3 Communicable Diseases



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3.1 Transmission dynamics of malaria in tribal areas

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Status : Ongoing project (May 2004 - November 2007)

Funding Agency : ICMR (Extramural)

Objectives

1. To study the dynamics of transmission of malaria in villages as well as at places of their occupational activities.
2. To study the bionomics of vector species and to develop appropriate control strategies.
3. To study the parasitological parameters in relation to transmission and treatment.
4. To study the population movement in relation to forest based economy.
5. To study the socio-cultural aspects related to disease and health.

Methodology

The study is being carried out in Baigachak (Dindori district) and Kanha (Mandla district) areas. These two districts are highly malarious areas in Madhya Pradesh. Together, these districts contribute for 29% of the state's malaria and 40% of *P. falciparum* infection while their population is only 2.4% of the state's population. Four villages have been selected in each of these areas. Fortnightly fever survey and spleen survey were carried out. Blood slides were collected .

Vector density and composition were monitored using hand catch, light trap and animal bait method. Sibling species of vectors were identified by polytene chromosome preparation method.

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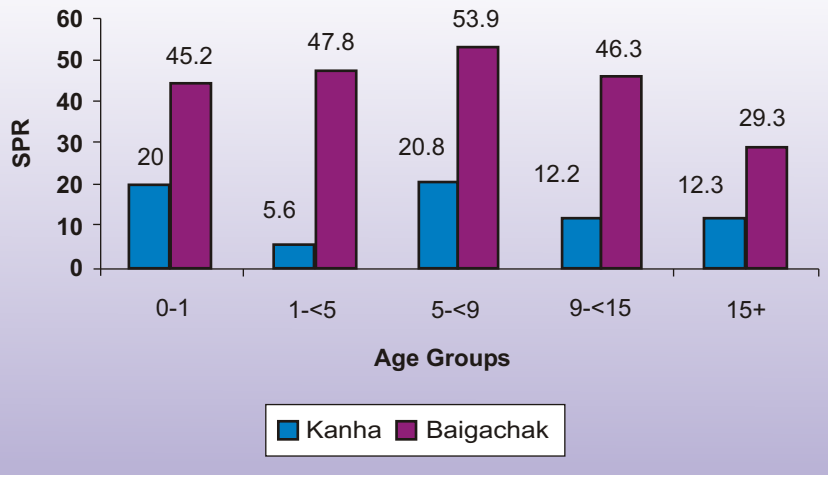
Salient Findings

Fever surveys revealed that overall slide positivity rate (SPR) & slide falciparum rate (SFR) in Baiga villages was 38% and 34.9% respectively, which was significantly higher than the corresponding figure in Kanha villages i.e. 12.6% and 12.3% ($P<0.05$). *P. falciparum* contributes for 92% and 98% of total malaria cases respectively in these two areas. Age group wise record revealed that people of all ages were infected with malaria and in Baigachak villages, positivity was higher in all the age groups. Infant and child parasite rate in Baigas were 45.3 & 41.2 respectively, while these indices were low in Kanha villages (Fig 3.1.1) where malaria infection was recorded in younger age groups in October month only.

Fig. 3.1.1 Age group wise slide positivity rate (SPR) in study areas



Spleen Survey: In each of the areas, 135 children between the ages of 2-12 years were examined for splenomegaly. Spleen rate of 66.7 and 15.5 were recorded from Baigachak and Kanha respectively during July-August. Slide positivity rate in splenomegaly cases were 70 and 0 respectively.

Entomological monitoring: Overall MHD of anophelines were higher (36.9) in Kanha villages than the Baiga villages (32.9) so was the density of *An. culicifacies* ($Z=2.44, p<0.05$). *An. culicifacies* was seen only in monsoon months in Baigachak while in Kanha, it was prevalent throughout the year. Resting density of *Anopheles fluviatilis* remains less at both the sites, as 0.8 & 0.14 and constitute 2.4 and 0.4% of total anophelines at Baigachak and Kanha villages respectively. However, light trap catches revealed that per night per trap catch of *An.fluviatilis*

was higher in Baiga villages (3.5) than Kanha villages (2.4) and constitute 23.6 and 8.5% of the total anopheline trapped. In Kanha area, all the *An. fluviatilis* were recorded in October month only.

Insecticide Susceptibility Test : *Anopheles culicifacies* was tested against DDT (4%) and Deltametherine (0.05%) as per the WHO standard method. Mortality of 15% against DDT (4%) after 24-hour recovery period was recorded from Baigachak area. Ninety-five percent mortality was observed against Deltametherine (0.05%) within one hour of exposure period and cent percent within recovery period of five hour.

Sibling Species : Specimens of *Anopheles culicifacies* ovaries were sent to Malaria Research Centre Delhi for sibling species identification. Specimens were examined by Polytene chromosome preparation. Species C is the predominant species at both sites followed by B in Kanha area and D in Baigachak. Only few specimens were identified as species A from both the areas (Table-3.1.1). *Anopheles fluviatilis* specimens are also being investigated using PCR and cytological techniques.

Table 3.1.1. Sibling species of *Anopheles culicifacies*

Area	Sibling species				
	A	B	C	D	Total
Kanha	2(3.0%)	9(10%)	77(86%)	-	88
Baigachak	1(5.5%)	1(5.5%)	46(89%)	3	59
Total	2(3.4%)	10(8.5%)	123(88%)	3	139

Host feeding preference: A total of 239 blood elutes of *An. culicifacies* were examined by gel diffusion method at MRC Delhi which includes 26 specimens collected from human dwellings during peak transmission season. Only one specimen was found positive for human sera (from Baigachak, sub species C). Rest all were positive for bovine sera. Fifteen blood elutes of *An. fluviatilis* were also examined from Baigachak area. One specimen showed positive reaction with human sera.

The study is in progress.

3.2 Prevalence of dengue in Jabalpur city

Gyanchand, Dr. Lalita Kabilan* Dr. N. Arunachalam*

Status : Ongoing project (November 2003 - October 2005)

Objectives

1. To study the prevalence of dengue vectors in different seasons and their potential breeding habitats.
2. To determine virus infection in vector species in study area.
3. To determine the prevalence of dengue infection in humans using ELISA and Haemagglutination inhibition techniques.

Methodology

Four areas of Jabalpur city were selected randomly covering all directions and socio-economic groups. Every fourth house in each area was searched for water holding containers and presence of *Aedes* larvae in those using flashlight. Larvae from these containers were collected and reared up separately to adult stage to identify the species and its breeding preferences. *Stegomyia* indices like House index (HI) Container index (CI) Breteau index (BI) and Pupal index (PI) were calculated

Salient Findings

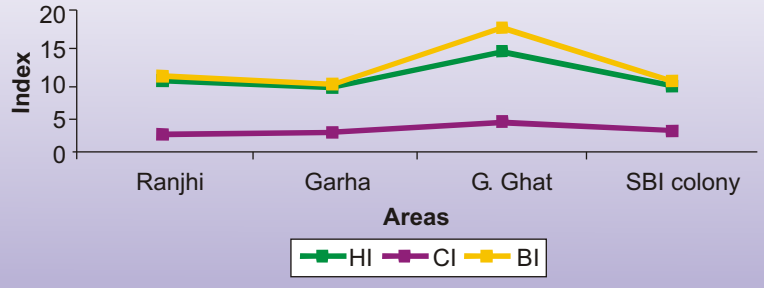
In all, 3515 household were surveyed in different months and 12897 water holding containers were checked. Overall HI, CI and BI were 10.8, 3.3 and 12.1 respectively which varied from month to month. Overall *Aedes aegypti* infestations were higher than threshold level of 5% of HI and below threshold level of BI (>20%).

Area-wise house, container and breteau index : Distribution of *Aedes* species was not uniform in all the areas. All the indices were higher in Gwari Ghat area

* CRME, Madurai

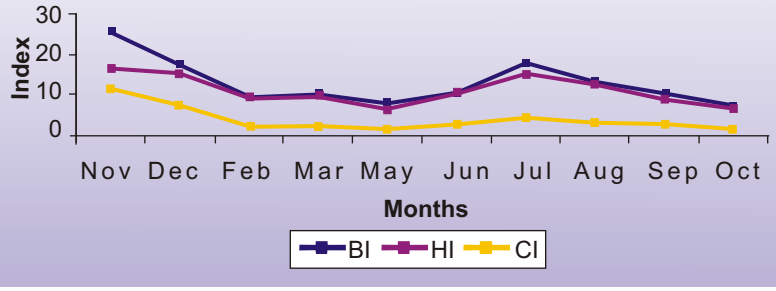
and lowest in SBI colony (Fig 3.2.1). Overall BI was below threshold level while HI was above the threshold level in all areas.

Fig. 3.2.1 Breteau, House and Container index in different areas of Jabalpur city



Month wise prevalence of *Aedes aegypti*: Overall Breteau index, Container index and House index showed typical seasonal trend with its peak during November and gradual decline till May and thereafter again in monsoon months (Fig 3.2.2).

Fig. 3.2.2 Breteau, Container index in Jabalpur



Aedes aegypti breeding habitat: Tyre, cement tank, under ground tank, cement cistern and ceramic drum were observed to be the major breeding sites for *Ae. aegypti*. Mud pot, metal drum and plastic containers also contribute significantly as breeding sites. During summer (May), coolers were also found having *Aedes* larvae. Among infested containers, mud pots constituted 28% followed by cement tanks and cement cisterns.

Species composition: A total of 1329 specimens of Aedes genera were

emerged. *Ae. aegypti* was the commonest species (94.9%) followed by *Ae. vittatus* and *Ae. albopictus* (Table 3.2.1).

Table 3.2.1 Species composition of *Aedes*

Month	*Ae. aegypti*		*Ae.albopictus*		*Ae. vitattus*		Total	%*Ae.aegypti*
	Male	Female	Male	Female	Male	Female		
Nov	91	108	9	13	0	0	221	90
Dec	89	99	0	2	0	0	190	99
Feb	44	68	0	0	0	0	112	100
Mar	71	101	0	0	0	0	172	100
May	28	32	0	0	0	1	61	98
Jun	83	93	0	0	5	2	183	96
Jul	90	101	0	0	3	12	206	93
Aug	24	29	0	0	10	5	68	78
Sept	31	30	0	0	2	2	65	94
Oct	27	24	9	0	0	0	51	100
Total	578	685	9	15	20	22	1329	95

Indoor resting density: Limited efforts were made to collect adult *Aedes* mosquito to determine man-hour density and species composition. Overall man-hour density of *Ae. aegypti* and *Ae. albopictus* was 2.3 and 0.06 respectively. *Aedes aegypti* constituted 96% of *Aedes* genera. Species composition of resting adults was similar to the composition of laboratory emerged adults (Table 3.2.2)

Table 3.2.2 Man Hour density of *Aedes aegypti* in Jabalpur

Months	*Ae. aegypti (N)*	*Ae. albopictus (N)*	*Ae. aegypti (MHD)*	*Ae.albopictus (MHD)*
Jan	*8*	*1*	*2.0*	*0.25*
Feb	*2*	*0*	*0.5*	*0.0*
Mar	*14*	*0*	*3.5*	*0.0*
May	*0*	*0*	*0.0*	*0.0*
Jun	*27*	*0*	*6.5*	*0.0*
Aug	*12*	*0*	*3*	*0.0*
Sept	*14*	*1*	*1.6*	*0.1*
Oct	*7*	*0*	*1.8*	*0.0*
Total	*84*	*2*	*2.33*	*0.06*

The study is in progress. Blood samples from school children and adult mosquitoes are being collected. Dengue virus isolation will be carried out at CRME, Madurai.

3.3 Epidemiologic and etiologic study of acute diarrheal diseases in pre-school children of tribal areas of Madhya Pradesh

Dr. Anup Anvikar, Dr. C. K. Dolla, Dr. Tapas Chakma, Dr. V. G. Rao

Status : Ongoing project (May 2004 - November 2005)

Funding Agency: ICMR (Extramural)

Objectives

1. To assess the incidence of acute diarrheal diseases in under 6 children.
2. To identify organisms responsible for acute diarrheal diseases and know their antibiotic susceptibility pattern.
3. To characterize *E. coli* strains into various diarrheagenic types by using PCR.

Methodology

Study design, site and participants

This community-based study is being carried out in 8 tribal villages of Jabalpur district. The study group comprised of 866 tribal preschool children (age 6 years) having acute diarrhea. Chlildren are being followed up everyday for presence of diarrhea. A standard protocol was used to assess the clinical history. Detailed characteristics of each episode such as number of motions per day, whether feces are liquid, semisolid, or solid, presence of blood/mucus, h/o fever, pain in abdomen and vomiting were noted. Stool samples were collected from children having diarrhea. They were immediately transported to the laboratory.

Investigation of stool samples

The samples were cultured on appropriate media to isolate and identify *Salmonella* sp, *Shigella* sp, *Vibrio*. Antimicrobial susceptibility testing was done using disc diffusion method. The samples were also examined for the presence of trophozoites, ova and cysts of parasites by direct microscopy. Rotavirus was detected by using ELISA.

Detection of diarrheagenic E. coli by PCR

The *E. coli* isolates were grown in Luria broth and subjected to PCR for identifying various genes of diarrheagenic *E. coli*.

Salient Findings

This community-based study is being carried out in 8 tribal villages of Jabalpur district. The study group comprised of tribal preschool children (age 6 years) having acute diarrhea. Eight hundred and sixty six children are being followed up every day for the presence of diarrhea. Stool samples were collected from children having diarrhea and were immediately transported to the laboratory.

In all, 589 episodes of diarrhea were recorded during the study period. Stool samples could be collected from 408 children. Maximum children having diarrhea were in the age group of 6 months to 1 year followed by those in age group of 1 to 2 years. Bacterial enteropathogens, except diarrheagenic *E. coli* were isolated from 12 children. *Shigella* sp was isolated from 7 samples, *Vibrio cholerae* from 2 and *Salmonella* sp from 2 samples. No *Plesiomonas shigelloides, Aeromonas* species, *Campylobacter* or *Yersinia* species were isolated. *Giardia lamblia* was identified in 31, *Entamoeba histolytica* in 5, and *Entamoeba coli* in 8 children (Figure 3.3.1).

E. coli was isolated from 106 samples. *E. coli* strains were typed into various diarrheagenic types using multiplex PCR (Table 3.3.1, Figure 3.3.2). Enteroaggregative *E. coli* (EaggEC) was isolated from 40 samples. Enterotoxigenic *E. coli* (ETEC) was isolated from 2 samples and Enteropathogenic *E. coli* (EPEC) from 9 samples. From 48 children, non-diarrheagenic *E. coli* was isolated. Mixed infections of diarrheagenic *E. coli* with non *E. coli* pathogens were not observed. However, mixed infection of various diarrheagenic *E. coli* was observed. In 4 children, both EPEC and EaggEC were present. EPEC, EaggEC and ETEC were present in one child, while ETEC and EaggEC were present in one child. Both Shigatoxin *E. coli* as well as ETEC were present in one child. The rates of detection of diarrheagenic *E. coli* in children with diarrhea are shown in Figure 3.3.1.

Antimicrobial susceptibility pattern showed that diarrheagenic *E. coli* strains were highly resistant to Co-trimoxazole (75.9%) and Ampicillin (72.4%). These

strains showed 63.8% resistance to Cephalexin, 55.2% to Norfloxacin and Ciprofloxacin, 46.6% to Chloramphenicol, 43.1% to Furazolidone and 41.4% to Nitrofurantoin.

Table 3.3.2 shows the clinical profile of children with diarrhea caused by diarrheagenic *E. coli*. More than two third of the cases occurred in the age group of 6 months to 2 years. *E. coli* diarrhea cases were equally seen in both male and female children. EAggEC diarrhea was associated with high fever in 4 children and that due to ETEC with one child. Two children with EAggEC infection presented with bloody diarrhea.

Table 3.3.1: Types of diahhreagenic *E. coli* and primer sequences

Type of *E. coli*	Target gene
Entero toxigenic *E. coli*	elt, est
Entero pathogenic *E. coli*	eae, bfpA, eaf
Shigatoxin producing *E. coli*	stx1 , stx2
Enteroinvasive *E. coli*	ipaH
Enteroaggregative *E. coli*	eagg, east

Figure 3.3.1: Detection of various pathogens in stool samples (n=114)

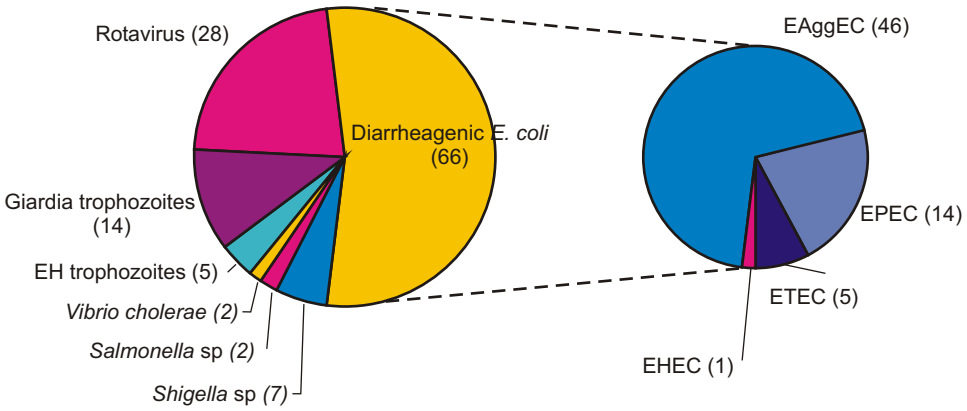


Figure 3.3.2 PCR for detection of genes of EAggEC

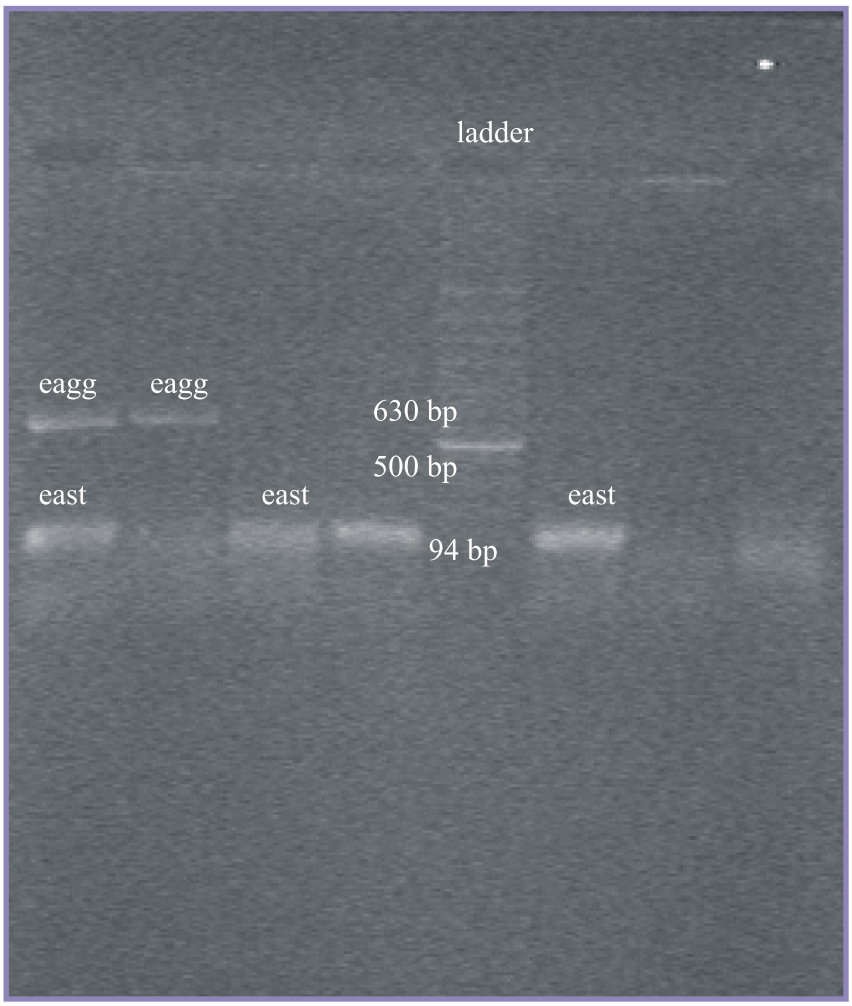


Table 3.3.2: Clinical profile of children with diarrhea caused by diarrheagenic *E. coli*

		EaggEC (n=46)	EPEC (n=14)	ETEC (n=5)	EHEC (n=1)
Age	<6 months	4	0	0	0
	6 months -3 years	37	13	5	1
	> 3 years	5	1	0	0
Sex	M	24	6	4	1
	F	22	8	1	0
Associated parasitic infection		10	2	0	0
Duration	Upto 3 d	13	4	3	0
	3-7 d	16	6	1	1
	7+d	17	4	1	0
No. of episodes	Upto 5	16	9	3	0
	5+	30	5	2	1
Stool character	Watery	39	12	5	1
	Mucoid	7	2	0	0
	Bloody	2	0	0	0
Associated symptoms/signs	Fever (>38^{0}C)	4	0	1	1
	Vomiting	7	1	0	0
	Pain in abdomen	6	1	0	0
	Dehydration	12	2	1	1

3.4 A study of markers of hepatitis B, C and HIV in tribes of Orissa

Dr. Anup Anvikar, Dr. Tapas Chakma, Dr. D. Das, Dr. A. P. Dash

Status : Ongoing project (July 2004 - January 2006)

Funding Agency: ICMR (Extramural)

Objectives

1. To find out the prevalence of viral markers in different tribes of Orissa.
2. To know the subtypes of hepatitis B & C viruses and HIV responsible for infection in the tribes.

Methodology

The study is being conducted in *Bondo* tribe of Orissa. The tribe is located in inaccessible, interior areas of Khairput block of Malkangiri district of Orissa. Total population of the tribe is around 5,129 (according to 1991 census), which is distributed in small groups; the population of each group ranging from 50 to 200.

Serum samples will be collected from 1000 individuals. Serology for HIV, HBV and HCV will be conducted by using commercial ELISA kits. Sub typing of HBV and HCV will be done by PCR.

The study is in progress.



3.5 Concomitant infection of intestinal parasites with filaraisis

Dr. D. Das, Dr. Surendra Kumar, Dr. Anup Anvikar, Dr. A. P. Dash

Status : Ongoing project (September 2003 - August 2005)

Funding Agency : ICMR (Extramural)

Objectives

1. To estimate the prevalence of filariasis by antigen detection method.
2. To estimate the infestation of intestinal parasites in the study population.

Methodology

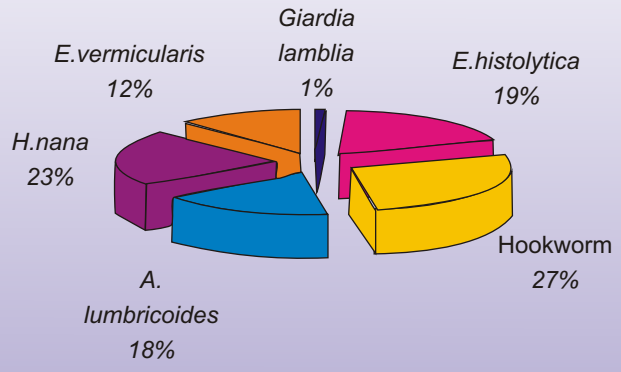
The subjects of five villages in Ajaygarh block, Panna district were screened for filariasis. The individuals were clinically examined for filarial diseases like adenolymphangitis, hydrocele (in males) and lymphoedema following WHO guidelines. About 2 ml blood was collected from each individual by venepuncture, Sera were separated and stored at -20^0 C until tested. The presence of Circulating Filarial Antigen was estimated by using Og4C3 ELISA. Stool samples were collected in the morning from these individuals and wet smears were examined under microscope for the presence of intestinal parasites. A part of the stool sample was also preserved by adding formalin and later examined for the presence of intestinal parasites by sugar flotation method.

Salient Findings

It was observed that out of 544 individuals screened for filariasis, 36.2% showed the presence of CFA by Og4C3 ELISA, reflecting active filarial infection in them. The prevalence of filarial disease (lymphangitis, hydrocele, lymphoedema) was found to be 26.1% in this population. Stool samples could be obtained from 265 of these 544 individuals. It was observed that about 50% of this population was infected with at least one intestinal parasite. The commonest helminths

observed were Hook worm (14.3%), Round worm (9.1%), followed by *E. vermicularis* (6.4%). Commonest protozoan seen was *E. histolytica* (9.8%). *G. lamblia* was seen in one stool sample (Fig 3.5.1).

Fig. 3.5.1 Distribution of various helminths (n=265)





W. bancrofti **microfilariae**

3.6 Community prevalence and etiology of sexually transmitted infections in tribal area of Madhya Pradesh

Dr. V.G. Rao, Dr. Anup Anvikar, Dr. K.B. Saha, Dr. C. K. Dolla

Status : Ongoing project (July 2004 - October 2005)

Funding Agency: ICMR (Extramural)

Objective

To know the community prevalence of sexually transmitted infections in tribal area of M.P.

Methodology

The study is being conducted in Kundam block of Jabalpur district. As per the prevalence obtained in earlier studies, a sample of 10000 has been drawn for the study. Individuals in the age group of 15-49 years were screened for STDs. Various samples like blood, urethral discharge, endocervical swab, vaginal swab & urine were collected and processed as under:

Table 3.6.1 Processing of samples

Sr. No.	Sample	Investigation performed	Organism detected
1	Blood	ELISA	HBV, HIV, HSV
		TPHA	*T. pallidum*
2	Vaginal Swab	Gram Stain	*G. vaginalis*
3.	Urethral Swab Endocervical Swab	Gram Stain, Culture	*N. gonorrheae*
4.	Urine in males Endocervical Swab in females	PCR	Chlamydia

Salient findings

A population of 1174 from four tribal villages was screened for the presence of various STDs. Fifty two individuals were having at least one STD giving a community prevalence of 4.4%. In males, the prevalence was 3.8%, while in females, 5.5%. In males, the commonest STD was gonorrhea followed by syphilis. Bacterial vaginosis was the commonest STD in females, followed by trichomoniasis, gonorrhea and candidiasis (Table 3.6.2.)

The study is in progress.

Table 3.6.2 STDs in study population

Sr. No.	STD	Males (n=449).	Females (n=725).
1	Gonorrhea	5	4
2.	Syphilis	4	2
3.	Bacterial Vaginosis	-	12
4.	Trichomonasis	-	7
5.	Candidiasis	-	6
6.	HIV	2	-
7.	HSV	5	6
8.	Chlamydia	1	3
9.	Total	17	40*

*Five women had multiple STDs