito nets has been taken up for the first time. The periodical information on vectorial status for malaria transmission like identification of vectors and its species complex, bionomics, feeding habits and susceptibility status in these districts of Orissa, are also essential to help evaluation of efficacy measures in the continuing Programme. Hence, with request from NVBCD Programme of Govt Of Orissa, the study with above broad objectives in five identified districts of Orissa will be undertaken. Sixteen district now taken under GFATM where the bed net will distributed.

Work Methodology

1. To assess preparatory activities including survey and measures for the involvement of Panchayat Raj Institutional (PRIS), NGO and Self help group (SHGS) for bed net impregnation.
2. To access training and IEC activities.
3. To access the willingness of the community to pay for the insecticide.
4. Operational detail of camp organized for treatment of nets.
5. Action taken for the procurement of insecticide for treatment of the nets.
6. Coverage level achieved.
7. To access the involvement of PRIs/NGOs.

Work Progress

Out of five districts Mayurbhanj district was surveyed. One-time mosquito collections were done. This will be repeated in all seasons.

1. Vector survey at Mayurbhanj district

The study was under taken in eight PHCs are of Mayurbhanj districts, they are PHC –Rangamatia, Badasahi, Badampahar, Jasipur, Raruban, Sukruli, Bisoi and Bijatala. Fifteen species belonging to four genera of mosquito were collected from thirteen villages. From the total mosquito collected Anopheles are 61.2%, Culex 38.0%, Armigeris 0.41% and Mansonia 0.27% (Fig 1). Ten species were from genus *Anopheles*, the rest were *Culex, Armigeris* and *Mansonia.* Total 717 mosquitoes were collected among which 439 were Anophelines. Among nine anopheline species collected, three were identified vectors of malaria they are *An.annularis, An.culicifacies* and *An.fluviatilis.* (Fig 2). (The abdomen of major species showed half gravid (HG), full fed (FF), and unfed). The main malaria vectors were collected from both cattle shed and human dwelling.

Fig. 1

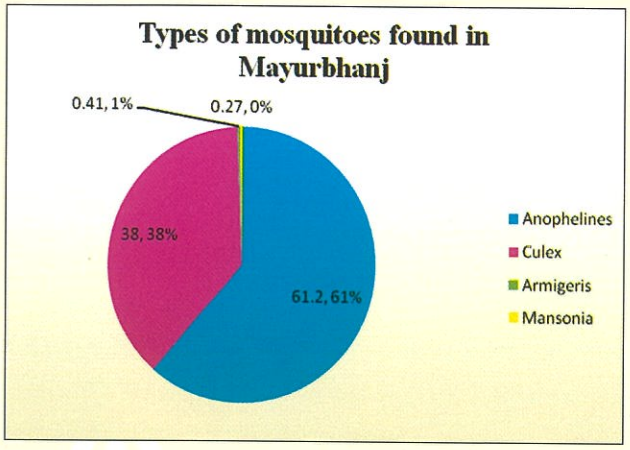
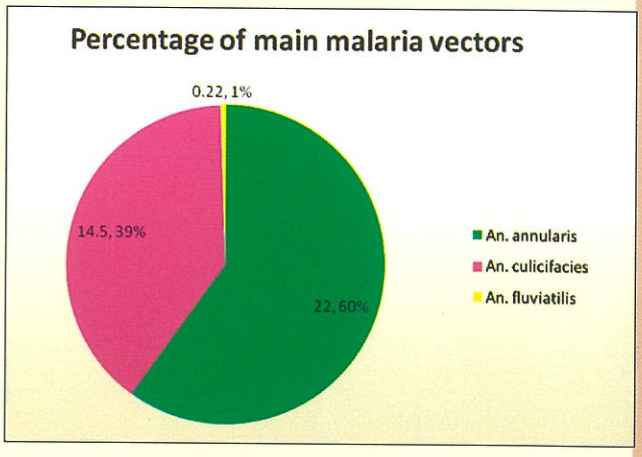


Fig. 2



3.2 Epidemics of cholera in Kashipur (Raygada dist)and Dasmantpur block (Koraput dist.) of Orissa (July-Sept, 2007)

Investigators :

Dr. B. B. Pal
Dr. H. K. Khuntia
Mr. S. K. Samal
Mr. B. K. Swain

On request from DHS, Govt. of Orissa, investigation was done on the different diarrhoea affected villages. The epidemics of cholera was reported during the month of July-Sept, 2007 in Rayagada (Kashipur, Kolnara), Koraput (Dasmantpur and Laxmipur), Kalahandi (Th. Rampur) and Gajapati (Mohana) districts of Orissa. Stool samples were collected from the indoor diarrhoea patients and water samples were collected from different river, nala, chua, pond and stream water for bacteriological analysis. A total of 6 blocks, 358 villages, 8206 diarrhoea cases, 162 deaths with attack rate 6.38% and case fatality rate 1.97% were reported. Both from the diarrhoea and environmental water samples (stream, river, nala, chua) *V. cholerae* O1 Ogawa biotype El Tor was isolated which was identified as hybrid strain showing mixed character of classical and El Tor biotype with altered antibiogram. The multiplex PCR assay revealed that both clinical and water isolates were *ctx*A and *tcp*A positive showing biotype El Tor. The

MAMA PCR assay revealed that most of the *V.cholerae* isolates were hybrid of *V.cholerae* O1 Ogawa biotype El Tor with *ctx*B classical biotype.

3.3 Outbreak of cholera in the Rayagada town during January, 2008

Investigators :

Dr. B.B.Pal

Dr. H.K.Khuntia

Mr. S.K.Samal

A localized outbreak of cholera, reported in Rayagada town affecting adjacent villages was reported during the month of January, 2008. The causative organism of this outbreak was eltor variant strain of *V.cholerae* O1 Ogawa biotype El Tor with *ctx*B gene of classical strain. The same strain was isolated both from diarrhoea patients and from the water samples of Jhanjabati river, which flows nearby the town indicating presence of the strain in environment.

3.4 Service Rendered in Filaria OPD, Capital Hospital, BBSR

Investigators :

Dr. A. S. Kerketta

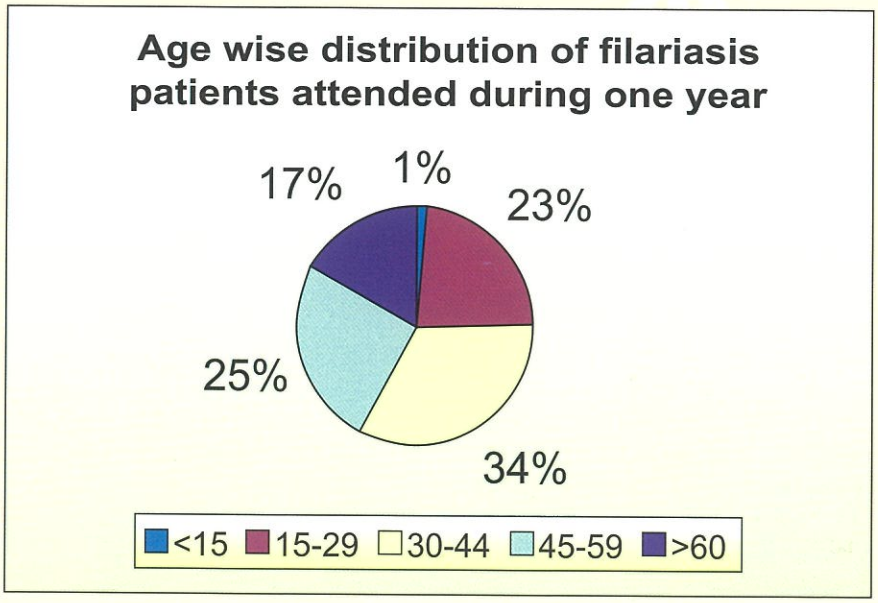
Dr. B. Dwivedi, Dr.L.Ho, Mr R. K Das

Mr. K Dhal

During the reporting year a total of 1085 filariasis patient attended the filaria out patient department (OPD), at State Capital Hospital. Of which 617(56.9 %) cases are follow-up cases, comprising of 54.3% male and 45.7% female. Total 468 (43.1%) new cases attended the OPD. Of which 271 (57.9%) are male and 197 (42.1%) are female. The age distribution of the filariasis cases shows around 33% of the cases belong to the age group 30-44 years followed by 25.4% in age group 45-59 and 23.3% in age group 15-29 years. The prevalence of filarial disease found among the patients above 60 years is 17.1%. A merely 1.3% cases of age group less than 15 years reported to the OPD. The age distribution of the cases given in (Fig-1). The commonest clinical presentation encountered was filarial lymphoedema of different grades, which was marked in 320 (68.4%) cases. The lymphoedema grade- I was found in 184 (39.3%) patients. The lymphoedema Grade- II found in 53 (11.3%), Grade III in 81 (17.3%) and elephantiasis in 2(1.2%) of the cases. Cases with acute Adenolymphangitis (ADL) attack found in around 97(20.7%) of patient which includes both ADLA and ADL. A total of 26(5.5 %) male patients reported with hydrocele or orchitis. The other symptoms like filarial nodule, myalgia, arthralgia urticaria in 0.2%, 4.3%, 0.2%, and 0.2. % of patients respectively. All the lymphoedema patients were given advise on proper foot care management, limb elevation and bandaging. Around 32 cases were given pneumatic decompression therapy.

Pattern of clinical presentations of cases attended filariasis OPD in last one year

Clinical presentations	N	%
Lymphoedema Grade- I	184	(39.3%)
Lymphoedema Grade- II	53	(11.3%)
Lymphoedema Grade- III	81	(17.3%)
Elephantiasis	2	(1.2%)
Acute ADL	97	(20.7%)
Hydrocele	26	(5.5 %)
Others	25	(5.3%)
Total	468	



Out of the data generated from OPD, two papers have been developed. One paper has been published in indexed journal i.e *Transaction of Royal Society of Tropical Medicine and Hygiene* and one paper has been communicated to *Indian Pediatrics*.

3.5 CD4 counts on HIV Positive patients referred from State AIDS Cell

Funding :
State AIDS Cell, Govt. of Orissa
Principal Investigator :
Dr. B. B. Pal
Co-investigators :
Dr. H. K. Khuntia
Starting Period :
March, 2007

Background:

On request of State AIDS Cell (OSACS) and NACO, New Delhi CD4 count of HIV positive patients was undertaken at this centre from March, 2007 to May, 2007 and continued from August, 2008. 175 blood samples from HIV positive patients were received anonymously and tested.

Work Progress:

One hundred seventy five HIV positive blood samples were referred from ART centre, Berhampur; VCCTC, Capital Hospital, Bhubaneswar and from other centers of Orissa for the CD4 counts of HIV positive patients. The reports were submitted to OSACS, Govt. of Orissa for treatment, care and management of HIV positive patients in this region.

3.6 Frequency of Microalbuminuria in tribal population of orissa: A preliminary survey

Funding :
Intramural
Investigators :
Dr. B. Dwibedi
Dr. S. K. Kar
Dr. S. K. Das

SUMMARY:

Chronic kidney diseases are becoming a growing burden of end stage diseases because of rising prevalence of diabetes and hypertension mostly without treatment or irregular treatment. Most of these conditions are detected late when treatment becomes difficult and cost effective. Hence, the mainstay of preventing these remains in diagnosing the disease at an early stage with certain markers, which can be good indicators of early kidney disease.

The state of Orissa with tribal population of 22% and low life expectancy of the tribal population, no detailed study has been addressed towards the cause of death or terminal morbidity. An attempt was taken to find out the prevalence of microalbuminuria, hypertension and glycosuria in the tribal population of Orissa, as an one time study with an idea to step towards early diagnosis of chronic kidney disease.

In this study 2186 numbers of sampled adults with age 18 + years tribal population were included from 17 tribes of 13 districts of Orissa. All the individuals were examined clinically and urine tests were performed by rapid strip test (Roche diagnostics) in the field.

Systolic hypertension (>140mmHg) was detected in 5.4% people where as diastolic hypertension (>90mmHg) was detected in 68% people studied. Urine sugar was detected in 20% of individuals and urine albumin was positive in 18% cases. Microalbuminuria was present in 27% of the tribal population. Among the hypertensives, 37% has shown microalbuminuria. The detail analysis of work is in progress.

Though this study has shown high prevalence i.e. 27% of microalbuminuria in the tribal population, it needs to be repeated including creatine clearance and to correlate other parameters with this observation.

3.7 Investigation of reported outbreaks of suspected chikungunya in Orissa (2007-08)

Funding : Intramural
Investgators:
Dr. B. Dwibedi
Dr. A. S. Kerketta,
Dr. S. K. Kar
Technical staff :
K. Dhal, J. Sabat, S. Dixit,
T. Moharana, R. N. Nayak,
B. N. Sethi.

SUMMARY

First outbreak of chikungunya virus infection was reported from the district of Sundergarh during February 2006, which was confirmed by serological tests. Since that period many episodes of chikungunya like illness affecting large numbers of people were reported from the state. Confirmation of the cases from the suspected outbreaks was essential for management of the outbreaks. Hence upon request from the state health department, investigation of the reported epidemics were carried out by the centre during the year 2007-08. Clinico-epidemilogical investigation of two large epidemics was carried out by the investigating team during the year. Referred blood samples from outbreaks from other affected areas collected through State Health Surveillance Cell were also tested for sero confirmation.

The study has shown that Population from 11 districts were affected by outbreaks of chikungunya like illness during the year 2007, covering 28 revenue blocks. Individuals of all ages and both sexes were affected with symptoms of sudden unset of fever and joint pain with associated symptoms like rash, lymph node enlargement, conjunctival congestion, pain abdomen etc. The clinical case affection rate was 5-30% in the different outbreaks and no case of fatality was noted. The acute symptoms persisted for 5-7 days and were responsive to analgesic and antiallergics.

Collected blood samples and referred samples were subjected to ELISA test for detection of IgM antibody specific to chikungunya and Dengue virus for sero confirmation. The test results has shown 10-42% sero positivity for Chik IgM from different outbreak areas and none of the samples were positive for Dengue IgM antibody. The results of investigations were intimated to the State Health Department, which was useful for taking steps for effective outbreak management.

3.8 Mid term evaluation report of the malaria mitigation measures in the command area of the rengali left bank canal system.

Funding :
Extramural
Investigators :
Dr. A. Mahapatra,
Dr. A. S. Kerketta &
Dr. R.K.Hazara
Closing date : 2009

Background:

Rengali Left Irrigation canal of Dhenkanal District of Orissa, is being funded by the external Banking funds from Japan, as it was mandatory to take Malaria mitigation measures, under the JCB-Bank guide lines; The Canal Authorities have extended the financial assistance to the District Health administration, Dhenkanal in 2006. The Base Line Survey and the concurrent survey were conducted by the VHAI- Voluntary Health Association of India, New Delhi.

The Mid-term evaluation and the End –Line survey were entrusted to Regional Medical Research Centre, Bhubaneswar in 2007. The Mid term evaluation was conducted and the End Line will be followed in the year 2009 in time.

Study area :

The study was conducted in the four blocks – PHCs of Dhenkanal district. The villages studied were selected from these blocks/ PHCs based on malaria endemicity, proximity to the canal system and availability of health facility etc. All these parameters were considered in selection.

Sampling :

Stratified random sampling procedure was adopted for selection of the villages from the specified blocks on either side of the Left Bank Canal (LBC). All the households from the selected villages are included in the survey. Vector collection was carried out from 25% of the sampled households and examination of the vector breeding sites is performed in and around all the probable breeding spots in the village, in all seasons.

Detailed research Plan :

Secondary data: Malaria morbidity and mortality secondary data collection was carried out from the state health department, and from the specified PHCs.

Primary data collection procedures include

Fever survey (house-to house visit in the selected villages) :

Method adopted: During the fever survey, cases of fever at the time of visit or during the past two weeks will be recorded. The survey will be carried out for 2-3 consecutive days for an accurate estimation of the quantum of

malaria in the community. From all the villages surveyed, from 3 different PHC – CHC reveals that, out of a total of fever cases were screened for malaria in this house to house survey, a total of 24% were positive for malaria; among these 70% were positive for Pf and the rest 23% were Pv and the rest cases were of mixed infection. The Parasite identification was carried out at RMRC Laboratory.

Cross Checking:

For the purpose of Cross Checking 10 Pf positive slides from all the PHC-CHCs Hospital laboratory were brought to RMRC laboratory for cross-examination. This measure is to enhance the capability of the laboratories. The results are as follows :

	CHC/PHC Reported Pf	RMRC	
		Pf	PV
PHC, Anlaberini	10	6	4
CHC, Mathakerala	10	9	1
CHC, Parajanga	10	8	2

This suggests that the Technicians need little orientation / training. In this regard the MO & ADMO-PHC had been apprised & they had promised to send them to Bhubaneswar for training. However, the ADMO-Ph had suggested it would be nice if the technicians training can be organized at the District Head quarters, in this regard, he had been pursuing the matter at Joint Director, Malaria& Filaria, level.

The Evaluation team in the field village

Knowledge, attitude, belief and practice (KABP) survey :

The General KABP of the population regarding Malaria is assessed under these following lines. The results reveal that:

- People do have a misconception about the cause of Malaria. Only 26% of the population does have the correct knowledge about the cause of malaria. Rest of the population does possess a fallacious knowledge, about the cause of malaria.
- People do possess a negative (scared) attitude towards malaria, with a degree of indifference among 37% of the respondents. Almost half of them were not having any clear/ systematic knowledge about the cause, prevention and treatment of malaria (59.0%). That is the reason; People have an in-different attitude about malaria control or mitigation measures taken in the studied areas.

- This can be mitigated by the use of local - Folk media (Dance song) use for dissemination of the right knowledge. Print-media like leaf lets and poster carry less impact on this population under report, due to illiteracy and lack of interest. Hence selection of proper media is important at this juncture.

    - This was informed to the local health authorities, who are implementing the IEC and to the JBIC Official too in the last quarterly review meeting in the presence of VHAI representatives.

- People do have an indoor sleeping habit for about 5 months (rain & winter) and rest 7 months they sleep out side the house. Cattle sheds are near the house. Mostly (43%) in the front yard and rest in the back yard. This enhances the men-vector contact.

- Use of mosquito nets is good and protects from mosquito bite. This knowledge is known to almost 78% of the population. However, they have not developed the habit of sleeping under the net; only 36% of the people use net at night. Suffocating feeling is the main cause for not using nets. Added to this it is not affordable, nets are costly is the notion (27%) of the people. Those who are supplied (free by social marketing) with the net they do use the net and adjusted themselves to the net use.

- Consultations with the quacks become compulsory, for the people affected with malaria, due to non-availability of health personel in time and the other reason is the demanding nature of the people in Govt. service in health sector. This attitude has to be changed, and a people friendly attitude has to be inculcated. On the other hand, People feel free to approach a quack, than a health personel.

DDC- FTD / AWC :

Angan Wadi workers was the DDC. They were declared FTD but practically we have observed that none of the FTDs were collecting slides. However, they were treating around 20 to 30 fever cases per day. Many of the cases are going to the pvt. Practitioners/ quacks. Choloroquine stock was maintained. However primaquine RT was not carried out by any ANM of these villages, as scheduled in the National programme. The main reason as studied is lack of interest and delay in Slide report adds a lot to this problem. Besides this the supply of primaquine at the DDC/ FTD level may be ensured.

Time lag in slide examination :

It was observed that the slide transport from periphery to the microscopic center and reporting back takes minimum of 15 – 21 days. Efforts should be made to minimize this time-lag in slide collection, examination and reporting.

For this little supervision and monitoring can help in improving the situation.

Rapid Test Kit:- availability of Rapid Test Kit is a problem round the year; it was observed that the Rapid Test Kit availability at the -FTDs was not sufficient and smooth.

Microscope & Training of LT:

The Dam authorities and the project office had sanction microscopes for the laboratories. This is definitely a welcoming step. These microscopes were purchased and distributed to different microscopic centers. This was even recorded in the 3rd quarterly review meeting held on 29.11.2007.

The cross checking results suggests that the Technicians need little orientation training. In this regard ADMO-PH had promised to send them to Bhubaneswar. However, the ADMO-PH had suggested it would be nice if the technicians training can be organized at the District Head quarters, as it had the entire infrastructure. This suggestion was only to save time of the technicians, as they are vital at the PHC level.

Renovation of Laboratories: There is a mitigation component or provision for renovation of laboratories. In Two PHCs namely Amlaberinae and Srimula the lab renovation work has completed, as per the 3rd quarterly report and these two were physically verified by the team. The process has been initiated and soon it will be completed in other PHC-CHCs too.

Spray & Bed Net:

Spray: Spray quality, coverage and concentration were consistent in the two villages as the team had observed. Spray was carried out in a little delayed schedule. This can be rectified with advance planning at the State level.

Bed Net:

Bed net procurement orders are in process at the district level. In the recent quaterly review meeting it was decided and the CDMO-in-charge has promised to process the same at the earliest. Once the consignment is received, the distribution can be carried out.

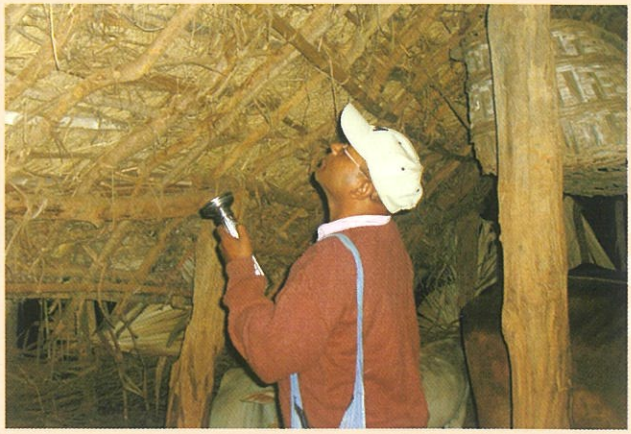
Impact of IEC :

The efforts of the IEC programme for the malaria mitigation measures, initiated by the project through the Local State Govt. Health authorities are commendable. The posters and leaflets in the form of prescriptions pads are a good innovative idea. Besides these, IEC campaigns were carried out in several villages, by meetings and camgains. It was evident from the KABP data generated by the District Health Office. But it was observed that, the general public is not well aware of the facts of the IEC massages on cause, prevention and treatment of malaria programmes.

Hence it is suggested that an effective mode may be adopted; i.e. focal - Folk media (Dance-Song) may be used for dissemination of the right knowledge. Print-media like leaf lets and poster carry less impact on this population under report, due to illiteracy and lack of interest, in going through line by line. Hence selection of proper media is important at this juncture.

Vector Collection:

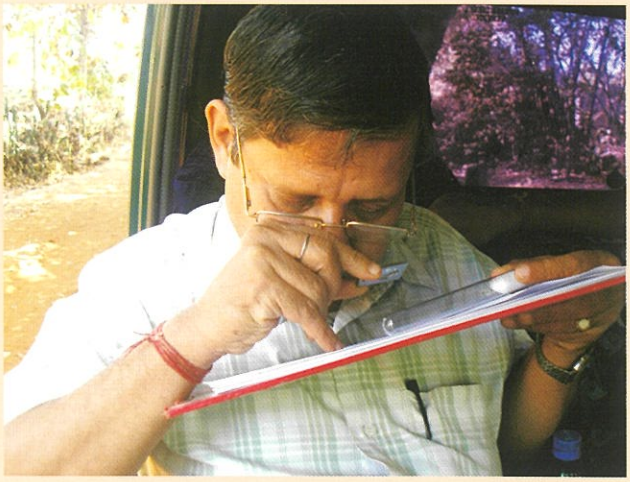
Collection of the adult vectors from 25% of the house holds: The collection of adult mosquitoes will be done with the help of mechanical aspirator / sucking tube in torch light in morning hour i.e. from 6 AM to 9 AM, and in the evening 6 PM to 9 PM. The collection sites include unsprayed a) human dwelling; b) cattle shed; c) mixed dwellings.



The entomological team looking for vectors

Vector Identification:

After field collection, mosquitoes will be first identified in field condition on the basis of their morphology. After that they were carried to the lab immediately and were identified under the microscopes, using standard reference.



Spot identification of the vector by the entomologist of the team

Mosquito density:	**PMHD**
An. Aconitus - 10	3.
An. Annularis – 5	1.7
An. Culicifecis – 2	0.67
An. Subpictus – 5	1.67
An. Hyrcanus – 3	1.0
An. Varuna - 6	2.0

Habitat:

Vectors are collected from human dwelling, cattle shed & out door resting habitats like of the vector, like rest sheds at the yard for paddy harnessing (Khala), using standard method and procedure & entomological expertise.

Molecular identification:

(The molecular identification of the sibling species of mosquito vector will be done by PCR method using allelic specific primer, designed already). An.aconitus, An.varuna and An.culicifacies was reconfirmed by PCR method.

Resistant status of vectors by PCR method: The KDR gene will be amplified by PCR method. I Iomozygote KDR gene of sodium channel block hetrozygote not found.

Breeding sites :

Examination of the breeding sites of the vectors like indoor resting in human dwelling, cattle shed resting, and out door collection was carried out.

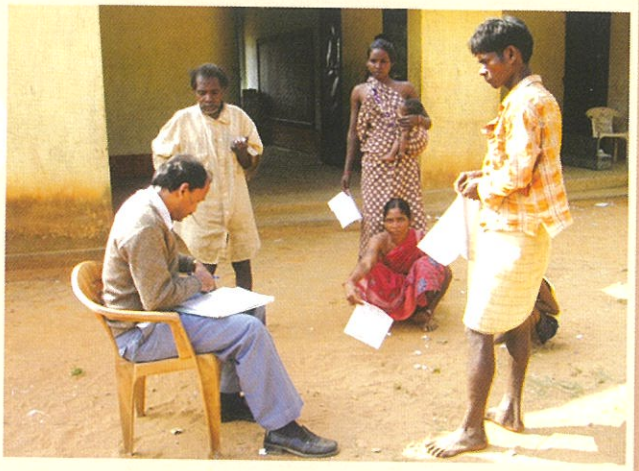
Collection of the larvae: Larvae were collected from the breeding sites and carried to the lab. In the Laboratory, their identification is carried out using standard identification procedure.

Sensitisation of Health Workers: This sensitization of Health worker is on the cards. Soon it will be carried out. The ADMP-PH had promised in the 3rd quarterly meeting, besides promising the team. This will help in Door-to-Door visit of the Health worker too.

Hatcheries:

The construction of new hatcheries and repair of the old ones is ongoing. The concerned MO's were instructed to, coordinate with the JE responsible in the concerned PHCs. The SDO & Asst. Eng of the area attended the quarterly meeting and noted down the points. Only One hatchery could be made functional as on date, however, all other PHCs are taking positive steps and very soon these will be operational.

After the malaria mitigation measures were taken in 2007; this is the Mid-Term evaluation report of 2008. The End-Line report will be available in the year 2009 in time soon after the water is released and the mitigation measures are over. It may be noted that, it was decided (during the last quarterly meeting) to create a model sub-center in Amlaberini CHC and all concentration will be paid for this purpose. The Japan Bank People will be visiting the Dam site next year due to the Centenary celebration of the Bank.



RMRC field study on Hepatitis in th Deogarh District

3.9 Hepatitis B and C virus infection in thalassemic children receiving multiple transfusion

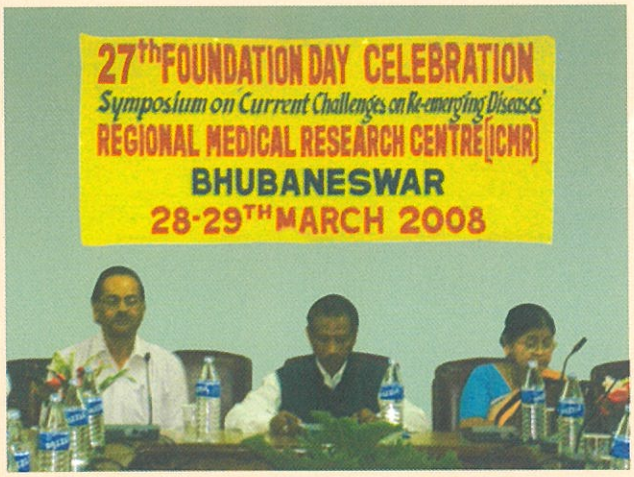
Investigators:
Dr. B. Dwibedi, L. Das
J. Sabat, Dr. S. K. Kar

SUMMARY

This is a well recognized fact that Hepatitis B and C viruses can be transmitted through blood transfusion. Thalassemia patients are a high risk group for HBV and HCV infection from the early childhood due to exposure to multiple transfusions. Though good donor screening and testing procedures have reduced the transmission, still many countries could not adequately protect chronically transfused patients from this complication. One of the causes is some percentage of HBsAg negative bloods do carry the virus which might be detectable by DNA PCR. Information on the magnitude of the HCV infection in the thalassemic children in this region is not known. Thus a pilot study has been undertaken to know the prevalence of HBV and HCV infection among thalassemia patients.

One hundred and seventy four children of 8 months to 18 years of age, from Thalassemia unit of Central Red Cross Blood Bank, Cuttack were included for the study. History of blood transfusion and clinical history was recorded. Blood samples from the enrolled children were tested for serological diagnosis of HBV and HCV infection. One of the above samples was positive for HBsAg, whereas anti HBc positivity was 17.8%, majority of which were also positive for anti HBs. 36% of the children without history of Hepatitis vaccination were positive for anti HBs. Prevalence of sero markers for HBV infection was found to be higher in the children who received >80 units of transfusion. Antibody to Hepatitis C infection was found in 6 subjects: 3 of them had anti HBs antibody from which 2 were vaccinated for HBV.

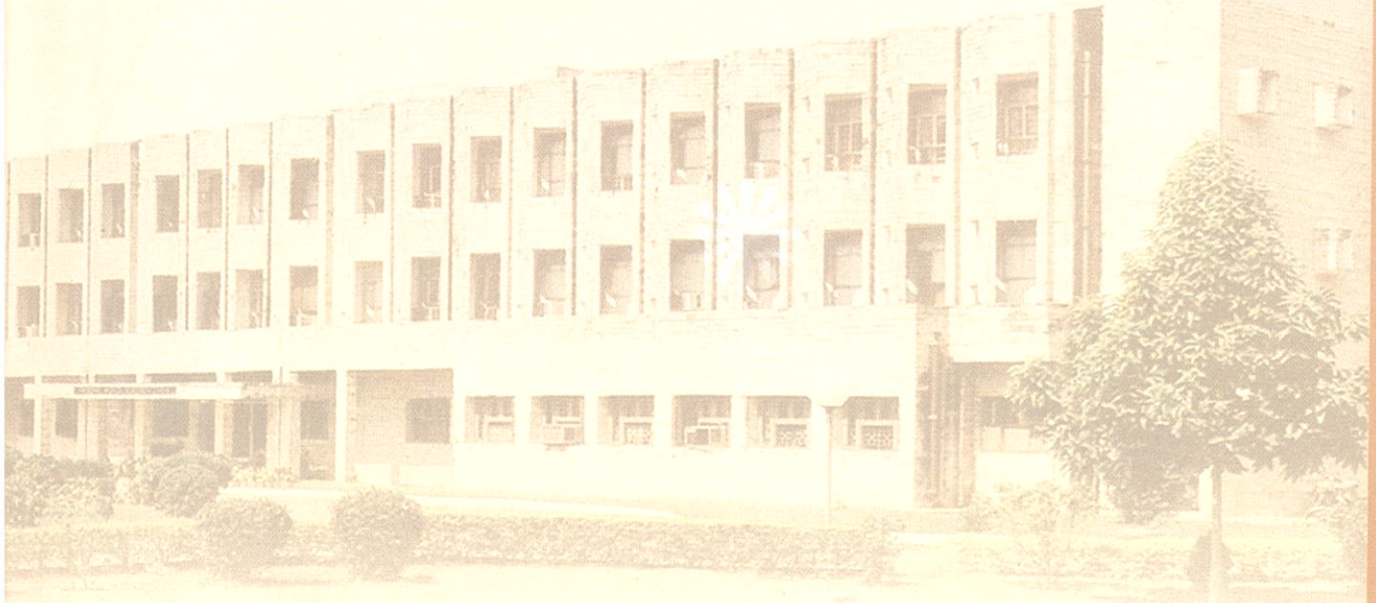
The above pilot study has shown high prevalence of HBV and HCV infection among multitransfused thalassemic children.



27th Foundation Day celebration & Symposium on curent challengers on re-emerging diseases

General Information and Publications

4



General Information and Publications

4.1 Publications- 2007

1. Babu B V, Nayak A N and Kerketta, A S. A survey on foot care practices among filarial lymphoedema patients from Orissa, India. *Tropical Biomedicine*. 2007; 24 (2): 7-14.

2. Babu B.V., Mishra, S., Swain, B.K. Personal-protection measures against mosquitoes: a study of practices and costs in a district, in the Indian state of Orissa, where malaria and lymphatic filariasis are co-endemic. *Annals Trop Med Parositol.* 2007; **101** (7): 601-609.

3. Balgir RS Aberrant Heterosis in Hemoglobinopathies with Special Reference to â-thalassemia and Structurally Abnormal Hemoglobins E and S in Orissa, India. *Journal of Clinical and Diagnostic Research*. (2007); 1: 122-130.

4. Balgir RS Genetic Burden of Red Cell Enzyme Glucose-6-Phosphate Dehydrogenase Deficiency in Two Major Scheduled Tribes of Sundargarh District in Northwestern Orissa. *Current Science*. 2007; 92 : 768-774.

5. Balgir RS Identification of a Rare Blood Group "Bombay (Oh) Phenotype" in Bhuyan Tribe of Northwestern Orissa, India *Indian Journal of Human Genetics*. 2007; 13: 109-113.

6. Balgir RS (2007) Infant Mortality and Reproductive Wastage Associated with Different Genotypes of Haemoglobinopathies in Orissa, India. *Annals of Human Biology*. 2007; 34: 16-25.

7. Bulliyya G, Mallick G, Sethy GS, Kar SK. Haemoglobin status of non-school going adolescent girls in three districts of Orissa, India. *International Journal of Adolescent Medicine and Health*. 2007; 19(4) : 395-406.

8. Kerketta AS, Babu BV, Swain BK. Clinicians' practices related to management of filarial adenolymphangitis and lymphoedema in Orissa, India. *Acta Trop*. 2007; 102(3):159-64.

9. Mishra K, Raj DK, Hazra RK, Dash AP, Supakar PC. The development and evaluation of a single step multiplex PCR method for simultaneous detection of Brugia malayi and Wuchereria bancrofti. *Mol Cell Probes*. 2007; 21(5-6):355-62.

10. Mohanty A, Kar P, Mishra K, Singh DV, Mohapatra N, Kar SK, Dash A P and Hazra R K. Multiplex PCR assay for the detection of anopheles fluviatilis species complex, Human host preference and plasmodium Falciparum sporozoite presence, using a unique mosquito processing method. *Am J Trop Med and Hygiene*. 2007; 76(5) : 837- 843.

11. Rath K, Nayak AN and Babu BV. Community's knowledge and perceptions about filarial elephantiasis and hydrocele in coastal Orissa, India. *Asia Pacific Journal of Public Health*. 2007; 19(1) : 28-33.

12. Sethy PGS, Bulliyya G, Mallick G, Swain BK, Kar SK. Prevalence of goitre and iodine deficiency among school children in urban slums of Bhubaneswar, Orissa. *Indian Journal of Pediatrics*. 2007; 74 (10): 917-921.

Publications – 2008

1. Babu BV, Mishra S. Mass drug administration under the programme to eliminate lymphatic filariasis in Orissa, India: a mixed-methods study to identify factors associated with compliance and non-compliance. *Trans R Soc Trop Med Hyg*. 2008;102 (12) 1207-1213.

2. Babu BV, Swain BK and Kar SK. Primary Health Care Services among a migrant indigenous population living in eastern Indian City. *Journal of Immigr Minor Health*. Sept. 2008.

3. Bulliyya G, Dwibedi B, Mallick G, Sethy GS, Kar SK. Determination of iodine nutrition and community knowledge regarding iodine deficiency disorders in selected tribal bloks of Orissa, India. *Journal of Pediatric Endocrinology & Metabolism*. 2008; 21(1): 79-88.

4. Kerketta AS, Mohapatra SSS, and Kar, SK . Assessment of the therapeutic efficacy of chloroquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in a tribal block of the Kalahandi district of Orissa, India. *Tropical Doctor*. 2008; (38) 82-83.

5. Kerketta AS, Bulliyya G, Babu BV, Mohapatra SSS, Nayak RN. Health status of the elderly population among four primitive tribesof Orissa, India: A clinico epidemiological study. *Z Gerontol Geriatr*. 2008; Apr 10 (E pub).

6. Kerketta AS, K Dhal, Nayak RN. A successful outcome of gross haematuria treated with diethylcarbamazine and Ivermectin. *Trans R Soc Trop Med Hyg*. 2008; 102(5): 506-7.

7. Khuntia HK , Pal BB and Chhotray GP . *Vibrio cholerae* O139 may be the progenitor of cholera outbreak in coastal district of Orissa, India, 2000: A molecular evidence. *American Journal of Tropical Medicine & Hygiene*. 2008; 78 (5): 819-822.

8. Khuntia HK , Pal BB, Chhotray GP (2008). Quadriplex PCR Assay simultaneous for detection of biotype, serotype, toxigenic potential and central regulating factor of *V.cholerae*. *Journal of clinical Microbiology*. 2008; 46 (7):2399-401.

9. Khuntia HK, Samal SK, Nayak SR, A. K.Sarangi AK, Mohanty P, Kar SK and Pal BB Incidence, Serotype, antibiogram and Toxigenicity of *V.cholerae* during 2000, six month after the Super Cyclone, 1999 in Orissa, India. *Journal of Pure and Applied Microbiology*. 2008; (2)187-204.

10. Mishra S, Swain BK, Babu BV. Sexual risk behaviour among migrant tribals living in urban slums of an eastern Indian city: implications on the spread of HIV. *Coll Antropol*. 2008; 32(1):1-4.

11. Nishank SS , Ranjit MR and Chhotray GP. First report of non-sense mutation at codon 15 (TGG'→ TAG) in exon 1 of β-globin gene in a β thalassaemia trait in state of Orissa (India*). Hematology*. 2008; 13;1:65-67.

12. Nishank SS, Chhotray GP, Kar SK & Ranjit MR (2008) Molecular variants of G6PD deficiency among certain tribal communities of Orissa(India). ***Ann Hum Biol.*** 2008; 35(3):355-61.

13. Sahu BR, Mohanty MC, Sahoo PK, Satapathy AK, Ravindran B. Protective immunity in human filariasis: A role for parasite specific IgA responses. ***Journal of Infectious Diseases.*** 2008; 198 :434-43.

14. Khuntia HK, Samal SK, Sarangi AK, Nayak SR, Kar S K, and Pal BB. Ecological interaction of toxigenic vibrio cholerae in aquatic environment. ***Current world Environment.*** 2008; 3: 109-113.

15. Samal SK, K. Khuntia HK, Sarangi AK, Nayak SR, Sahoo N, Chhotray GP and Pal BB (2008). Incidence of bacterial enteropathogens among hospitalized diarrhoea patients from Orissa, India. ***Japanese J. Infectious Diseases.*** 2008; 61(5): 350-355.

16. Mandal NN, Bal MS, Das MK, Beuria MK .Protective efficacy of filarial surface antigen in experimental filariasis.***J Helminthology.*** 2008; 16 : 1-4.

17. Khuntia HK, Samal SK, Sarangi Ak, Nayak SR, Sahoo D, Kar Sk, and Pal BB. Sprtum of Multiple antibiotic resistance among clinical strains of vibrio cholerae O1 and O139 isolates during 1999-2003 in Orissa, India. *Biochemical & Pharmacology Journal.* 2008; 1 (1): 177-184.

Papers in Press:

18. Purohit Bishwaranjan and Mahapatra Amarendra. "A Review on high Burden of Malaria during Pregnancy: Need of Social Science Intervention". ***Journals of Ethno Medicine.*** 2008.

19. Das Amitav, Manickam P., Yvan Hutin, Pal B.B, Chhotray G.P, Kar S.K and M.D.Gupte. Two sequential out breaks in two villages illustrate various modes of transmision of cholera ***Epidemiology & Infection.*** 2008.

20. Purohit B, Mahapatra A and Hansdah D. P. "High infant mortality due to malaria during pregnancy & the effect of weekly choloroquine chemoprophylaxis: a case study from Nayagarh district, Orissa", ***National Medical Journal of India.*** 2008.

21. Bal, M.S., Beuria, M. K., Mandal, N.N. and Das, M.K. Antigenaemia in young children living in Wuchereria bancrofti endemic areas of Orissa, India. ***Trans. Roy. Soc. Trop. Med. Hyg.*** 2008.

22. Swain S, Mohanty M, Mahapatra N, Parida SK, Marai NS, Tripathy HK, Kar SK and Hazra RK. The development and evaluation of a single step multiplex PCR for simultaneous detection of *Anopheles annularis* group, human host preference and *Plasmodium falciparum* sporozoite presence. ***Acta Tropica.*** 2008.

23. Ranjit MR, Sahu U, Khatua CR, Mohapatra B N, Achraya A S & Kar S K. Chloroquine-resistant p falciparum parasites and severe malaria in Orissa. ***Current Science.*** 2008.

24. Das A,. Das T. K,.Sahu U,.Das, Kar S. K. &. Ranjit M. R. CD36 T188G gene polymorphism and severe falciparum B.P malaria in India. ***Trans R Soc Trop Med Hyg.*** 2008.

25. Rout Ronnaly, Mohapatra B N, Kar S K & Ranjit M R Analysis of the genetic complexity of P falciparum isolates circulating in mild and severe malaria patients in Orissa, India. ***Acta Trop.*** 2008.

26. Majhi Gunanidhi D, Mohapatra B N, Kar S K & Ranjit M R. The short form of CCTTT microsatellite in inos promoter influences the clinical outcome in P falciparum infection. ***Parasitol Res.*** 2008.

4.2 Workshop/Conference/Seminar/Training Program conducted/ organised:

1. A meeting was organized for establishment of Virology Laboratory in the Centre on 20th June 2007 at RMRC, Bhuaneswar. A team of scientists comprising Dr. U.C.Chaturvedi and Dr. D.A.Gadkiri from National Institute of Virology (NIV, Pune) have visited RMRC for setting up diagnostic Laboratory for Viral diseases at RMRC , Bhuabneswar.

2. Pre-SAC Project Review meeting of RMRC, Bhubaneswar was held on 13-14 Sept 2007 under the Chairmanship of Dr.S. Pattnayak. The other members are Dr.S.P. Tripathy, Dr.R.Prabhakar, Dr.K. Ramachandran had participated the meeting for discussing on on-going research projects of the Centre.

3. RMRC, Bhubaneswar had organized a TB Culture facility meeting on 17th September 2007. Dr. P.R. Narayanan , Director, Tuberculosis Research Centre, Chennai, and two senior scientists Dr. V. Kumarswami and Dr. Shelva kumar of TRC Chennaihad participated the meeting. Other state Govt. Officials like Dr.J. Mohanty, Medical Officer, State TB Cell & Dr.P.N. Mohanty, SCB Medical College, Cuttack had participated the meeting.

4. Human Ethical Committee meeting held on 24th Sept. 2007 under the Chairperon of Dr.(Mrs)Justice A.K. Padhi, for discussing on ethical issue related projects of the Centre. The other experts like Dr.S.K. Mohapatra, Dr.(Mrs) Manorama Das, Dr.(Mrs) Kasturika Pattnayak and Dr.S.K. Kar, Director of the Centre were present in the ethical meeting.

5. A meeting was organized 3rd Oct. 2007 by RMRC, Bhubaneswar by inviting Dr. Gourinath Sastri, eminent statistician and Deputy Director, NIN, Hyderabad for discussing the statistics related issues on various research projects of the scientists.

6. 21st SAC Meeting: The 21st Scientific Advisory Committee (SAC) Meeting of Regional Medical Research Centre for the year 2006-07 was held during 11 -12 October, 2007. Dr. Sandip K. Basu was the Chairman of 21st SAC and Dr. S.K. Kar, Director of the Centre was Member Secretary.

7. The SAC sub-committee meeting was held on 17th and 18th November 2007 with the experts to discuss on the continuing research program. Dr.Sandip K. Basu, Chairman of SAC and other members like Dr.D.S. Agarwal, Dr.Ira Ray, Dr.Satish K. Gupta. Dr.K. Ramachandran, Dr.V. Kumaraswami had participated the sub committee meeting.

8. Regional Medical Research Centre, Bhubaneswar organized a training Program on **"Epidemic Preparedness on Vector Borne Diseases"** for state Medical officers from 21st to 23rd February 2008 and 25th –27th March 2008.

9. A Symposium on **"Current Challenges on re-emerging diseases"** was organized by RMRC, Bhubaneswar on 28th March 2008. Honorable minister of Health and Family welfare, Govt. of Orissa, shri Sanatan Bishi inaugurated the symposium. Eminent scientists and doctors of the country had participated in the symposium. Dr. M.S. Jawahar from Tuberculosis Research Centre, Chennai had delivered a talk on "Current Challenges on Tuberculosis". Dr. K. Ghosh from IIH, Mumbai talked on Sickle cell and heamoglobinopathy, Prof. P. K Das, SCB Medical College, Cuttack talked on "Life style diseases",

Dr. Saroj Mishra, from IGH, Rourkela talked on "Severe Malaria". More than 30 scientists and experts have participated in this brain storming session of the symposium.

10. The Centre Organised International training workshop on behalf of NBVDCP, New Delhi on Trainers Training of Malaria technical supervisors and strategy of operation in world bank assisted districts of Orissa on Malaria control . The training was conducted from 21st April to 2nd May 2008. The participants are from Medical officers, Malaria inspectors and Lab technicians from Orissa, Madhya Pradesh and Chhatisgarh.

11. A meeting on "Prevention of HIV/AIDS amongst tribals an operational study, in Orissa" was organised on 17th April 2008 . Dr. S.P.tripathy, Ex- DG ICMR, Director ORSAC and Social scientist Prof.. Rita Ray and Director Tribal Welfare have attended the meeting.

4.3 Seminar/ workshop attended

Dr. S. K. Kar

1. Delivered talk on "Current malaria situation in Orissa" in the review meeting on Malaria, organized by NRHM & RNTCP at Hotel New Marrion, Bhubaneswar on 28th April 2007.
2. Participated as an expert in the meeting of "State level monitoring action for IRS and Anti-malaria month campaign-2007 at the conference hall of Directorate of Health Services, Orissa on 5th May 2007.
3. Participated in investigators meeting on Multicentric Study on " Hepatic Viral infection in Primitive tribes", ICMR Tribal Task Force Project and presented "Viral hepatitis in primitive tribes of Orissa" on 1st May 2007.
4. Addressed the participants of 7th Professional Development course on "Filariasis elimination programme-Current Challenge" at RMRC, Bhubaneswar on 29th May 2007.
5. Delivered talk on "Malaria Situation in Orissa" to participants of Professional Development Course organized by State Institute of Health & Family Welfare on 7th June 2007.
6. Participated in the Press sensitization meeting on Elimination of Lymphatic Filariasis and delivered talk on "Mass Drug administration in filariasis" on 13th June 2007 at Hotel Pushpak, Bhubaneswar.
7. Participated as expert in Kalyani Programme by Door Darshan (live phone-in programme "*Hello Kalyani*" on "Chikunguniya and Dengue infection", organized by Doordarshan on 28th June 2007.
8. Participated in the State level Symposium on "The Half way mark and State of Realization on MDGS in Orissa" and talked on "Malaria Perspective in Orissa through MDGs on 7th July'07 at IDCOL Auditorium, Bhubaneswar. The Symposium was organized by CYSD, Orissa.
9. Participated in Scientific Advisory Group Meeting of ICMR at New Delhi on 12th- 13th July 2007.
10. Participated as an Expert in Pre SAC Consultative meeting at TRC, Chennai on 4th August 2007.

11. Delivered talk on " New achievements through Science" at Science Exhibition "SCIENORENA", organized in the DAV school, Bhubaneswar on 28th September 2007.

12. Delivered talk on "Health & Nutritional problems of women- A socio-legal analysis". at a National Seminar, organized by Emarti Devi Woemen's College, Cuttack on 29th September' 2007.

13. Attended Annual Day Celebration of ICMR & Oration function at ICMR Hqrs on 5th October 2007

14. Participated as a Member in Scientific Advisory Group meeting of ICMR held at New Delhi and presented the scientific achievements of the Centre during 12th -13th October 2007.

15. Participated in Governing Body Meeting of ICMR at Nirman Bhawan, New Delhi on 24th October, 2007.

16. Delivered talk on " Scope for improving MDA regimen in LFE" at the 7th Joint Conference on malaria and other communicable Diseases held at Desert Medicine Research Centre, Jodhpur on 27th October 2007.

17. Dr. S.K. Kar, Director of RMRC, attended ICMR/WHO(SEARO) Brain stroming meeting on malaria in Orissa on 12th & 13th Nov. 2007, at Hotel Suryansh, Nalco Square, Chandrasekharpur, Bhubaneswar.

18. Addressed CDMOs/District level Officers on "Patho-physiology and transmission of lymphatic filariasis" at training programme on MDA organized by State Health Department at RMRC on 14th November 2007.

19. Participated in the meeting of the Indian Lymphatic Filariasis Research Group as an Expert held at Tuberculosis Research Centre, Chennai on 19th November 2007.

20. Participated in the international Conference on "Lessons from Microbial World" at KIIT University, Bhubaneswar- 2007 on 23rd November 2007.

21. Delivered talk on "Non-alcoholic Fatty Liver Disease Challenges ahead" as guest speaker at Hotel Swosti Plaza, Bhubaneswar at 2nd Annual Meet of Orissa, organized by Indian National Association for Liver Diseases on 24th November 2007.

22. Attended 3rd Annual convention at Central Clinical Auditorium, college of Veterinary Science & Animal Husbandry, OUAT on 25th Dec, 2007.

23. Attended press sensitization workshop on MDA2007 for elimination of Lymphatic Filariasis on 5th Dec,2007 at Hotel Puspak, Bhubaneswar.

24. Addressed the doctors & Health professionals of BMC area at Capital Hospital on 5th December 2007.

25. Attended Informal meeting of research Group working on Lymphatic Filariasis in India at TRC, Chennai on 19th December 2007 and spoke on "Ongoing trials of DEC in MDA in lower doses".

26. Addreesed the students and guests of DAV School, Chandrasekharpur on the occasion of Annual function on 28th December 2007.

27. Participated a meeting on Resolving Critical Challenges in the GPELF being co-hosted by WHO and the Global alliances Gates grant at Geneva, Switzerland from 9th & 10th January, 2008.

28. Participated in Technical Action Committee meeting to develop strategy for distribution of mosquito net at State Institute of Health & Family Welfare, Bhubaneswar on 28th Jan,2008.

29. Attended Symposium on "MDA on Filariais" at RMRC, Bhubaneswar on 30th January 2008.

30. Addressed the participants of Vulnerable Community Plan (VCP) Workshop for dissemination of implementation plan for malaria control, Kalazar strengthening in tribal areas organized at RMRC, Bhubaneswar on 7th February, 2008 and spoke on "Malaria situation in Orissa in southern districts with reference to vulnerable group.

31. Participated in Hepatitis meeting at Council on 14th Feb,2008.

Dr. N.Mohapatra

1. Attended Brain storming meeting on Malaria in Orissa , on 12th and 13th November 2007

2. Attended workshop on formation of Model District Action plan 2008-09 for control of vector Borne Diseaases on 6th and 7th December 07.

3. Attended IX International Symposium on Vector and Vector Borne Diseases from 15th to 17th February 2008 and presented paper entitled "Polymerase Chain reaction diagnosis of malaria vectors, malaria epidemiology and reemergence of Anophels minimus and An.philippinensis in Baudh district of Orissa."

Dr. A.S.Kerketa

1. Attended meeting on malaria, TB and NRHM Implementation in Orissa at Hotel Maryon, Bhubaneswar on 28.04.2007.

2. Attended meeting on review of malaria and other health programme by Addl.Secy.MOH & FW, GOI and the team, at Hotel Presidency, Bhubaneswar, on 05.10.2007.

3. Attended technical Action Committee Meeting on Malaria and other vector borne disease control on 28.01.2008 at SIH & FW, Orissa.

4. Attended symposium on current challenges on re-emerging diseases at RMRC. Bhubaneswar on 28.3.2008.

Dr. B.B.Pal

1. Attended The International conference on "Opportunistic pathogens in AIDS" at AIIMS, New Delhi, 27-29th Jan, 2008 and presented a paper on "Epidemiology of HIV/AIDS in Orissa".

2. Attended JICA/NICED training course on Molecular epidemiology of diarrhoeal diseases with special reference to cholera from 12 to 23rd Nov, 2007 at NICED, Kolkata.

Dr. B. Dwibedi

1. Participated international conference of microbiology and microbial world at KIIT University, Bhubaneswar 2008. Paper Presented – Hepatitis Virus infection in Primitive Tribes of Orissa, India.
2. Participated in PEDICON–2008, National Conference of Indian Academy of Paediatrics.

Dr. G. Bulliyya

1. Attended meeting on Vitamin A estimation by DBS method during 10-14th March, 2008 at National Institute of Nutrition, Hyderabad.

Dr. M. K. Beuria

1. Attended symposium on Lymphatic filaraisis in pediatric population: age specific prevalence in an endemic region of Orissa, India. International conference on "Lessons from the Microbial world" 23-24 November 2007, KIIT University, Bhubaneswar, Orissa.
2. Attended sumposium on Induction of IgG4 antibodies in endemic normals-a risk factor for acquiring filarial infection. IX International symposium on vectors and vector borne diseases, 15-17 February, 2008, Puri, India.

Dr. M.R.Ranjit

1. Attended the IX International Symposium on Vectors and Vector Borne Diseases held at Puri from 15-17th February 2008 and presented the paper entitled Relationship of drug resistant mutations with pathogenesity of P falciparum parasites in a malaria hyperendemic area.
2. Visited different PHCs of Koraput district from 26th Sept 2007 to 28th Sept 2007 to assist the Medical team deputed by DGHS to study the cause of rising incidence of Acute Diarrhoeal Disease/ Cholera after the flood.
3. Delivered a guest lecture on "Molecular aspects of severe malaria" at the Department of Biotechnology, GIET, Gunupur on 8th March 2008.
4. Delivered a guest lecture on "Challenges and Opprtunities in Malaria Research"at Regional Institute of Education, Bhubaneswar on 20th December 2007.

Dr. R. S. Balgir

1. Participated by presenting a paper entitled "Current Status of Sickle Cell Disease in Orissa" in a workshop on "Thalassemia and Sickle Cell Anemia" organized by TASWELS ORISSA, Bhubaneswar on 2nd May 2007.
2. Participated by presenting a paper entitled "Dimensions of Hemoglobin Disorders in Orissa" in a State level Seminar on "Thalassemia and Sickle Cell Anemia: Care Till Cure organized by Center for Catalyzing Community and Indian Red Cross Society, Bhubaneswar during 7th and 8th September 2007.

Dr. R. K. Hazra

1. Attended Brain storming meeting on Malaria in Orissa, on 12th and 13th November 2007.

2. Attended workshop on formation of Model District Action plan 2008-09 for control of vector Borne Diseaases on 6th and 7th December 07.

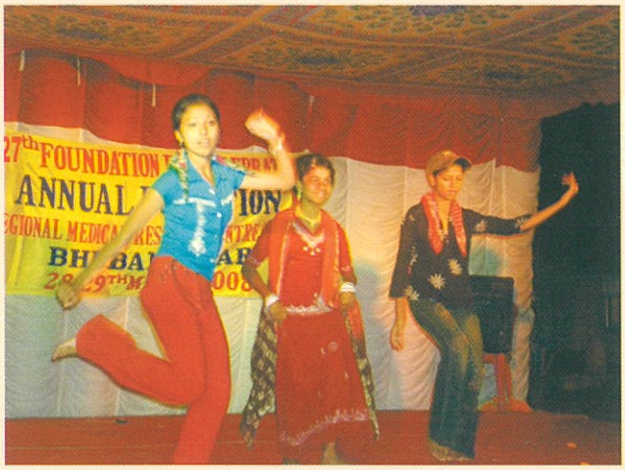
3. Attended IX International Symposium on Vector and Vector Borne Diseases from 15th to 17th February 2008 and presented paper entitled "Polymerase Chain reaction diagnosis of malaria vectors,malaria epidemiology and reemergence of Anoph minimus and An. philippinensis in Baudh district of Orissa".

Training imparted

1. Training on "Malaria Epidemic preparedness" for medical officers of Government of Orissa from 21.02.08 to 23.02.08 and from 25.03.08 to 27.03.08
2. Training on malaria for PHC Medical officers and block level programme officers from 28.04.08 to 1.05.08.
3. Training of M.O.s on Epidemic preparedness at RMRC, Bhubaneswar for 16 GFATM district from18.03.08 to 20.03.08

Field Investigation (Thalasaemia test among NCC cadets)

Regional Medical Research Centre carried out a survey of prevalence of beta thalassaemia amongst the NCC school children, camp at Sainik School, Bhubaneswar on 8th June 2007. Mr. Sudhansu Sekhar Nisank, SRF and Mr. B. N. Sethy, Lab Tech. has collected the blood samples and beta Thalassaemia test was carried out in Pathology laboratory. Out of total 182 samples collected from students, 14 were found to be *NESTROFT* positive and only six students (frequency of 3.2%) were found to be beta thalassaemia carriers by electrophoresis test .This 3.2% frequency of beta thalassaemia carrier correspond to national level of prevalence of beta thalassaemia (3 to 4%) as reported by earlier studies (Verma et al.1997, Vaz et al.2000, Agarwal et al 2000, Colah et al.2004).



Cultural Programme on eve of Foundation Day

Students reading in the Library

4.4 Human Resource Development

List of Ph.D scholars undertaking the studies :

Sl. No.	Name	Date of Joining Ph.D program	Funding	Title of the Research Topic	Guide/Co- guide	Status
1.	Mr. Alok Das Mohapatra	7/1/2003	CSIR (SRF)	A study of apoptosis in filariasis	Dr. B. Ravindran	Registered
2.	Mr. Sudhansu Sekhar Nisank	29/1/2004	CSIR (SRF)	Molecular characterization of Thalassemia and its clinical significance in Orissa	Dr. G.P.Chhotray	Registered
3.	Mr. Aditya K Panda	24/2/2005	CSIR (SRF)	Genetic Polymorphism in Malaria and Filariasis.	Dr. B. Ravindra.	Registered
4.	Mr. Santos K Panda	22/1/2004	CSIR (SRF)	Innate and adoptive immunity in experimental and Human Filariasis	Dr. B. Ravindran	Registered
5.	Ms. Upasana Sahoo	4/8/2005	RMRC (SRF)	Role of Microparticles and ABCA1 transporter in the pathogenesis of cerebral malaria	Dr. M. R. Ranjit	Registered
6.	Ms. Prajyoti Sahu	3/8/2005	RMRC (SRF)	Prevalence of HBV & HCV infection and their genotypes among acute/ chronic symptomatic hepatitis patients in hospital.	Dr. S.K.Kar	Registered
7.	Mr. S.K.Samal	16/8/2005	RMRC (SRF)	Isolation characterization and diagnosis of A. hydrophilia isolated from freshwater fishes	Dr. B. B.Pal	Registered
8.	Ms. Madhumita Panda	29/7/2005	CSIR (GRF)	Immuno regulation of regulatory cells in human and experimental malaria.	Dr. A.K.Satapathy	Registered
9.	Mr. Basanta Kumar Swain	1/8/2005	RMRC (SRF)	Impact of Health and Nutrition Education Intervention by Peripheral Health Institutions among Pregnant Women in Tribal Orissa, India	Dr. B.V.Babu	Registered
10.	Ms. Sunita swain	16/8/2005	Lady TATA Memorial fund (JRF)	Moleculaar Identification of an Annularis comples of Orissa	Dr.R.K.Hazra	Registered
11.	Ms. Ronaly Rout	23/5/2006	UGC (JRF)	Role of Pf. EMPA in Severe Clinical Manifestation off. Malaria	Dr. M.R.Ranjit	Applied for Registration
12.	Mr. Biswaranjan Purohit	19/9/2005	RMRC (SRF)	Malaria Preventive Intermittent Treatment of Choloroquine among the Pregnant Women- an Anthropological Perspective	Dr. A.Mohapatra	Registered
13.	Ms. Asima Tripathy	31/8/2006	RMRC (SRF)	Factors affecting the vectorial competence of anopheles vectors in Orissa and its impact in Malaria.	Dr. N.Mohapatra	Applied for Registration
14.	Mr. Gunanidhi D Majhi	17/10/2006	CSIR (SRF)	Role of iNOS in the pathogenesis of severe P. Falciparum Malaria	Dr. M.R.Ranjit	Applied for Registration
15.	Ms. Swati Kumari	31/8/2007	Lady Tata Memorial Fund (JRF)	Molecular Identification of Anopheles subpictus and its role in malaria transmission in different eco- zones of Orissa, India Dr. N. Mohapatra	Dr. N.Mohapatra	Applied for Registration

Ph.D Award : Dr. Bikash Ranjan Sahu has been awarded Ph.D in Zoology under Utkal University, Bhubaneswar on the topic " Role of Antibody in protective immunity in human and Experimental Filariasis" under the guidance of Dr. B. Ravindran, Ex- Deputy Director (SG), RMRC and presently, Director, ILS, Bhubaneswar in 2008.

M. Sc. Dissertation works: During this period more than 60 M.Sc and three M.Phil. Students from various Universities/ Institutions have undertaken their dissertation program in various departments under the scientists of the Centre as project work for their dissertation program.

Summer Training : During this period the Centre has organized a summer training program for B.Tech (Biotechnology) for the period of one month. More than 50 students from various universities/ Institutions of the country have participated in the training program.

Vigilance Awareness Week : RMRC, Bhubaneswar observed vigilance awareness week from 7th – 11th November 2007 in its premises. A meeting was held on at the center on 12th Nov. 2007 at 3.30 P.M at Seminar Hall of the Centre. All the officials in the seminar also took the pledge as directed by Central Vigilance Commission.

Retirement:

1. Dr. M.K.Das, DD (SG) and Dr. G.P.Chhotray, DD(SG) have supernuated from Council's service w.e.f 28th Feb. 2008 and 31st December 2007 respectively.

4.5 Facility

Library & Information:

Library & Information Centre of Regional Medical Research Centre, Bhubaneswar houses an exclusive collection of books, foreign and Indian journals, databases, reprints, etc. on various subjects of biomedical sciences. For the calendar year 2008, the library subscribed 41 foreign journals and 42 Indian journals and procured 250 books. The library provides services like reference, inter-library loan, on-line literature/database search through Internet and off-line MEDLINE services. The reprint request is also provided to the scientists through ICMR Librarians Group Mail services (icmrlibrarians@yahoo.com) and through JCCC@ICMR. The Local Area Networking (LAN) facility has been provided to all scientists, researchers and office staff along with INTERNET connection to all computers (presently 30 connections) through BSNL broadband.

ONLINE JOURNALS:

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. These journals are RMRC IP activated. All scientists and library users can access the journals in any computer node in RMRC building which are connected to RMRC LAN system.

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

NML- ERMED Consortia

The National Medical Library, New Delhi in collaboration with ICMR and DGHG constituted NML-ERMED consortium for online journal access along with NML library reprint request. In this consortium total 1515 Medical journals are accessible through IP activation. Any scientist can access the journals in RMRC library (www.nmlermed.in). The participating libraries are National Medical Library (NML), all 26 ICMR libraries, AIIMS library, and other DGHS libraries.

Publication Cell:

The library & Information Centre is doing publication activities of the Institute. RMRC News Bulletin and Library News Letter are being published from this division. IEC materials on various diseases are also made on local languages for distribution to the public. Besides, the division looks after the publication of Centre's Annual Report. Head of the library acts as editor of Library News Letter and Asst. Editor of RMRC News Bulletin.

The following are the publications from RMRC being published from library division.

1. RMRC News Bulletin
2. Library News Letter
3. IEC Materials on various diseases on Malaria, Filariasis, Sickle cell diseases, IDD, in regional language.
4. Posters on recent advances in Fiariasis, Malaria, Sickle cell diseases, and diarrhoea for children.

HRD Activities:

RMRC conducts M.Sc. dissertation program for M.Sc. Biotechnology and M.Sc. Microbiology students in two batches per year i.e January- June and July- December. Library acts as Coordinator for six monthly M.Sc. dissertation program of the Centre from Various Universities of the Country . Besides that the Summer Training program for B.Tech (Biotechnology) students also conducted by the institute in April-May.

b. Insectorium

The Centre maintains the insectorium facility at the Entomology division by rearing of various stages of vectors used for laboratory studies. Cyclic colonies of three mosquito species i.e. *Aedes aegypti* (black eyed Liverpool strain), *Anopheles stephensi* and *Culex quinquefasciatus* are being maintained. This year attempt is being made to rear *Aedes albopictus* and *Anopheles culicifacies.* Cyclic colonies are maintained for conducting different experiments such as development of different strains and species of filarial worms that will help in the selection of proper animal model and conducting bio-assays of different plant products for observation of its insecticidal properties. Different stages of mosquitoes are also supplied to Universities, other laboratories and state Govt. for giving training for identification of species. Students from various universities, Research laboratories also come for training to know the technique of rearing of mosquitoes.

c. Animal house

Animal facility in the center continues to be used for all relevant on-going projects. Currently M. Coucha, Balb/c mice, Guinea pigs and Rabbits are available for experimentation. All the projects concerning animal use/ experimentation are discussed in Animal ethical committee of the center and work progress also reviewed periodically by the committee. Staffs have maintained periodic records of animal house. This facility is maintained regularly with periodic inspection and health monitoring by vetenary doctor.

d. OPD Facilities:

The filariasis OPD has been initiated and continuing at capital hospital, Bhubaneswar since last seven years. The filariasis cases referred by the physicians from different areas of the state are being rendered the service of diagnosis, treatment advice, and compression therapy using pneumatic decompression machine and instruction on foot care management of lymphoedematous limb. Another room has been asked to the capital hospital authority where the diagnostic facility of malaria and hepatitis will be made available.

Activities of NNMB Unit, Orissa, Bhubaneswar

The NNMB Unit, Orissa under NIN, Hyderabad functioning at RMRC, Bhubaneswar has completed the second Repeat Tribal Survey for "Assessment of Diet & Nutritional status of Tribal population along with assessment of Prevalence of obesity and hypertension among adult men and women of >= 20 years of tribal population."

The objectives of the survey are as follows:

1. To assess the food and nutrient intake among different age/sex/physiological groups of tribal population living in the Integrated Tribal Development Agency/Project (ITDA/P) areas in NNMB functioning states.
2. To assess their nutritional status of all the available individuals in terms of Anthropometry, clinical examination and to study the time trends in the nutritional status.
3. To assess the history of morbidity during previous fortnight among all the individuals covered for anthropometry.
4. To assess the prevalence of obesity and hypertension among the adult men and women (>= 20 years) in tribal population.
5. To assess awareness about hypertension and Diabetes among adults (>= 20 years) of the tribal community.

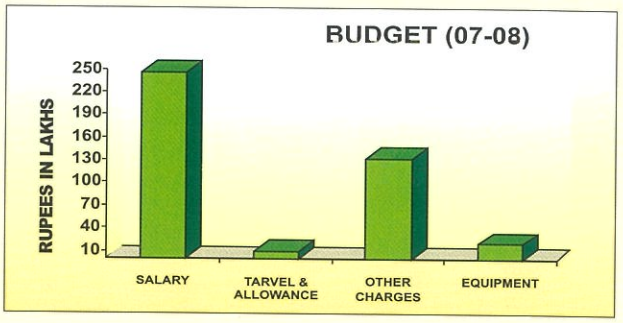
All 120 villages were selected in 11 tribal districts of the state were completed by June 2008. Total 4,808 households were covered during the survey. Demographic Profile and Socio-economic status of each household was collected in a predesigned schedule. 15603 individuals were covered for clinical Examination, Anthropometry and to know their morbidity status.

Diet Survey was completed in 1200 households using Individual Dietary Food Intake method. KAP on Hypertension and Diabetes Mellitus with Blood Pressure measurement, waist circumference and Hip circumference measurement was covered among 3144 individuals out of which 1580 are males and 1564 are females. The data was sent to NIN, Hyderabad for analysis.

The Unit has also done Survey for "Early detection of chronic renal diseases among tribal population of Orissa where some important data like the tribe to which he or she belongs, his/her age at marriage, any infant mortality, history of olignria, anuria, Haematuria, Pyuria, Diabetes Mellitus, Hypertension are collected along with Blood Pressure measurement and examination of Urine for Albumin/ Sugar/ Micro-albumin among >= 15 years individuals. All total 1984 individuals are covered in this survey. The data was handed over to clinical Division of RMRC, Bhubaneswar for analysis."

Budget and Resource Generation:

The total sanctioned Budget in respect of the Centre (Non-Plan & Plan) for the year 2007-08 is 4.10 Crore. The head-wise expenditure of budget is shown below in the graph. The resource generation during the period is 1 Crore 50 Lakh from extramural grants and Ph.D program through UGC , CSIR and others.



4.6 22nd Scientific Advisory Committee

Dr. Sandip K. Basu — Chairman
Professor Eminence
National Institute of Immunology
Aruna Asaf Ali Marg
New Delhi 110 067

Dr. Satish Gupta — Member
Staff Scientist-VII and Chief
Gamete Antigen Laboratory
National Institute of Immunology
Aruna Asaf Ali Marg,
Delhi 110 067

Dr. K. Ramachandran — Member
No.8, G-2, Visveshapuram
Mylapur, Chennai 600 004

Dr Amit Ghosh, Director, — Member
Indian Institute of Advanced Research
The Puri Foundation,Block No.2,
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Gandhinagar 382 011

Dr. V. Kumarswami — Member
Deputy Director(SG) & OIC
Tuberculosis Research Centre
Mayor V.R. Ramanath Road
Cheput, Chennai-600031

Dr. D. S. Agrawal — Member
B-24, Swasthya Vihar,
Delhi110092

Dr.Subrat K. Acharya — Member
Prof,. & Head Dept of Gastroenterology
AIIMS, New Delhi 110 029

Dr S. K. Kar, Director — Member Secretary
RMRC, Bhubaneswar.

ICMR Representative
Director, Health Services — Special Invitee
Govt. of Orissa, Bhubaneswar.

Dr. P. K. Das — Special Invitee
Ex-Director, VCRC, Pondicherry

Human Ethical Committee:

1. Justice (Mrs) A.K.Padhi - Chairperson
   Former Judge, Orissa High Court
   10, Bhasakosh Lane, Nimchouri
   Cuttack 753 002
2. Mrs Kasturika Pattanayak - Member
   Ex-Chair Person
   Social Welfare Board
   Govt. of Orissa, 1, Lewis Road
   Bhubaneswar.
3. Dr (Mrs) Manorama Das - Member
   Retd. Prof. of Pharmacology &
   Addl. Secretary H&FW
   Santiniketana, Mathasahi
   Station Bazar
   Cuttack 753 003
4. Dr P. K. Acharya - Member
   N-1 A/10 IRC Village
   Near CRP Square
   Bhubaneswar 751 015
5. Dr.B.B. Tripathy - Member
   Retd. Prof. Of Medicine
   Saradiya Mission Road
   Cuttack 753 001

6. Dr (Mrs) P.Mohanty Hejmadi - Member
Ex-V.C., Sambalpur University
GM-8, V.S..S.Nagar
PO: Vani Vihar, Bhubaneswar 751 004

7. Dr.S.K. Kar - Member Secretary
Director
R.M.R.C., Bhubaneswar

Institutional Ethical Committee for Animal Experimentation

1. Biological Scientist :	Dr M.K.Beuria, RMRC,BBSR
2. Two scientists from different Biological disciplines :	Dr R.C.Patra, Prof. & Head Dept. of Veterinary Medicine OUAT, Bhubaneswar
	Dr R.K. Hazra RMRC,BBSR
3. Veterinarian	Dr S.K. Ray, Ex-Principal Orissa Coll. of An. Husb & vet. Sc. Bhubaneswar
4. Scientist In-charge :	Dr A.K.Satapathy Animal facility RMRC, BBSR.
5. A biological scientist from Outside the Institute :	Prof. G.B.N.Chainy Prof. of Zoology, Utkal University, BBSR
6. A non-scientific socially aware member :	Mrs.Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, BBSR
7. Nominee of the CPCSEA, Chennai :	Mr N. R.Mansingh, Inspector, SPCA O/o CDVO Office, Puri
8. Member – Convener :	Dr S.K.Kar, M.D., Director, Regional Medical Research Centre Bhubaneswar.

Technical Equipment Purchase Committee

1. Dr. A.K. Sahoo — Chairman
Principal Scientist
CIFA, Kausalya gang
Bhubaneswar- 751 002

2. Dr. P. Das — External Member
Sr. Scientist
CIFA, Kausalya gang
Bhubaneswar- 751 002

3. Dr. N.K. Debata — External Member
Prof. Microbiology
SUM-Hospital, Bhuabnesware

4. Dr. M.K.Beuria — Member (Sub. Specialist)
Scientist-D
RMRC, BBSR

5. Dr. B. Dwibedi — Member
Scientist-B
RMRC, Bhuabneswar

6. Mr. G. Behera
Accounts officer
RMRC, BBSR — Member

7. Dr. (Mrs.) N. Mohapatra
Scientist D
RMRC, BBSR — Member -Secy

12. Technical Building Maintenance Committee :

1. Mr. D.N. Tripathy Retd. Chief Engineer, CPWD	Chairman
2. Mr. P.K. Pattanik Retd. Sup. Eng. (Elect.), CPWD	Member
3. Mr. P. Kapoor Retd. Jt. Director (Agriculture)	Member
4. Dr. M.R. Ranjit Scientist-D, RMRC	Member
5. Mr. A.K.Mohapatra Admn. Officer, RMRC	Member

BIO-SAFETY COMMITTEE

1. Prof.G.B.N. Chainy Zoology Department Utkal University, Vaini Vihar Bhubaneswar 751 004	Chairman
1. Dr.M.R. Ranjit, Scientist-D RMRC, BBSR	Member
2. Dr.M.K. Beuria, Scientist-D RMRC, BBSR	Member
3. Dr. R. K. Hazra, Scientist-C RMRC, BBSR	Member

Scientists in Research Group

Filariasis

Dr. (Mrs.) N. Mohapatra, M.Sc., Ph.D.	Scientist-D
Dr. M.K. Beuria, M.Sc., Ph.D.	Scientist D
Dr. A.K. Satapathy, M.Sc., Ph.D.	Scientist-D
Dr. Bhagirathi Dwibedi, M.B.B.S, M.D	Scientist-B

Malaria

Dr. M.R. Ranjit, M.Sc., Ph.D.	Scientist-D
Dr. (Mrs.) A.S. Kerketta, M.B.B.S.	Scientist-C
Dr. A. Mohapatra, M.Sc., M.Phil., Ph.D.	Scientist-D
Dr. R.K. Hazra, M.Sc., Ph.D.	Scientist-C

Diarrhoea

Dr. B.B. Pal, M.Sc., Ph.D.	Scientist-D

Haemoglobinopathy:

Dr. A. Moharana, MBBS, M.D	Scientist-B

Nutrition:

Dr. G. Bulliyya, M.Sc., Ph.D.	Scientist D

Tuberculosis

Dr. Dasarathi Das, Ph.D.	Scientist-C

Statistical support group:

Mr.P.K. Jangid, M.Sc,	TO
Dr.A.S. Acharya, Ph.D.,	RA
Mr.R.C. Parida, M.Sc	SA

RMRC Foundation Day held on 29th March, 2008

Staff position (As on 1st July 2008)

Scientists:

DR. S.K. Kar, MD, Dip. Clin. Epid.	Director (Scientist-G)
Dr. (Mrs.) N. Mohapatra, M.Sc., Ph.D.	Scientist-D
Dr. M.K. Beuria, M.Sc., Ph.D.	Scientist-D
Dr. M.R. Ranjit, M.Sc., Ph.D.	Scientist-D
Dr. A. Mohapatra, M.Sc., M.Phil., Ph.D.	Scientist-D
Dr. A.K. Satapathy, M.Sc., Ph.D.	Scientist-D
Dr. G. Bulliyya, M.Sc., Ph.D.	Scientist-D
Dr. B.B. Pal, M.Sc., Ph.D.	Scientist-D
Dr. R.K. Hazra, M.Sc., Ph.D.	Scientist-C
Dr. Dasarathi Das, Ph.D	Scientist-C
Dr. (Mrs.) A.S. Kerketta, M.B.B.S.	Scientist-C
Dr. Bhagirathi Dwibedi, M.B.B.S, M.D	Scientist-B
Dr. A. Moharana, MBBS, M.D	Scientist-B

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Dr. S.K. Parida, M.Sc., Ph.D.	Technical Officer
Mr. P. K. Jangid, M.Sc.	Technical Officer
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Dr. A.S. Acharya, M.Sc., M.Phil, LL.B.,Ph.D	Research Assistant
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Mr. R.C. Parida, M.Sc.PGDCA	Research Assistant
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Mr. D.P. Hansdah, M.Sc.	Research Assistant
Mr. N. Mandal, M.Sc., M.Phil., B.Ed.	Research Assistant
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Dr. (Mrs.) M.S. Bal, M.Sc.,M.Phil., Ph.D.	Research Assistant
Dr. H.K. Khuntia, M.Sc. Ph.D	Research Assistant
Miss. Sujata Dixit, M.Sc	Research Assistant
Mr. H.K. Tripathy, B.Sc, PGDME	Technical Assistant
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Mr. R.N. Nayak, B.A.	Census Taker
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Mr. B.N. Sethi, Dip. MLT	Lab. Technician
Mr. S.C. Rout	Lab. Technician
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Mr. K. C. Dalai, B.A., ITI	Lab. Assistant
Mr. B. K. Kanhar	Lab. Assistant
Mr. G. D. Mansingh	Insect Collector
Mr. B. Pradhan	Insect Collector

Mr. C.S. Tripathy, B.Com. LL. B.	Insect Collector
Mr. S.S. Beuria	Insect Collector
Mr. G. Simhachalam	Insect Collector
Mr. K.C. Parichha	Insect Collector
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Mr. N.N. Pattnaik	Laboratory Attendant
Mr. K.C. Jena	Laboratory Attendant
Mr. S. K. Mallick	Lab. Attendant
Mr. H.K.Jena	Field Attendant
Mr. Banamali Nayak	Field Attendant
Mr. Baburam Behera	Sweeper- cum- Attendant
Mr. K.C. Nayak	Sweeper

STUDENTS:

Mr. Santosh Kumar Panda, M. Sc.	SRF (UGC)
Mr. Aditya Kumar Panda, M. Sc.	JRF (CSIR)
Miss. Madhumita Panda, M.Sc.	JRF (UGC)
Mr. Sudhansu Sekhar Nishank, M. Sc.	SRF (CSIR)
Miss. Ronali Rout, M.Sc.	JRF (UGC)
Mr. Gunanidhi D Majhi, M.Sc.	JRF (UGC)
Miss Upasana Sahoo, M.Sc.M.Phil	SRF (RMRC)
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Basant Kumar Swain , M.A	SRF (RMRC)
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Biswa Ranjan Purohit , M.A	SRF (RMRC)
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Sunita Swain M.Sc.	JRF (Lady Tata)
Swati Kumari, M.Sc	JRF (Lady Tata)

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Mr. Chakradhar Naik	Sweeper-c-Attendant
Mr. R.S. Bahadur	Watchman

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Mr. R. Rath	L.D.C.
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Mr. D.C.Rao	Sweeper

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Mr. K.G. Samal	Attender
Mr. R.K. Hembram	Field Attendant

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Mr. S.K. Satapathy	U.D.C.
Mr. Sankar P Sharma	Watchman

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Mr. B.K. Moharana	Plumber-c-Carpenter
Mr. Banamali Sahoo	Gardener
Mr. Sankar Bisoi	Cook-cum-Guest House Attd.

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Mr. S.K. Das	Animal House Attendant
Mr. Jaladhar Naik	Animal House Attendant
Mr. Pandav Sahoo	Animal House Attendant

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Mr. R. Pradhan	Driver (Grade-I)
Mr. Anakar Nayak	Driver (Grade-II)
Mr. A.R. Khan	Driver (Grade-II)
Mr. P.K. Behera	Driver

NNMB STAFF

Dr. S.K. Das, MBBS	Research Officer (Medical)
Mrs. S. Paikray	Asst. Research Officer
Mrs. Haraprava Sahu	Social Worker
Mr. D.K. Mohanty	Steno-C-Office Asst.
Mr. R.K. Sahoo	Driver

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