

Message

I am delighted to keep on record of the excellent progress and contribution of Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna for significant scientific achievements made towards the goal of prevention, control and treatment of Visceral leishmaniasis in Bihar. The contribution of the Institute in conducting clinical trials of anti-leishmanial drugs like Miltefosine and Paromomycin in collaboration with ICMR/WHO/TDR is remarkable.

The Council will make all endeavors to help this Institute to achieve its ultimate glory.

Prof. N.K.Ganguly
Director-General
I.C.M.R., New Delhi.



Rajendra Memorial Research Institute of Medical Sciences
2002-2003

Preface

A series of landmark events marked the year 2002-2003. Revitalization of the Institute at different levels became evident as the WHO/TDR expressed its willingness to make it an important center for the clinical trials on Kala-azar in this region.

Staggering amount of capital works were accomplished in the year 2002-03. Building a new Tropical Disease Hospital (Samrat Ashoka Hospital), an extension of the existing 50-bedded hospital, is an enormous undertaking. The preliminary work of site preparation at the construction site was accomplished during this period. Preparation and processing of the EFC document was approved. It is envisaged to provide a state of the art medical research center for tropical diseases on the pattern of Mahidol Tropical Disease Research Center, Bangkok and School of Tropical Medicine, Calcutta.

Application of remote sensing technology (GIS) for identification of Kala-azar epidemic foci was pursued for the last three years bore fruitful and encouraging results. The satellite data of study areas have already been extracted. FCC map and Land use map, generated by RRSC, Kharagpur, were compared to find out the correlation of geographical distribution of vector, sandfly with environmental and land cover variables.

PCR based diagnosis of Visceral Leishmaniasis was initiated to compare its usefulness with conventional diagnostic methods. Biochemical and molecular characterization of isolates from SAG responsive and unresponsive Kala-azar patients from different parts of Bihar are being continued to study the strain variations of *Leishmania donovani*.

Comparative efficacy of DDT and malathion for vector control in selected endemic villages of Bihar were undertaken. *P.argentipes* was prevalent in both endemic and non-endemic districts. Insecticide susceptibility test in district Vaishali demonstrated presence of DDT resistant foci.

A study on role of Grass-root Level PHC's functionaries was initiated with objectives to assess the functioning of health care services in Kala-azar endemic regions and identifying limitations, if any.

The constant guidance and support received from Prof. N.K.Ganguly, Director-General, ICMR and other officials of ICMR, New Delhi is gratefully acknowledged. The sincere and dedicated works of all scientific, technical and administrative staff deserve my special appreciation.

S.K.Bhattacharya
Director

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Rajendra Memorial Research Institute of Medical Sciences
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1. Hospital based surveillance for Kala-azar.

Hospital based surveillance of Kala-azar has been carried out since 2001 for establishing a Kala-azar monitoring and research support database at the RMRI hospital in relation to clinical, epidemiological and socio-economic characteristics. A total of 427 parasitologically confirmed VL patients were admitted during January 2001 to December 2002. The age-distribution of Kala-azar patients is presented (Fig.-I). Male: Female ratio was 1.9:1. Most of the VL cases (89%) came from the rural areas of nearby endemic districts. Newly diagnosed or fresh VL cases were nearly 90%. About 36% patients had per-capita income of Rs. 4000 per month.

Distribution of type of houses is presented (Fig.-II). Most of the patients (64%) were illiterate. Domestic animals like cow, ox, buffalo, goat etc. were found in more than 69% houses, which were surrounded by dense vegetations like banana, bamboo, small creepers and seasonal crops. More than fifty percent of houses were made of mud or brick plastered with mud.

Fig.-I: Age distribution of Kala-azar patients

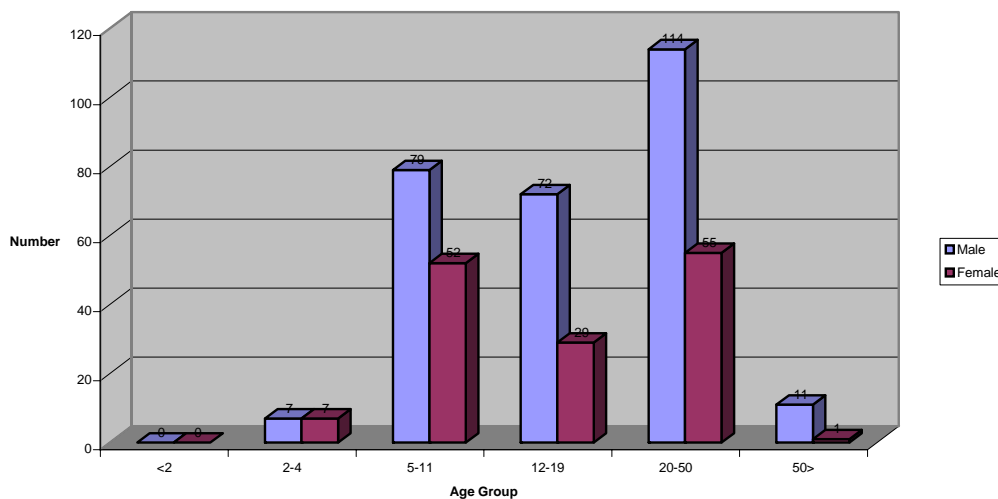
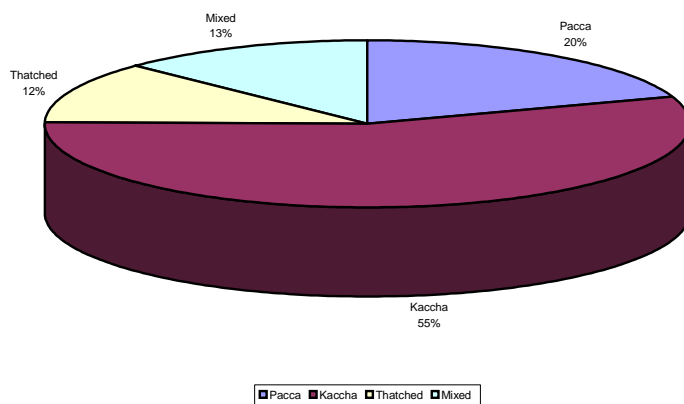


Fig.-II: Distribution of House Type

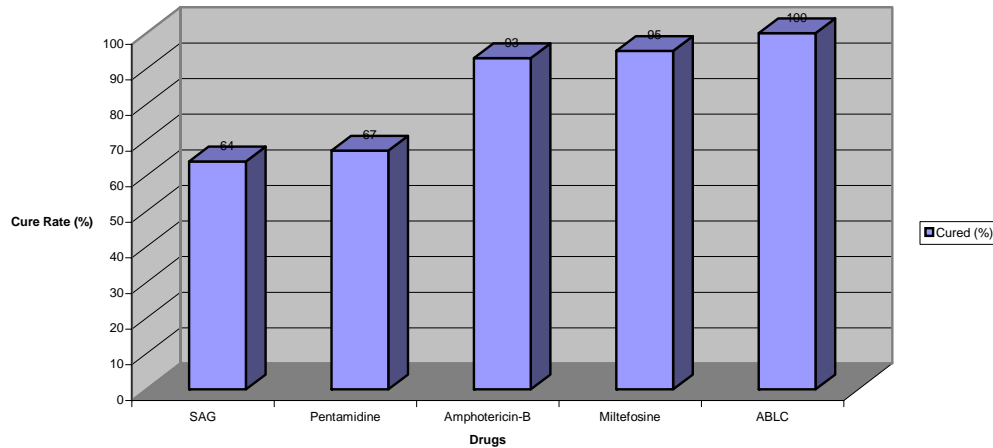


Clinical and laboratory characteristics were compared between two groups of VL patients i.e. <12 years and >12 years of age groups. High Grade fever (>104 degree F) with chill and rigour was recorded in 65% of cases in both groups. There was splenomegaly and hepatomegaly in 89% and 82% of cases in age group <12 years and 83% and 73% of cases in age group >12 years respectively. Leucopenia was recorded in 52% cases and anaemia (Hb<10gm%) in 96% cases. Platelet count was below normal range in 70% of VL cases in age-group >12 years as compared to 37% in <12 years age. SGPT and SGOT were within normal value in 80% cases respectively. Na⁺ and K⁺ level was below normal in 20% and 26% cases respectively.

Cure rate of various drug regimens at the end of treatment showed that number of cases admitted were either resistant to SAG and pentamidine or both (Fig.-III). These cases were

treated with Amphotericin B, success in 93% of the cases at the end of treatment and 100% of the cases responded to Amphotericin B Lipid Complex (ABLC).

Fig.-III: Cure Rate of various drug regimens



2. Phase IV trial of Miltefosine in the treatment of Visceral leishmaniasis (ICMR/WHO/TDR/Zentaris sponsored).

A WHO/TDR/Zentaris sponsored Phase IV clinical trial of orally administered Miltefosine was started in 13 clinical centres spread over 6 VL endemic districts in Bihar with objectives to evaluate the use of Miltefosine in patients with confirmed VL in recommended doses in an outpatients setting with facilities for admission, if necessary and to examine whether the high efficacy and low toxicity seen in hospitalised patients can be replicated in an outpatient setting. After completion of all the preliminary formalities, the patient recruitment started from January 2003. A total of 701 clinically and parasitologically confirmed VL cases have been recruited in 13 clinical centers till March 2003. The study is being continued.

3. Immuno-phenotyping of cellular infiltrates in dermal tissue and peripheral blood from PKDL cases.

The objectives of the study were to examine the distribution of different sub-population of T-lymphocytes in dermal lesions and peripheral blood of PKDL patients by using phenotype markers for CD4+ and CD8+ T-cells and to correlate the distribution of T-cell subpopulation in different PKDL lesions with their cytological changes and treatment response.

Fifteen PKDL cases aged between 12 to 57 years were recruited for this study. Clinical information regarding past and present history of Kala-azar, duration, type, sites and size of skin lesions and other relevant information were recorded. Diagnosis was confirmed by demonstration of leishmania parasite in the imprint smear of skin biopsy of PKDL cases. Further immuno-histochemical staining was done by indirect APAAP method for identification of T-cell subpopulation of CD4 & CD8 surface phenotypes.

Samples of peripheral blood of 15 PKDL cases (Before treatment – 15; After treatment – 6), 5 fresh kala-azar cases and 5 healthy individuals, used as control, were collected in EDTA. DAT was used for determination of Antibody titre for leishmania. Ficoll Hypaque density gradient centrifugation method was used for isolation of mononuclear cells from the blood. Flowcytometry was used for counting of T-cells subpopulation in blood.

Lymphocytes circulating in peripheral blood reflected that the absolute number of circulating CD4+ and CD8+ T-cell subpopulation and surface expression during active infection in PKDL cases were reduced (mean value 631/ μ l and 401/ μ l respectively) as compared to those of healthy controls (mean value 1064// μ l and 699// μ l). However, levels of CD4+ and CD8+ cell surface expression were much lower in active kala-azar cases than the PKDL cases. CD4+ T-cell increased up to mean 1126// μ l following treatment. On the other hand CD8+ T-cell did not differ much in PKDL patient.

Cases	No.	CD4+ / μ l	CD8+ / μ l	CD4 : CD8
Active PKDL cases	15	631	401	1.57 : 1
Active KA cases	5	408	218	1.87 : 1
Healthy contacts	5	1064	699	1.60 : 1

4. Reactive nitrogen and oxygen intermediate metabolism in patients with visceral leishmaniasis.

The objectives were to evaluate the parameters associated with oxygen intermediate metabolism in order to know the effector mechanism of macrophages in VL patients and to study the molecular mechanism of oxygen intermediate mediated pathogenesis that may help administering suitable antioxidant therapy in VL patients.

The non-enzymatic proteinaceous antioxidants, non- enzymatic small molecular antioxidants were studied in 30 patients suffering from VL both before and after treatment. Plasma albumin, iron, glucose, uric acid, bilirubin were investigated both before and after successful chemotherapy.

Table: Levels of plasma albumin, uric acid, iron, glucose, and bilirubin in control subjects and kala-azar patients in ranges

Groups	Before Treatment	After Treatment
Albumin (g/dl)	2.97±0.53	3.64±0.42
Uric acid (mg/dl)	2.12±0.26	3.63±0.25
Iron (µg/ml)	40.40±9.62	73.95±11.28
Glucose® (mg/dl)	81.06±14.91	92.43±12.38
Bilirubin (mg/dl)	0.51±0.31	0.25±0.18

The low value of albumin observed in subjects before-treatment showed the possible defect in the metal binding capacity, however, there was an increase in the albumin value after the successful treatment. The observed low value of uric acid in subjects before-treatment might be due to the defective chelating of metal ions. The other non-enzymatic antioxidants and non-enzymatic small molecular antioxidants, i.e., glucose, bilirubin, uric acid, albumin and iron showed low value in subjects before-treatment and increased after the successful treatment indicating that pro-oxidant mechanism within the body were more active at the time of infection than the antioxidant mechanism (Oxidative stress) and this contributed to the pathogenesis of the disease

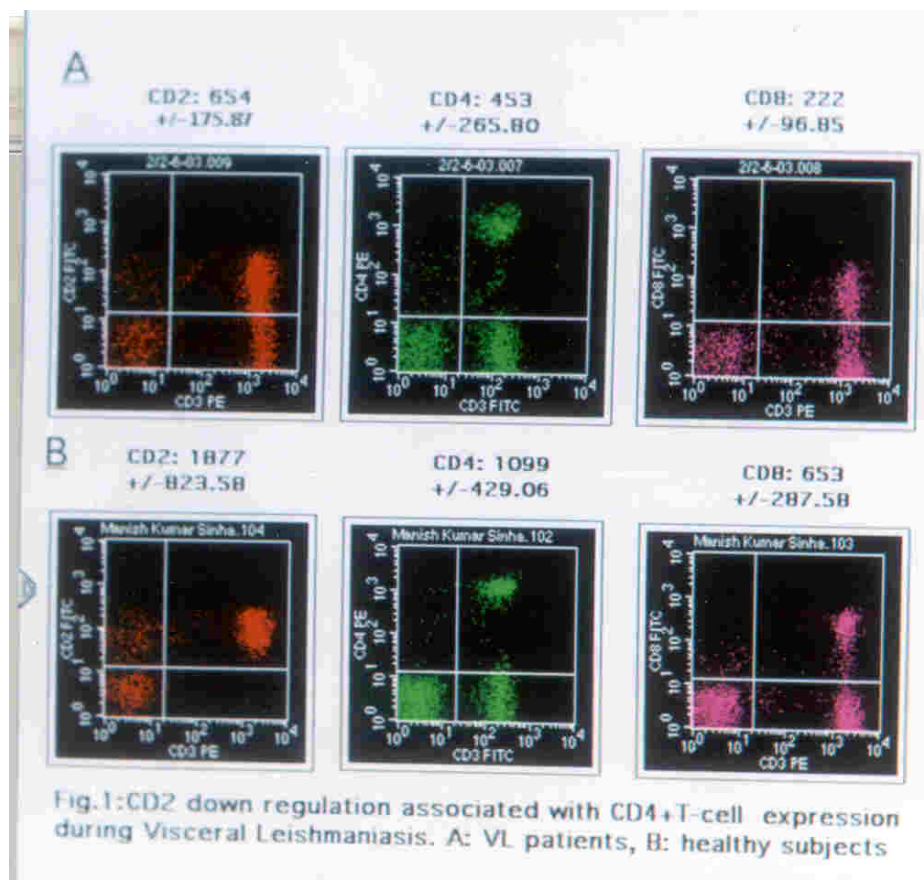
5. Role of CD2 Antigen in T-cell signal Transduction pathway in Visceral Leishmaniasis.

The objectives were to understand the CD2 pathway of T-cell activation and to find out the possible means for modulation of this pathway as a mechanism to ensure protective cytokines (Th1) in patients with Visceral Leishmaniasis.

Seventeen (17) subjects were included in study and clinically classified as asymptomatic early (n=7), symptomatic fresh VL (n=10), cured VL (n=10) and control (n=10). CD2+T lymphocytes was estimated by enumerating in PBML using FACS-Calibur (BD) for cell surface antigens and their relation with IFN- γ (Th1) & IL-4 (Th2) activity in conditioned

medium of PBMNC unstimulated or anti-CD2 stimulated antibody. To define mechanism of CD2 dependant T-cell activation, Protein Kinase-C activation was assayed following culture of 20×10^6 T-cells/ml in conditioned medium in presence of anti-CD Ab and their relation with changes in Th1 & Th2 profile was also determined.

The kinetics of CD2 expression of T-cells prior to infection (healthy) was $1981 \mu\text{l}$ that sequentially declined during early infection ($898 \mu\text{l}$) & further reduced to $437.75 \mu\text{l}$ when infection attained peak level. IFN- γ & IL-4 were reciprocally produced during the extremes of Leishmaniasis. A direct relation of CD2 deficiency & Th1 (IFN- γ) dysfunction was shown. Distinct relation of CD2 deficiency with signaling pathway that might lead to PKC activation during antigen mediated T-cell activation was shown during extremes of disease (% CD4+ cell positive for PKC activity = 5.08%). Exogenous CD2 exposure to T-cell however showed the ability of CD2 to manipulate the PKC signals (13.07%) that enabled CD4+ T-cells to switch over to Th1 effector pathway (IFN- γ : $90.96 \text{pg}/1 \times 10^6$ cells, unstimulated: 42.53pg).



6. In Vitro, Role of *Leishmania* isolates of Responsive and Unresponsive patients in IFN- γ & IL-4 production by similar sets of T-cells.

The objectives were to evaluate the protective cytokine (IFN- γ) and the disease promoter cytokine (IL-4) production by Flow Cytometry method when similar sets of T-cells stimulated *in vitro* by *Leishmania* isolates of responsive and unresponsive patients of Sodium Antimony Gluconate as well as to find out the statistical significance of cytokines production between isolates (responsive and unresponsive) treated samples of mononuclear cells.

The consecutive patients of febrile spleno-hepatomegaly coming for treatment in RMRIMS from endemic area of Kala-azar had been investigated for confirmation of Kala-azar by demonstration of *Leishmania donovani* bodies in Giemsa stained smear of bone-marrow / spleen. Only Parasitologically confirmed cases were considered for inclusion in the study.

For isolation of parasites and their subsequent adaptation, the bone-marrow / spleen aspirates from Kala-azar confirmed patients had been collected in modified NNN medium with 30% pooled rabbit blood, Schneider's and Grace's insect tissue culture media with 20% Fetal Calf Serum (FCS) and had been incubated at $24\pm 1^\circ\text{C}$ in BOD. The adapted parasites had been used in experiments, as stimulant.

The heparinized peripheral blood samples for study were collected from patients and healthy controls after taking informed consent. The samples were coded for double blind study. The peripheral blood mononuclear cells (PBMCs) were separated from blood samples. The lymphocytes were stimulated by *Leishmania* isolates with adjuvant PHA (phyto-haem-agglutination) and Golgi stop. The lymphocyte cell surface was stained with Phycoerythrine (PE) labeled anti-human CD4 monoclonal antibody (BD Pharmingen) and fixed by cytofix / cytoperm solution. The cells were permeabilized by perm / wash solution (BD Pharmingen). The cells accumulated cytokines (IL-4 & IFN- γ) were stained by Allophycocyanin (APC) labeled anti-human IL-4 monoclonal antibody (BD Pharmingen) and Fluorescein Isothiocyanate (FITC) labeled anti-human IFN- γ monoclonal antibody (BD Pharmingen) respectively.

The cells were acquired by Flow Cytometer (BD FACs CaliberTM Becton Dickinson, San Jose, CA, USA) and the results were analyzed for standardization of technique using BD cell quest proTM software (Becton Dickinson).

7. Bacterial Infections in visceral leishmaniasis.

This study was initiated with an objective to find out the incidence of bacterial infections associated with VL. Out of 50 Kala-azar patients studied, 16 patients had complaints of burning during micturition, of which 7 were found positive for *E.coli* infection by urine culture. Eight (8) patients, who had cough and productive sputum along with suspect X-ray chest P.A. view, underwent blood PCR for Koch's. Seven patients were found PCR positive. All these eight patients were examined for sputum for acid-fast bacilli. Five, out of eight patients, were tested positive for acid-fast bacilli (Ziehl Neelsen's staining).

8. PCR based diagnosis of Visceral Leishmaniasis from suspected cases of Kala-azar in Bihar

The objectives were to develop a new Gene target (Non Transcribed Spacer region of rRNA gene) for the diagnosis of visceral leishmaniasis and to compare PCR results of aspirates and blood with the results of conventional diagnostic methods using aspirates. A sample of 113 clinical aspirates (90 Splenic & 23 Bone marrow) were collected in 1.5 ml. eppendorf tube containing T.E. buffer from suspected cases of Kala-azar in the ward / O.P.D. Peripheral blood samples (5 ml) were also collected from same 88-patients during night (9 P.M. approx.) in EDTA vial.

Smear of the aspirates was examined after Giemsa staining for the presence of amastigotes. After inoculation of aspirates in biphasic culture medium (BHI agar medium having 30% defibrinated pooled rabbit blood and Locke's solution with peptone & glucose as overlay), aspirates were incubated at 25°C. The wet smear of cultures were examined microscopically for the presence of promastigotes at the interval of 2-3 days up to at least 4 weeks before considering the samples as negative.

DNA from all aspirates was isolated by physio-chemical method with the treatment of proteinase K, 10% SDS, incubated at 65°C for 1 hour. DNA of all blood samples was isolated by commercially available Qiagen kit. In every batch of samples, positive and negative controls were randomly included for checking contamination and inhibition.

Amplification reaction was performed in volumes of 50 µl containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM MgCl₂, 0.2mM of each dNTPs, 0.6 µM of each primers and 1.5 units of Taq polymerase and 5 µl DNA sample. Each reaction mixture, overlaid with 2 drops of mineral oil to prevent evaporation and amplification, was performed in thermal cycler as initial denaturation at 94°C for 2min., followed by 35 cycles consisting of denaturation at 94°C for 1 min., annealing at 48°C for 1 min., and extension at 72°C for 2 min.; this was followed by a final extension cycle at 72°C for 5 min.

Amplify products (Amplicons) were subjected to electrophoresis in 1% agarose containing ethidium bromide (0.5µg/ml) at 50 volts in 1 TBE buffer (89mM Tris-borate, 02mM EDTA, pH – 8.0) for 2 hours and bands were visualized under ultra violet light. The techniques have been standardized on clinical samples. The results of the experiment will be evaluated after some modification in primer design for meaningful conclusions, which may help in diagnosis of visceral leishmaniasis.

9. Biochemical and molecular characterization of SAG responsive and unresponsive Kala-azar isolates of Bihar

The objective was to observe the variation in SAG responsive & unresponsive isolates of Kala-azar cases of Bihar using biochemical & molecular methods. After primary isolation & culture adaptation, mass culture of different isolates was done in monophasic media. After microscopic examination, promastigotes of log phase were harvested and washed three times with sterile normal saline at 3000 rpm for 20 min to get sufficient amount of pure culture.

For isolation of DNA, the parasites were suspended in TE buffer. DNA was isolated by physio-chemical method i.e. using proteinase K treatment, 10% SDS, N-Cetyl N,N,N, trimethyl ammonium bromide (CTAB / NaCl), 5M NaCl, deproteinization by equal volume of chloroform : isoamyle alcohol (24:1, v / v). Precipitation of DNA with 0.6 vol. of isopropanol, air-drying, resuspension in T.E. buffer (10mM Tris, 01mM EDTA, pH-8.0), and storage at -20°C was done for further use. NTS region of the rRNA gene was amplified from all the isolates. The PCR products (amplicons) were digested using Restriction Enzymes *Hha* I, *Hae* III, *Hinf* I. Electrophoresis was done in 1% agarose at 25 volts in 1xTBE buffer (89mM Tris-borate, 02mM EDTA, pH – 8.0) for 5hours. After staining for 15 min.in ethidium bromide (0.5µg / ml), bands were observed under UV light.

Some variations were observed in few isolates by RFLP with the Restriction Enzymes *Hha* I, *Hae* III, *Hinf* I. But these observations need further repetition of whole procedure and confirmation. Some other restriction enzymes are under trial.

For SDS-PAGE the parasites were suspended in PBS, pH 7.2. Then promastigotes were lysed by freezing thawing using liquid Nitrogen and 37°C for 4–5 times. This suspension was centrifuged at 8,000 rpm for 40 min at 4°C. The supernatant was distributed in small aliquots and stored at –70°C till use. Electrophoresis was done in 12.5% gel at 60/120 volts. Gel was stained with Coomassie brilliant blue solution for over night. After destaining, polypeptide pattern of *Leishmania* strain was observed and the molecular weight was calculated with the marker. SDS – PAGE of different isolates are being done again, although no clear cut marked differences in their protein profile was observed.

10. Removal of bacterial and yeast contamination from *Leishmania* promastigotes culture by Agar plating (Adhoc Project).

The laboratories working in leishmaniasis frequently face the problem of contamination of *Leishmania* promastigotes culture by various bacteria and yeasts. Removal of such contamination is of prime importance for maintaining pure culture. The existing remedy is the use of various antimicrobials, though it is much preferred to avoid because antimicrobials might exert selective pressure on sensitive promastigotes that possibly leads to primary cloning. Beyond certain concentration, antimicrobials are inhibitory to the parasite itself and some of them have strong antileishmanial effects. Initially effective concentration of antimicrobials later become ineffective due to increasing antimicrobial resistance both in bacteria and yeast. Antifungal agents like 5-Fluorocytosine create mutation. Most of the antimicrobials being used are very expensive (5-Fluorocytosine, Gresiofulvin) and hence unaffordable for many laboratories of the developing countries. Continuous sub-passaging of culture with antimicrobials is required for the complete removal of contamination – which is very laborious and time-consuming task, so the present study is focused on a method, based on the capacity of *leishmania* to multiply and to form macroscopic colonies on agar-based media. This method overcomes all the drawbacks of classical methods and moreover it is easy to perform, economical and at a single attempt, both bacterial and yeast contaminants can be removed without any adverse effect on promastigotes and without using any antibiotics.

In this new method, semi solid agar based medium was successfully used to separate promastigotes physically from their contaminants to get individual isolated colonies. The agar containing solid media provides a firm surface on which individual cells or colony forming

unit (CFU) can form discrete colonies and are advantageous for isolating a particular type of microbe from contaminants. Quadrant streaking of small amount of culture on semi solid media by inoculation loop, gradually thin out the sample and separate the cells spatially from each other. Though attempts were previously made to culture the *L. donovani* promastigotes in semi solid medium with different media, no one has reported the possibility of removing contamination by simple quadrant streaking. Several methods are being practiced for the removal of contamination from *L. donovani* culture and in similar protozoan but none of them is completely reliable and free from the risk of cell loss. This reported approach reduces the cumbersome culture of leishmaniasis, especially in the removal of contamination and having several advantages over the existing methods. This method is simple and easy to perform, inexpensive, free from the risk of lethal effects of antifungal agents and also rapid (within 6 hrs.) in removing contamination.

Pointed arrows in the plate show the *Leishmania donovani* promastigotes colonies in contrast with contaminant bacteria

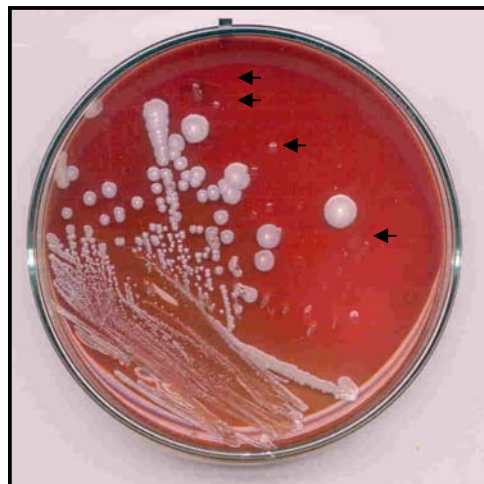


Table: Recovery of parasites at different time interval, in different overlay and percentage of agar interface.

Overlay on agar interface	Incubation Time (hrs)	Interface agar conce (%)	Microscopic observation
Locke's solution (kept on blood agar medium for 24 hrs)	16	1	Pure parasites
	30	1	With contamination
Locke's solution	16	1	Pure parasites
	30	1	With contamination
	16	1.5	A few parasites
	16	0.5	With contamination
	16	0.8	With contamination
	6	1	Pure parasites
	3	1	No parasites
HBBS	16	1	Pure parasites
	30	1	With contamination

11. Identification and characterization of *Leishmania donovani* (Promastigote) antigen of naturally and artificially infected *Phlebotomus argentipes*.

The objectives were the demonstration of parasite in guts of *P. argentipes*, comparison of its infectivity status with microscopic examination and immuno dot-Blot, and determination of peptide responsible for the infection in *P. argentipes* by observing their immuno reactivity against kala-azar.

Dot-ELISA

Lysates of individual *P. argentipes*, collected from the endemic areas, were prepared in PBS. Small piece of NCP were kept in the 48 well of tissue culture plate. The method described by Kaushal *et.al.* 1998 was applied for the immuno dot enzyme assay. Monoclonal antibody raised against *L. donovani* was used as primary antibody and HRPO as secondary antibody. Male *P. argentipes* were used as negative controls and reference strain (DD8) was used as positive control. After the addition of substrate, color development was observed for 15-30 minutes. Those who developed colors were considered positive.

Fourteen, out of 435, *P. argentipes* (3.22%) were found to be positive. *P. argentipes* (105) were dissected but except some bacterial infection, no flagellate infection was seen. Biuret method was used for determination of protein concentration in collected sand flies. The protein content ranged between 2200 ng / μ l to 2500 ng/ μ l.

Figure 1. End point determination of dot-immunoblot assay. Different concentrations of *L. donovani* promastigote protein (1 ug to 1.5 ng) in 2 fold (half) dilutions were reacted with 1:1000 dilution of monoclonal antibody. Wells 1-10 are; 1 (1µg), 2 (500ng), 3 (250ng), 4 (125ng), 5 (62.5ng), 6 (31.25ng), 7 (15.62ng), 8 (7.81ng), 9 (3.9ng) and 10 (1.5ng). Wells, +ve and -ve represent positive and negative controls respectively.



Figure 2. Specificity analysis of dot immunoblot. Lane A and B are the antigens collected from male *P. argentipes*. Lane C contains antigens of other sandfly species (lane C well, 1&2), *M. leprae* (lane C well 3&4), *M. tuberculosis* (lane C well 5&6), *P. falciparum* (lane D, well 1&2), *E. histolytica* (lane D, well 3&4), *G.labmlia* (lane D, well 5&6). Lane E and F are laboratory infected female *P. argentipes*. Signs +ve and -ve are positive and negative controls.

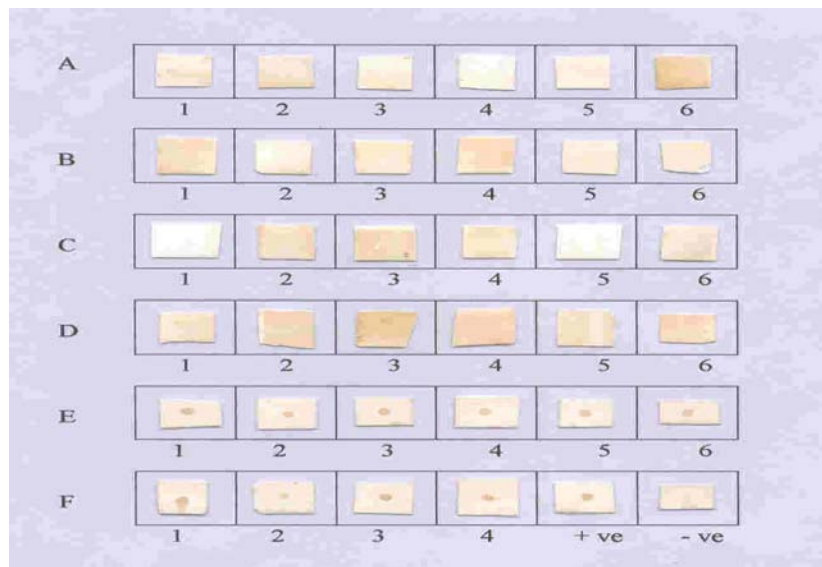
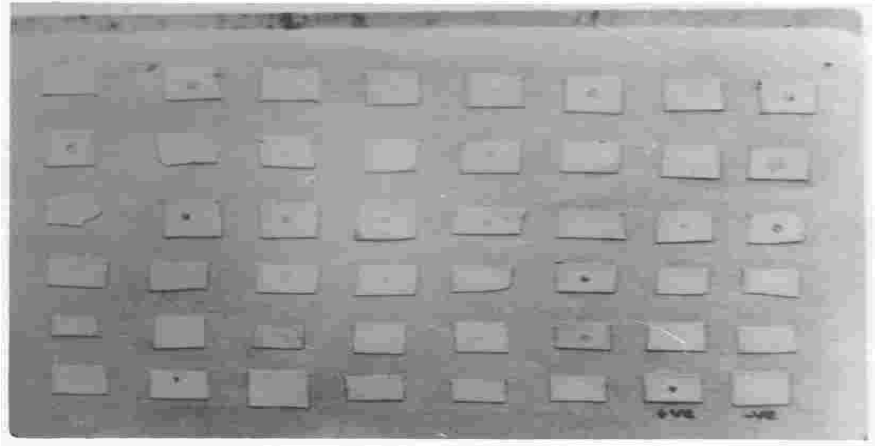


Figure 3. Evaluation of dot-immunoblot assay in detection of *L. donovani* infected *P. argentipes* in Kala-azar endemic districts of Bihar, India. Out of 435 female sandflies collected from two districts, 14 showed positive reaction, however, none of the 93 males were found positive.



Antigen study

Making a pool of Ten *P. argentipes* collected from the endemic areas of North Bihar, antigen study was performed. *P. argentipes* were washed thrice with cold phosphate buffered saline. After washing, a sample of 10-15 sand flies was kept in 80 μ l of PBS. Sand flies were crushed by sterile wooden sticks in 200ul eppendorf tubes. The tubes were centrifuged at 10,000 g for 10 min. and the resultant protein content of sandfly lysates was diluted in the sample buffer (1:1), boiled for 2 min. and electrophoresed in the discontinuous SDS-PAGE to identify immunogenic protein

The leishmania antigen derived from serum culture promastigotes and the reference strain DD8 were used as positive control. Lysates of male sandfly were used as negative control. The relative molecular weight of sandfly protein fraction was determined. The protein profile obtained from SDS- PAGE showed several major proteins. Direct comparisons of the protein fractions of sandfly and leishmania antigen were done to identify the immunogenic antigen in the sandfly gut. Total number of 35 pools, approximately (10 flies in one pool), homogenized lysates of 350 sandflies were analyzed so for by SDS-PAGE.

12. Evaluation of the impact of DDT and Malathion indoor residual spraying being used in Kala-azar control programme on the disease prevalence (ICMR task force project).

The aim of this study was to evaluate the efficacy of DDT indoor residual spraying in Kala-azar control. The different specific objectives were

1. To assess the susceptibility status of vectors to DDT and Malathion.
2. To study the impact of spraying on abundance and survival of the vector(s) population.
3. To determined the residual effect of the insecticide sprayed and

No spraying was conducted in the study area in the year 2002 and 2003. Man-hour density, abdominal stages and parity state were studied and compared with post spray period (conducted previous year). Data collected round the year on man-hour density, abdominal stages and parity status of *P.argentipes* are presented in Figure IV, V & VI.

Fig.-IV:Monthly distribution of man-hour-density

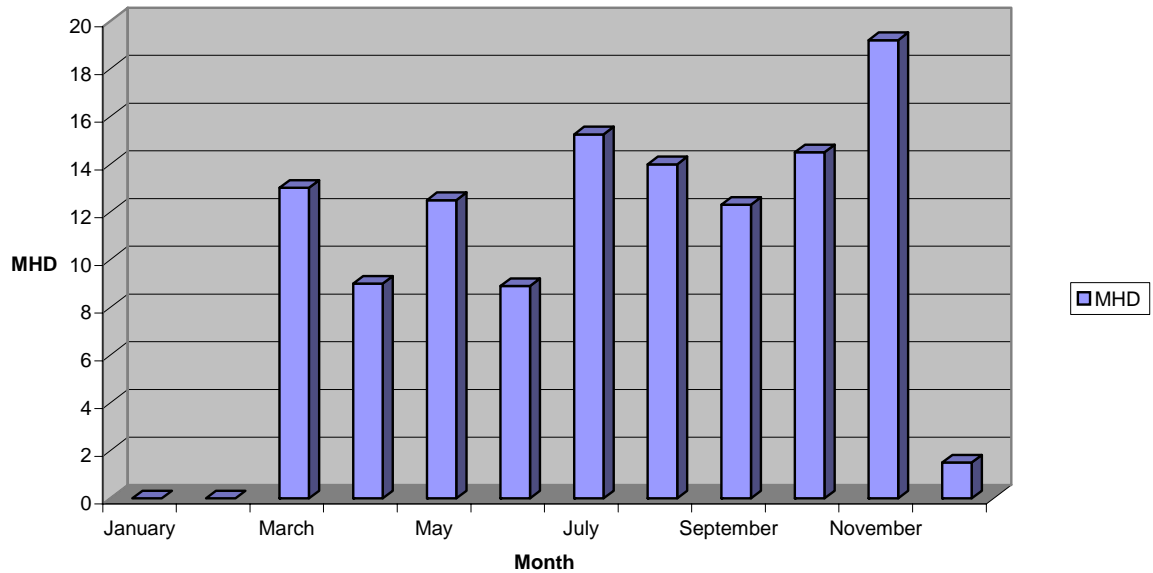


Fig.-V: Month wise abdominal stages of *P.argentipes*

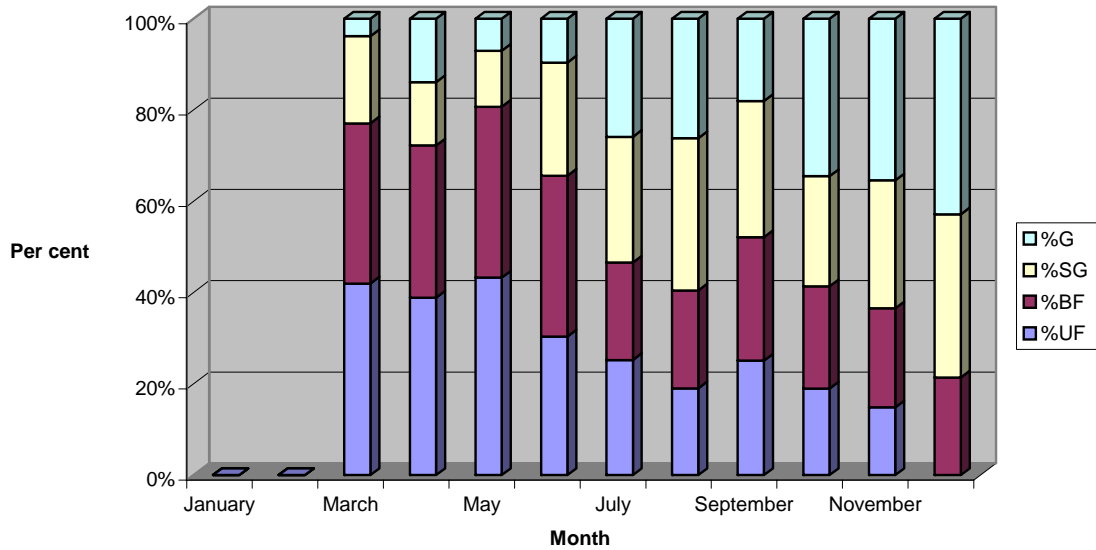
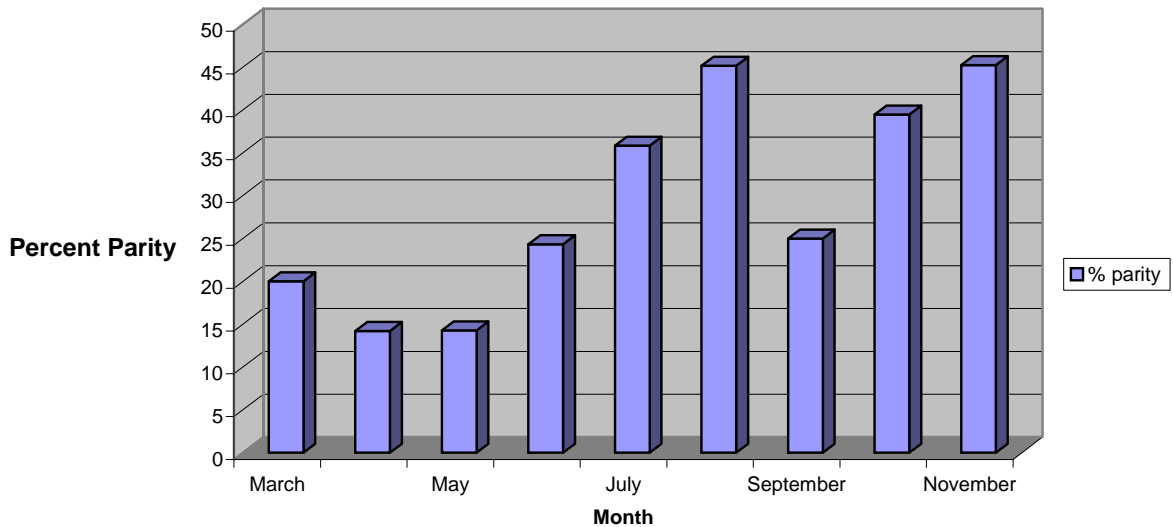


Fig.-VI: Month wise parity status of *P.argentipes*



Man-Hour Density (MHD), abdominal stages and parity status of *P.argentipes* were studied in Bochaha PHC, Muzaffarpur district and Patepur PHC, Vaishali district from January to December, 2003. *P.argentipes* population had normal pattern in the absence of any insecticide (Figure 1) and MHD was quite high ranging in between 13.01 to 19.2 from March to November respectively. Similarly, percentage of gravid fly was also high (25.9%) in comparison to post spray (5.8%). Only, 11.7% parous fly was recorded during post spray

where as it was 35% after one year of spray. This study clearly indicates that though *P.argentipes* has developed tolerance against DDT but still it is effective for containing sandfly density. It is understood that any insecticide, if used for a long period, would precipitate resistance in target species. Hence, as a policy, the insecticide in practice should be discontinued (insecticide holiday) or used rationally as per the need.

13. Control of Indian kala-azar vector, *Phlebotomus argentipes*.

This study was undertaken with objectives to isolate symbiotic bacterial flora of *P.argentipes* from different geographic regions of India and to develop a shuttle plasmid and transformation system for genetic modification of sandfly symbionts to be used in field condition.

Sand flies were collected from different endemic villages of Kala-azar viz. Wajitpur Majhauri of Muzaffarpur district, Majlishpur of Vaishali district and Mohanpur of Samastipur district in Bihar. The wild sand flies were dissected under sterilized condition. The head, wings and legs were removed. The alimentary canal was pulled out. The foregut and mid gut were removed and kept in normal saline. Motility of the bacteria was observed in mid gut under microscope. The pool of guts was homogenized and kept inside sterile vial. The primary isolation of bacterial contents was done by spread plate method in Trypticase Soya agar (TSA) medium and pure culture of bacteria was done by streak plate method on Nutrient Agar (NA). Morphologically different colonies were found appearing after 24 hours. Twenty different types of colonies were found morphologically arising. Each colony was grown separately and preserved in deep freezer for further characterization.

Bacterial colonies from the gut of sandflies



14. Application of remote sensing in identifying and mapping sandfly distribution in endemic and non-endemic Kala-azar foci in Bihar. (An ICMR Task Force Project in collaboration with RRSSC(ISRO), Kharagpur and MRC, New Delhi).

With increasing accessibility to new technologies viz. remote sensing, it has become possible to monitor land-use features on Earth's surface over various time interval to develop methods for rapid stratification of high susceptible areas and for the design of remedial measures. This study was taken up in collaboration with MRC, New Delhi, RRSSC, Kharagpur (ISRO) with the objectives as follows:

- i. To find out the association of vegetation, soil and sub-soil water in different environmental conditions with sandfly distribution in endemic and non-endemic foci of Kala-azar.
- ii. To map geographical distribution of vector in relation to VL using Remote Sensing and GIS technology and thus to define macro-ecosystem of sand fly and VL.
- iii. To monitor effect of specific vegetation cover, water bodies, human settlements and other land use features through conventional ground surveys as well as satellites on vector abundance in endemic and non-endemic foci to evaluate its role as an “Epidemic predictor”.

Earlier vector density was evaluated in both endemic (Patepur block, Vaishali district) and non-endemic (Lohardagga block, Lohardagga district) for each season for two consecutive years. Associations of eco-environmental factors were also looked for comparative study of vector density in both study areas. Ground and satellite data were gathered to locate landscape elements and probable sandfly breeding sites. Ground survey map of the almost all the selected villages under Patepur PHC of Vaishali district as endemic foci and Lohardagga Block of Lohardagga district as non-endemic foci has been completed. There are 18 villages in Patepur PHC and 12 villages in Lohardagga PHC. The respective FCC map and Land-use map have been generated by RRSSC, Kharagpur.

The collected land use data and FCC, prepared by RRSSC, Khargapur, were compared to find out the correlation of geographical distribution of vector and disease distribution in periodic fashion. The analysis of the facts and findings of this project is underway.

15. Study of grass root level functionaries of kala-azar in Bihar

The objectives were to study the existing pattern of functioning of health care services in terms of organization and implementation of facilities for diagnosis and treatment of Kala-azar at primary health centers (PHCs) and to identify shortfalls, if any, in above areas that requires strengthening.

Three PHC's each in three highly endemic districts were selected randomly for this study. Till date, 225 health personnel including Medical officers and para-medical staffs of nine PHC's were interviewed through semi-structured questionnaire. Among the respondents, female (81%) were significantly higher as compare to male (19%) and nearly 88% had rural backgrounds. The respondents had educational level of matriculation (60%), intermediate (30%) and graduation (10%). Only 17% had undergone some or the other training. The responses regarding symptoms were fever of long duration with cold (99%), weakness (63%), loss of appetite (63%) enlargement of spleen (45%) and blackening of skin (7%). The respondents were involved with activities related to kala-azar such as DDT spray (90%), case identification (82%), report for treatment at PHC (62%), suggestions for investigation (52%) and awareness activities (66%). The problems encountered by the health workers during their work were lack of diagnostic facilities (99%), lack of availability of medicine (99%), and lack of co-operation by PHC (51%). The other difficulties as told by them were sub-center running in rented building (61%), Sub-canter's condition poor/very poor (70%) and meeting of staff for Kala-azar (22%). The other problems were non-availability of audiovisual facilities (98%) and lack of IEC activities by district team. Nearly 99% respondents felt that audiovisual facilities were not required. Fieldwork for data collection is in progress.

16. Risk factors for Indian Kala-azar in endemic areas of Bihar.

There is not much quantitative information about the influence of socio-economic factors on Kala-azar in endemic areas of Bihar and whether these factors influence the occurrence of severe and complicated disease. The possible role of socio-economic variables has received little attention. Therefore, a study has been taken up to investigate whether socio-economic factors are important risk factors for kala-azar and , if so, identify those related to causation of disease, using the case-control approach.

An unmatched case-control design has been used for this study. A pilot study was conducted taking 60 kala-azar cases admitted in the ward during Jan-March2002 and 182 healthy controls from the endemic regions belonging to 60 VL patients. The potential socio-

economic factors for VL are investigated with the aid of a structured questionnaire by investigators to the patients and controls. In case of children, the attendants were interviewed.

Univariate-analysis was done to estimate odd ratio of all the factors and its significance using Mantel-Haenszel Chi-square, Yates-Corrected Chi-square, Fishers Exact Test. Out of 25 factors studied, seven factors were found significantly associated with disease. On the basis of significant factors, a sample of 134 VL cases and 406 healthy controls was estimated. The study is in progress.

17. Estimation of epidemiological inputs for computation of burden of visceral leishmaniasis.

This study was undertaken to estimate DALY's (Disability Adjusted Life Years) for visceral leishmaniasis in India.

Estimation of under-reporting: It is reported that the VL cases treated at PHCs during 1987-1991 was 57.4%(50.2 to 66.9 % CI) of the estimated cases and nearly 57% cases were treated by the private doctors (Bora et al 1995). An unpublished data of survey, conducted by the Institute in 10 villages of Muzaffarpur district in 21,000 populations during 2000-2001 for site preparation of vaccine trial (WHO collaborative study), reported that nearly Private Doctors treated 60% of VL cases. Taking these facts into consideration, the total number of estimated cases would be 2 to 2.5 times more than that of the actual cases reported in Bihar. In the year 2002, total incidence and deaths due to VL were 9510 and 148 respectively. If it were assumed that the extent of under-reporting of cases is constant over period of time and population sub-groups, which is very unlikely, then the estimated cases and deaths would be in the range of 19020-23775 and 296-370 respectively.

Estimation of incidence and deaths by age and sex: For the year 2002, a sample of 1165 VL cases reported in 25 PHCs and admitted at RMRI ward were collected and incidence by age and sex compiled (Table-2). Using the proportion of new cases in each age group and sex in the sample, age-sex wise distribution of total VL cases reported during 2002 in Bihar was estimated (Table-3). Average duration of illness and age at onset were estimated. Similarly, a sample of 30 deaths due to VL was collected from respective PHCs and proportion of deaths due to VL by age group and sex was used for estimating age-sex wise distribution of deaths due to VL (Table-4).

YLD and YLL of VL by age and sex were computed using the population of endemic districts by age and sex in Bihar using projected population figure using Census 2001 as baseline. The incidence by age and sex is also indicated and the pattern is very consistent as observed in the community. Finally, Disability-adjusted life years (DALYs) for VL in Bihar were estimated for 2002. (Table-5). The YLL component is significantly higher and goes down as the age advances.

Table-1: Age-sex wise distribution of population at risk of Kala-azar in endemic districts of Bihar-2002

Age	MALE	FEMALE	Total
0-4	4409763	4159127	8568889
5-14	9311001	8838144	18149145
15-29	9675383	8436597	18111979
30-44	6262769	6311201	12573970
45-59	4120420	3540730	7661151
60+	2216036	1940698	4156734
Total	35995371	33226496	69221867

Table-2 : Age-sex distribution of Kala-azar patients in sample collected from PHCs

Age	Male	%	Female	%	Total	%
0-4	21	2.75	25	6.22	46	3.95
5-14	312	40.89	171	42.54	483	41.46
15-29	180	23.59	90	22.39	270	23.18
30-44	164	21.49	82	20.40	246	21.12
45-59	71	9.31	28	6.97	99	8.50
60+	15	1.97	6	1.49	21	1.80
Total	763	65	402	35	1165	

Table-3: Age-sex wise distribution of Estimated Kala-azar cases in Bihar during 2002

Age (year)	Male	%	Female	%	Total	%
0-4	383	2.75	466	6.22	849	4
5-14	5688	40.89	3186	42.54	8873	41
15-29	3281	23.59	1677	22.39	4958	23
30-44	2990	21.49	1528	20.40	4517	21
45-59	1294	9.31	522	6.97	1816	8
60>=	273	1.97	112	1.49	385	2
Total	13909	65	7489	35	21398	

Table-4: Age-sex wise distribution of Estimated Kala-azar deaths in Bihar during 2002

Age (year)	Male	%	Female	%	Total	%
0-4	12	6	6	5	19	6
5-14	57	28	47	37	104	31
15-29	61	30	31	24	92	28
30-44	59	29	22	17	81	24
45-59	14	7	18	14	32	10
60>=	2	1	4	3	6	2
Total	205	62	128	38	333	

Table-5: Disability-adjusted life years (DALY'S, in thousands) of Kala-azar in Bihar – 2002

Age (year)	MALES			FEMALES			TOTAL		
	YLDs	YLLs	DALYs	YLDs	YLLs	DALYs	YLDs	YLLs	DALYs
0-4	67	942	1009	82	162	244	149	1104	1253
5-14	995	4029	5024	558	2335	2893	1553	6364	7917
15-29	574	3591	4165	293	978	1271	867	4569	5436
30-44	523	2611	3134	267	511	778	790	3122	3912
45-59	226	422	648	91	64	155	317	486	803
60+	48	29	77	19	16	35	67	45	112
	2433	11624	14057	1310	4066	5376	3743	15690	19433

18. Hospital based Sero-sentinel surveillance for HIV, HBV & HCV infection among STD & ANC in Bihar (India)

This study was carried out as a part of National AIDS Control Strategy under Annual Sentinel Surveillance Programme with main objectives to see the trend of HIV among STD and ANC attendees of hospitals in Bihar over a period of time and in different socio-economic status. A total 4726 sera sample was tested for HIV antibodies and VDRL. The distribution of sera sample and test result is presented below:

Type & No. of sites	Sample collected & tested	Positive for HIV-1	Positive for HbB	Positive for HbC	Positive for VDRL
STD (8)	2000	55 (2.75%)	64 (3.2%)	10 (0.54%)	251 (12.5%)
ANC(7)	2726	8 (0.29%)	50 (1.8%)	14 (0.54%)	681 (25%)
Total (15)	4726	63 (1.73%)	114 (2.41%)	24 (0.50%)	932 (19.72%)

19. Voluntary Counseling and Testing Centre (VCTC - under Bihar State AIDS Control Society).

A Voluntary Counselling and Testing Centre (VCTC), sponsored by the Bihar State AIDS Control Society, is functioning since 1st April 2001 at this Institute.

A total of 3184 blood samples were collected at VCTC for HIV screening till March 2003. Of these, 637 samples i.e. 20% were positive for HIV. The HIV positivity in females (23.14%) was higher as compared to males (18.76%). The maximum positivity rate was observed in the age-group 31-45 years (29%).

On the basis of occupation, the maximum HIV positivity rate was observed among truck /bus drivers (44%) followed by BMP/Military personnel (30%) and out-migrants labourers working in the metropolitans like Mumbai, Kolkata, Delhi, etc (25%). Among the housewives, 28% HIV positivity was observed, whom husbands were either truck drivers or migrant labourers.

Seminars / Symposia/ Meetings attended during 2002-2003

Dr.Kamal Kishore, Deputy Director

- ICMR Task Force meeting on “Remote Sensing” held at MRC, Delhi on 17th April 2002.
- ICMR Task Force meeting on DDT/ Malathion” at ICMR Headquarter on 10th July 2002.
- The Tenth International Congress of Parasitology (ICOPA X) held from 4th – 9th August 2002 at Vancouver, Canada.
- ICMR Task Force meeting on Remote Sensing at RRSSC, Kharagpur at IIT Campus on 20th August 2002.
- ICMR Task Force meeting on Remote Sensing at ICMR Headquarter on 11th Oct. 2002.
- Administrative Staff Orientation Training Programme, conducted from 09th - 10th Jan. 2003 by Indian Council of Medical Research at RMRIMS, Patna.
- Ist International Seminar of Medical Entomology organized by “The Literature Museum Society and Pathology Department, Gandhi Medical College” held at Bhopal from 19th – 20th Jan 2003.
- Meeting on “Alternative of Animal Use” conducted by Ministry of Forest & Environment and Ministry of Science & Technology at Scope Centre, New Delhi from 18th – 20th Feb. 2003.

Dr. P.K.Sinha, A.D.

- ICMR/TDR/WHO sponsored workshop of “Investigators of Phase IV trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May 2002.
- Technical Committee meeting under Directorate of National Anti-Malaria Programme, Govt. of India at New Delhi to discuss issues related to Inj. SSG under Kala-azar Control Programme on 1st July 2002.
- Training on Ethical Issues in Clinical Trials at Thamassat University, Bangkok from 29th July – 2nd August 2002.

- Group discussion with NASTAD (USA) delegates at RMRIMS on 18th Oct. 2002 in context with Voluntary Counseling Centre for HIV and Sentinel Surveillance Centre.
- A project meeting on “Haemoglobinopathies” at Institute of Immunohaematology (IIH), Mumbai on 07th Dec. 2002.
- Administrative Staff Orientation Training Programme, conducted from 09th - 10th Jan. 2003 by Indian Council of Medical Research at RMRIMS, Patna
- **Temporary Adviser** in “WHO/TDR, PDT meeting on Parmomycin Trial” at Bangkok, Thailand from 17th-18th Feb.2003.
- Meeting and field visits to “Implement Cross-boarder Collaboration Activities for the Prevention and Control of Kala-azar and Malaria”, organized by HMG Ministry of Health, Deptt. of Health Services, Nepal and Dept. of Health, Govt. of Bihar, India held from 22nd – 23rd Feb., 2003.

Mr. N. Kumar, A.D.

- ICMR/TDR/WHO sponsored workshop of investigators of "Phase IV Trial of Miltefosine for Treatment of Visceral Leishmaniasis" held at RMRIMS, Patna from 26th-28th, May 2002.
- Delivered a lecture in a Seminar on “Migration of Child Labour” at A. N. Sinha Institute for Social Studies, Patna during Aug., 2002.
- Meeting of “Task Force on Socio-behavioral Research on Kala-azar” held in ICMR HQ. On 28th Jan, 2003 and presented the Project "Prevention of Kala-azar through Socio-Behavioral Intervention"

Dr. Neena Verma, S.R.O.

- ICMR/ WHO/ TDR sponsored workshop for “Investigators of Phase IV Trial of Miltefosine”, held at RMRIMS, Patna from 26th - 28th May 2002.
- WHO meeting for “Assessment and follow up of Good Clinical Practice and Laboratories (GLP) in Bihar” from 28th - 29th Sept. 2002 for Phase IV trial of Miltefosine Treatment of VL at RMRI, Patna.
- 58th Joint Annual conference of Association of Physicians of India APICON- 2002 held at Chennai from 17th - 20th Jan. 2003.
- Annual conference of Association of Physician of India, Bihar chapter BAPICON- 2002, held at Rajgir.

- Annual conference of Indian Academy of Clinical Medicine , Bihar chapter held at Patna in June, 2002.

Mr. A.K.Gupta, S.R.O.

- ICMR / WHO / TDR workshop sponsored for the investigators of Phase IV Trial of Miltefosine for the Treatment of Kala-azar from 26th - 28th May, 2002, held at this institute.
- Re-orientation Training Programme for HIV Sentinel Surveillance – 2002 on 30th July 2002, organized by Bihar State AIDS Control Society, Dept. of Health, Medical Education & Family Welfare, Govt. of Bihar, at SIHFW Building, Patna.
- WHO workshop on phase IV trial of Miltefosine on 19th October at RMRI, Patna.
- Delivered a popular lecture entitled “Use of Modern Technology in Diagnosis of VL” in Hindi Seminar at this institute, held on the occasion of National Technology Day on 11th May 2002.

Dr. V. N. R. Das, S.R.O.

- ICMR/TDR/WHO sponsored workshop of Investigators of “Phase IV Trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May, 2002.
- Training on Ethical Issues in Clinical Trials at Thamassat University, Bangkok, Thailand from 29th July – 2nd August 2002.
- Training Programme on Epidemiological Aspects of Diseases held at NICED, Kolkata from 6th – 26th Nov.2002.
- Meeting and field visits to “Implement Cross-boarder Collaboration Activities for the Prevention and Control of Kala-azar and Malaria”, organized by HMG Ministry of Health, Deptt. of Health Services, Nepal and Dept. of Health, Govt. of Bihar, India held from 22nd – 23rd Feb., 2003.

Dr.K.Pandey, S.R.O.

- Workshop on “Statistical Computing and Clinical Trials” held at IRMS, New-Delhi from 15th – 19th April 2002.

- ICMR/TDR/WHO sponsored workshop of Investigators of “Phase IV Trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May 2002.
- Training on Ethical Issues in Clinical Trials at Thamassat University, Bangkok, Thailand from 29th July – 2nd August 2002.
- Training Programme on Epidemiological Aspects of Diseases held at NICED, Kolkata from 6th – 26th Nov.2002.
- WHO/TDR meeting on “Parmomycin Trial” at Bangkok, Thailand from 17th-18th Feb.2003.
- Meeting and field visits to “Implement Cross-boarder Collaboration Activities for the Prevention and Control of Kala-azar and Malaria”, organized by HMG Ministry of Health, Deptt. of Health Services, Nepal and Dept. of Health, Govt. of Bihar, India held from 22nd – 23rd Feb., 2003.

Dr. S. Bimal, R.O.

- ICGEB Meeting and discussion during Theoretical and Practical Course on Molecular Biology of Leishmania, 3rd – 5th Oct., 2002 at Trieste, Italy.
- ICMR/TDR/WHO sponsored workshop of Investigators of “Phase IV Trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May, 2002.

Dr. C.S.Lal, R.O.

- ICMR/TDR/WHO sponsored workshop of Investigators of “Phase IV Trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May, 2002.
- Advance WHO/TDR Course on Immunology, Vaccinology and Biotechnology applied to Infectious Diseases organized at WHO Immunology Research and Training Centre, Laussane, Switzerland and Annecy, France from 12th Sept. - 25th Oct., 2002.

Dr. V.Kumar, R.O.

- Workshop on “Multicentric Evaluation of a Simple Technique for the Extraction of the DNA from *W. bancrofti* infected *C. quinquefasciatus* for PCR assay & rDNA PCR

assay for the identification of *A. fluviatilis*” from 24th- 28th March, 2003 at Vector Control Research Centre, (ICMR), Pondicherry.

Mr. M.Muniraj, R.O.

- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV Trial of Miltefosine” for the treatment of visceral leishmaniasis, conducted at RMRIMS, on 26th - 28th May 2002.
- Reorientation training programme on “HIV Sentinel Surveillance – Round V (2002-03)” conducted by Bihar State AIDS control society on 30th July, 2002 at SIHFW, Patna.

Mr. Alok Ranjan, R.O.

- WHO sponsored workshop on “Estimation of Burden of Identified Infectious Diseases”, held at National Institute for Research in Reproductive Health, Mumbai on 14th - 15th Sept., 2002 and presented the progress of work with regard to Estimation of Burden of Visceral Leishmaniasis in India.
- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV trial of Miltefosine” for the treatment of visceral leishmaniasis, conducted at RMRIMS, Patna-7 on 26th - 28th May 2002.

Dr. Nawin Kumar, R.O.

- Workshop on “Statistical Computing and Clinical Trials” held at IRMS, New-Delhi from 15th – 19th April 2002.
- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV Trial of Miltefosine” for the treatment of visceral leishmaniasis, conducted at RMRIMS, Patna-7 on 26th - 28th May 2002.

Mr. Dharmendra Singh, R.O.

- Meeting on ICMR funded project on “Genome Project of Leishmaniasis” and participated in discussion on Molecular Biology of Leishmaniasis on 17th Feb. 2002 at I.I.C.B. Kolkata.

- Presented a paper on “Application of ARDRA for Identification and Molecular Epidemiology of Microbes” at the “2nd National Conference of Laboratory Medicine” from 21st – 23rd Feb., 2002 at All India Institute of Medical Sciences, New Delhi.
- International Symposium on “Leishmaniasis” & Workshop on “Molecular Methods in Infectious Disease Diagnosis” at Deptt. of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, from 23rd-24th Feb.2002.
- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV Trial of Miltefosine” for the Treatment of Visceral Leishmaniasis, conducted at RMRIMS, Patna, on 26th to 28th May 2002.

Dr. S.Narayan, R.O.

- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV Trial of Miltefosine for the Treatment of Visceral Leishmaniasis”, conducted at RMRIMS, Patna-7 on 26th - 28th May 2002.

Dr. D.S.Dinesh, R.O.

- As Temporary Advisor on “Scientific Working Group on Insect Disease Vectors and Human Health” held at WHO, Geneva, Switzerland from 12th –16th August 2002.
- Workshop on “Multicentric Evaluation of a Simple Technique for the Extraction of the DNA from *W. bancrofti* infected *C. quinquefasciatus* for PCR assay & rDNA PCR assay for the identification of *A. fluviatilis*”, from 24th- 28th March, 2003 at Vector Control Research Center, (ICMR), Pondicherry.

Dr. S.K. Kesari, R.O.

- Workshop on “Information & Communication. Advanced Orientation” from 25th - 27th June, 2002 at NIOH, ICMR, Ahmedabad.

Mr. S. K. Singh, Research Assistant

- National Symposium on “Plant Biotechnology: Role of Sustainable Development” and 25th Meeting of Plant Tissue Culture Association (India) from 17th -19th Feb., 2003.

Dr.V.P.Singh, Sr.T.O.

- ICMR/WHO/TDR sponsored workshop for the investigators of “Phase IV trial of Miltefosine” for the treatment of visceral leishmaniasis, conducted at RMRIMS, Patna-7 on 26th to 28th May 2002.

Mrs. M.S.Roy, Technical Assistant

- “Advanced Level Training for Laboratory Technicians” at Institute of Pathology (IOP), ICMR, New Delhi from 24th March – 23rd April 2003.

Mr. S.B.Barman, Technical Assistant

- Training programme on “Haemoglobinopathies” at Institute of Immunohaematology (IIH), Mumbai on 7th Dec., 2002.

Mr.Brij Nath Prasad

- Workshop on “Information & Communication. Advanced Orientation” from 25-27th June, 2002 at NIOH, ICMR, Ahmedabad.

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1. Verma N., Bimal S., Gupta A.K., Das V.N.R., Kar S.K. and Bhattacharya S.K. Immunohistochemical staining of dermal lesions in diagnosis of Post Kala-azar Dermal leishmaniasis (PKDL) in compare to Biopsy Imprint Smear & Culture. Journal of Lab. Med., Vol. 3 (1); 41-42, 2002.
2. Kumar N, Siddiqui N.A., Verma R.B., Kar S.K. Women's Perception on Kala-azar. Journal of Parasitic Diseases, 26 (1); 38-41, 2002
3. Kishore K., Kumar V., Lal C.S., Kesari S.K., Das P. and Bhattacharya S.K. Estimation of efficacy of Malathion (SRES) against *Phlebotomus argentipes* the vector of Indian Kala-azar, with the help of bioassay and HPLC analysis. Tropical Biomedicine, 20(1); 13-18,2003.
4. Sinha P.K., Rabidas V.N., Pandey K, Verma N., Gupta A.K., Ranjan A., Das P. and Bhattacharya S.K. Visceral leishmaniasis and HIV coinfection in Bihar, India. Journal of Acquired Immun. Def. Syndrome, Vol. 32; 115-116, 2003.
5. Dasgupta B., Raichoudhary K., Ganguly S., Sinha P.K., Bimal S., Das P., and Roy S. Upregulation of Interferon- gamma receptor –1 in monocytes of Kala-azar patients & THP1 cells after treatment with anti-leishmanial drugs. Annals of Tropical Medicine & Parasitology, 97; 191-198, 2003.
6. Thakur C.P., Narayan S. and Ranjan A. Kala-azar (Visceral leishmaniasis) and HIV co-infection in Bihar – India: Is this combination increasing? Journal of Acquired Immun. Def. Syndrome, Vol. 32; 572-573, 2003.

Publication in Proceedings:

1. Kishore K., Kumar V., Kesari S., Dinesh D.S., Palit A., Lal, C.S., Ranjan A. and Bhattacharya S.K. Malathion Slow release emulsified suspension as an effective weapon against Phlebotomine flies – A pilot study. Proc. WHO Workshop on “Strategies for Control of Kala-azar and Malaria”, pp: 117-121, 2002.
2. AkbarA Md., Palit A., Bimal S., Dhar K.M., Ahmad N. and Das P. AFLP in detection of SAG unresponsive leishmaniasis caused by *Leishmania donovani* a legitimate approach or not? Strategies for control of Kala-azar and Malaria. Proc. WHO Workshop on “Strategies for Control of Kala-azar and Malaria”, pp: 139-142, 2002.
3. Palit A., Sudhakar S., Srinivash T., Kesari S., Ranjan A., Kumar V., Kishore K., Adiga S., Bhattacharya S.K. Remote Sensing and GIS in Kala-azar transmission prediction in Bihar – application of new tools. Proc. WHO Workshop on “Strategies for Control of Kala-azar and Malaria”, pp: 61-70, 2002.
4. Ranjan A. and Bhattacharya S.K. Epidemiology of Kala-azar in India. . Proc. WHO Workshop on “Strategies for Control of Kala-azar and Malaria”, pp: 11-18, 2002.

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Mr. Narendra Kumar (Liaison Officer)

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Dr. C.S.Lal (Member)
Mr. Naresh Kumar (Member)



Rajendra Memorial Research Institute of Medical Sciences
2002-2003

Distinguished Visitors

Date	Name
26.05.2002	Dr. T.K.Jha, Kala-azar Research Centre, Muzaffarpur Dr. U.C.Samal, Prof. & Head, Cardiology, PMCH, Patna Dr. Herbert Sinderman, ASTA Medica, Germany Dr. N.K.Pal, CSTM, Kolkata Dr. Nitali Pramarie, CSTM, Kolkata Dr. Shyam Sundar, IMS, BHU, Varanasi Dr. V.Vijayasekaran, WHO Clinical Monitor, Chennai Dr. Somboon Keitinun, Thamatsat University, Bangkok Dr. Juntra Karbwang, Clinical Coordinator, WHO, Geneva Dr. R.H.Jani, Director, Med. Affairs & Res., German Remedies, Mumbai
20.10.2002	Dr. Suman Rijal, B.P.K.I.H.S., Dharan, Nepal Dr. R.C.Pandey, Chapra Dr. S.Mukherjee, Samastipur Dr. M.P.Sharma, Samastipur Dr. A.K.Aditya, Samastipur Dr. Sandeep Mishra, Darbhanga Dr. K.Tiwari, Muzaffarpur Dr. D.Nath, Motihari
25.10.2002	Micheal Macgrath, Inst. for One World Health, San Francisco, USA Dr. Arvind Pandey, IRMS (ICMR), New Delhi

- 26.10.2002 Dr. S.E.Hasnain, Director, CDFD, Hyderabad
- 31.10.2002 Dr. Dilip Mahalanalis, Director, Society for App. Studies, Kolkata
Dr. S.P.Mukhopadhyay, Head, Indian Inst. of Scientist Welfare, Kolkata
- 22.11.2002 Dr. Rabindra Kumar Sinha, Univ. Prof. (Zoology), Patna University, Patna
Dr. M.Chaudhary, Chief Malaria Officer, Govt. of Bihar, Patna
- 20.02.2003 Dr. G.P.Ojha, Director, Epid. & Dis. Control, Ministry of Health, Nepal
Dr. R.K.Pokhral, Sr. P.H. Officer, Epid. & Dis. Control, Nepal
Dr. Ashok Sharma, Prog. Coordinator, Env. Health Project, Nepal



Rajendra Memorial Research Institute of Medical Sciences
2002-2003

Workshops/Seminars/Conferences/Meetings held at the Institute

1. ICMR/TDR/WHO sponsored workshop of Investigators of Phase IV trial of Miltefosine for Treatment of Visceral leishmaniasis” held at RMRIMS, Patna from 26th – 28th May, 2002.
2. WHO/TDR/ICMR sponsored meeting for the investigators of phase IV trial of Miltefosine for the treatment of visceral leishmaniasis on Good Clinical Practice and Laboratories (GLP) in Bihar from 28th –29th Sept. 2002.
3. A scientific meeting was organized regarding initiation of WHO/ One World Health/ ICMR sponsored Phase III clinical Trial of Paromomycin and this meeting was attended by Dr. Micheal McGrath, Inst. for One World Health, San Francisco, USA on 25th Oct., 2002.
4. Dr. S.E.Hasnain, Director, Centre for DNA Finger printing & Diagnostics, Hyderabad addressed the scientists of this institute and delivered a scientific presentation about molecular tools in medical research on 26th Oct., 2002.
5. A clinical training of Kala-azar was organized for WHO fellows from Nepal on 20th Feb., 2003.

LIST OF STAFF MEMBERS

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Dr. S.K. Bhattacharya,
M.D., F.N.A.Sc., F.N.A., F.A.M.S.

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3. Dr. K. Pandey, M.D.	Senior Research Officer
4. Dr. Nawin Kumar, M.D.	Research Officer
5. Dr. Neeraj Kumar, M.B.B.S.	Senior Research Fellow
6. Dr.(Mrs.) Vineeta Singh, M.B.B.S.	Senior Research Fellow
7. Mr.S.P.Singh	Technical Assistant
8. Mr. N. K. Sinha	Technical Assistant
9. Mr. S.B.Barman	Technical Assistant
10. Mr. Umesh Kumar	Laboratory Technician
11. Smt. Pushpa Raj	Staff Nurse
12. Smt. S.Lakra	Staff Nurse
13. Smt. Geeta Kumari	Staff Nurse
14. Smt Marry Shanti	Staff Nurse
15. Smt. Raina Sinha	Staff Nurse
16. Smt. Anita Kujur	Staff Nurse
17. Shri S. R. Sharma	Attendant
18. Shri R.K. Singh	Peon
19. Mr. Ramparvesh Verma	Peon
20. Mr. Ajeet Kumar	Attendant
21. Shri Ganga Prasad	Cook
22. Smt. Jhalkuri Devi	Attendant
23. Smt. Madhuri Kumari	Dai
24. Smt. Kunti Devi	Attendant
25. Smt. Baso Devi	Cook Attendant
26. Shri Shankar Kumar	Cook Attendant
27. Shri Rajendra Ram II	Sweeper
28. Shri Arjun Kumar	Sweeper

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1. Dr. R.K. Topono, MBBS	Research Officer
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3. Dr. Gaurav Verma, M.D.	Senior Research Fellow
4. Mr. R.N.Sah, M.Sc.	Technical Assistant
5. Mr. Devendra Prasad	Laboratory Attendant

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| 7. Smt. Mehar Devi | Sweepress |

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| 3. Mr. Arvind Prasad | Technical Assistant |
| 4. Shri Rajendra Prasad | Attendant |

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| 7. Mr. Baidyanath Rai | Laboratory Attendant |
| 8. Mr. C. Prasad | Peon |
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| 10. Mr. Rajendra Ram | Sweeper |

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| 2. Mr. M. P. Thakur | Technical Assistant |
| 3. Mr. Rambalak Sah | Animal Attendant |
| 4. Smt. Geeta Devi | Animal Attendant |
| 5. Mr. K. Chowdhary | Animal Attendant |
| 6. Mr. S. Paswan | Animal Attendant |
| 7. Mr. Madan Sah | Animal Attendant |
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| 3. Dr. S. Kesari, Ph.D. | Research Officer |
| 4. Dr. D. S. Dinesh, Ph.D. | Research Officer |
| 5. Mr. N. K. Sinha | Technician |

6. Mr. M. Prasad	Technician
7. Mr. A. K. Mandal	Insect Collector
8. Mr. S. A. Khan	Field Assistant
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3. Mr. N. A. Siddique, M.Sc.(Stat.), PGDCA	Research Assistant

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2. Mr. B.N.Prasad, B.Com., B.Lib.Sc.	Lib. & Inf. Assistant
3. Shri Ragho Saran Singh	Gestetner Operator
4. Smt. Saroj Devi	Library Attendant
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12. Mr. S.N.Rabidas	Stenographer
13. Mrs. S.Kumari	Stenographer
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26. Mr. Balmiki Ram	Peon
27. Mr. Ashok Kumar	Peon
28. Mr. Manoj Kumar	Peon
29. Mr. Ramsaran Mahto	Peon
30. Mr. Sunil Kumar Hansda	Peon
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