Rajendra Memorial Research Institute of Medical Sciences
(Indian Council of Medical Research)
Agamkuan, Patna – 800 007.

Annual Report

(2005-07)

PREFACE

The research activities on clinical, basic and applied aspects of Visceral leishmaniasis (VL), which has taken a new dimension in achieving the mission by the previous Director, was further continued and strengthened during the period under report. The main artery of the institute, the clinical medicine department, is rendering yeoman service to the society. After completion of miltefosine phase IV and Paromomycin phase III trial, phase II trial of Sitamaquine, a new oral investigational product for treatment of VL patients, was successfully conducted with highly encouraging results. A few other clinical trials viz. miltefosine for PKDL (WHO/TDR), Paromomycin Phase IV trial (iOWH), Combination therapy of miltefosine and amphotericin B are to be commenced shortly.

Many new challenges in basic research were addressed viz. PCR based diagnosis of VL and PKDL from blood samples has been developed; some plants' extracts useful in propagation of *Leishmania donovani* promastigotes and possible source of replacement for fetal calf serum (FCS) in the routine culture were identified; malnutrition as a risk factor in the severity of VL along with hypocholesterolemia and hypertriglyceridemia has been observed. The role of CD2 antigen in T-cell signal transduction pathway and proteophosphoglycan in immunity in VL has been undertaken.

Various projects on the control aspects viz. Evaluation of DDT indoor residual spraying for kala-azar elimination programme; Monitoring and supervision of DDT spray operation in endemic districts of Bihar; Efficacy, acceptability and cost effectiveness of long lasting insecticidal nets in VL prevention; and Kalanet study (sponsored by European Commission) has been undertaken. Application of remote sensing technology and GIS has yielded very encouraging results.

Implementation strategies for VL treatment in India (WHO/TDR) and In-depth review of current Kala-azar elimination programme (NVBDCP, Govt. of India) have been undertaken.

Funds are available for the establishment of a 100-bedded Tropical Disease Research Centre, an extension of already existing 50-bedded indoor ward, besides the construction of an International hostel in the RMRI premises.

The continuous guidance, support and constant encouragement received from Prof. N.K.Ganguly, DG, ICMR and other officials of ICMR like Dr. S.K.Bhattacharya, Addl. DG, ICMR are gratefully acknowledged. Last but not the least, the sincere and dedicated efforts of all the scientific, technical and administrative staff deserves my special mention and appreciation.

(Pradeep Das)
Director

Contents

A. Ongoing studies

1. Hospital based surveillance for Kala-azar.

Objective:

The main objective of this Institutional project is to:

a) monitor changes in disease patterns including therapeutic response and to collect other relevant information,
b) provide a data base on Kala-azar for researchers to generate and test hypothesis and to carry out clinical and epidemiological research,
c) provide a regular report to the government and other relevant agencies on kala-azar from a systematic sample of all kala-azar patients attending the hospital,
d) to develop an early warning system for forecasting an epidemic,
e) to improve care and introduce better preventive measures.

Progress:

The parasitologically confirmed VL patients, admitted in Indoor ward of this institute, were interviewed through pre-tested questionnaire to collect the information on their demographic, socio-economic status, current and past history of case, besides the therapeutic response based on clinical and laboratory parameters.

Since the inception of this study (Jan. 2001) to March 2007, a total of 1901 (Male 1245, Female 656) were taken into this study. Maximum VL patients were in the age group 5-14 years (39.2%) and male patients (65.4%) were higher as compared to the females (34.6%). About 90% of the patients were from the rural areas of nearby endemic district and 75.2% of the patients hailed from the poor socio-economic strata (Income range Rs. 1000-5000 per month). Nearly 56.4% patients were residing in mud and thatched houses with very poor light condition inside the bedroom and more than 52.1% of the patients kept domestic animals in their houses. Vegetations around the households were reported from 74.5% of the patients.

Clinical and laboratory characteristics were compared between two age groups i.e. ≤12 years (Gr. I) and >12 years (Gr. II). Fever >100^{o}F with chill and rigor was recorded in nearly 50.2% and 38.0% of cases in Gr. I and II respectively. Splenomegaly (>5 cm) was recorded in 70.0% of cases in Gr. I and 66.5% in Gr. II, whereas hepatomegaly (>5 cm) was in 41.1% and 36.1% of cases respectively. Leucopenia was recorded in nearly 74.1% and 51.8%; severe anemia (Hb <6.5g/dl) in 37.2% and 26.2%; SGPT within normal range in 84.3% and

72.8%; and SGOT within normal range in 64.2% and 54.2% of cases in Gr. I and II respectively.

Out of various therapeutic options viz. Sodium antimony gluconate (SAG), Pentamidine, Amphotericin B, Amphotericin B lipid complex, Miltefosine and Paromomycin, SAG and Pentamidine had a cure rate of about 58.5% and 66.7% respectively at our Indoor setting and hence, it is no longer in practice currently. Cure rate with Amphotericin B, Miltefosine and Amphotericin B lipid complex were 93.9%, 97.5% and 100% respectively. Under Phase III clinical trial of Paromomycin and Phase II clinical trial of Sitamaquine, initial cure rate observed were 93.6% and 100% respectively.

Table: Regimen wise cure rate

Regimen	Treatment completed	Cured	% cured
SAG	118	69	58.5
Pentamidine	9	6	66.7
Amphotericin B	1424	1338	93.9
Miltefosine	161	157	97.5
Amphotericin B Lipid complex	3	3	100
Paromomycin	110	103	93.6
Sitamaquine	7	7	100

2. A Phase II, multi-centre, open-label, randomised study to evaluate the safety, tolerability and pharmacokinetics of oral sitamaquine compared with amphotericin B in the treatment of visceral leishmaniasis caused by *L. donovani* in endemic areas. (Sponsor: GlaxoSmithKline; Study No. STQ 105938)

Objectives(s)

Primary objective:

- To characterize the pharmacokinetic profile of multiple doses of sitamaquine with or without food in subjects with visceral leishmaniasis.

Secondary objectives:

- To describe the safety and tolerability of sitamaquine in the treatment of subjects with visceral leishmaniasis, and to compare with amphotericin B.
- To evaluate the efficacy of a 21 day course of treatment with oral sitamaquine.

Progress:

Being one of the clinical center of this multicentric study, 62 patients were screened after obtaining proper written informed consent. After assessment of inclusion and exclusion criteria, only 11 subjects (target 60 subjects including all centers) were randomized for medication (7 in Sitamaquine group and 4 in amphotericin group). The subjects randomized in sitamaquine group (sequence 1-4, categorized as fed and fasting stage) received target dose of 2mg/kg once daily for 21 days and subjects under sequence 5 were treated with intravenous amphotericin B in the dose of 1 mg/kg every alternate days for 30 days. Out of 11 enrolled patients, one patient (amphotericn group) withdrew himself from the study in the mid of the treatment and rest (n=10) completed the treatment. Pharmacokinetics samples were collected as per the time point defined in the protocol. Patient enrollment in this study is over. Follow up of the patients for any AE/SAE, relapse is under way.

Special features:

All the screening, randomization, screening failure, withdrawal, and treatment completion were registered through "Registration and Medication Ordering System (RAMOS)". The ECG images were transferred to the eResearch Technology through fax for further evaluation. 4-chamber echocardiogram (Colour Doppler) images were captured in DICOM format and transferred through FTP/ CD. Electronic CRF (eCRF) was adopted for entering data of each subject of scheduled time points as well as resolving the queries, raised by Data Management Cell.

Observations:

Initial cure was observed in all the treated subjects. After assessment of clinical biochemistry parameters, it was observed that two subjects (sitamaquine group) developed acute glomerulonephritis like proteinuria at the end of treatment as per the laboratory reports, but it was without any clinical signs and symptoms. In order to further investigate the safety parameters, these two patients were re-hospitalized. It was observed that the elevated protein/ creatinine ratio, protein in urine, urine creatinine and albumin in urine reversed to normal

limit without giving any medicine. Data analysis to characterize the pharmacokinetic profile, assessment of safety, tolerability and efficacy of sitamaquine is being done by the Data Management Cell of the sponsor.

3. Susceptibility to Visceral Leishmaniasis (Kala-azar) in human beings - the role of Testosterone.

Objectives:

To evaluate the levels of testosterone in relation to Visceral Leishmaniasis (VL) infection in males.

Progress:

Under this observational pilot study, 20 fresh and parasitologically confirmed VL male cases (age group: 15-45 years) and 20 healthy controls within same age group range were included. After obtaining informed consent, each subject was subjected for estimation of testosterone levels by CIA& RIA technique. Blood samples were collected in fasting stage. Anti-testosterone antibodies were immobilized on microwell plates. This followed further incubation of serum samples from patients and control with HRP labeled testosterone. This was done to allow testosterone in the sample to compete with HRP labeled testosterone for binding to the immobilized antibodies. After washing, enzymes substrate was added and colour development was monitored. It was observed that the amount of testosterone in the samples was inversely proportional to the enzyme activity. The absorbance was measured at 450 nm on ELISA plate reader.

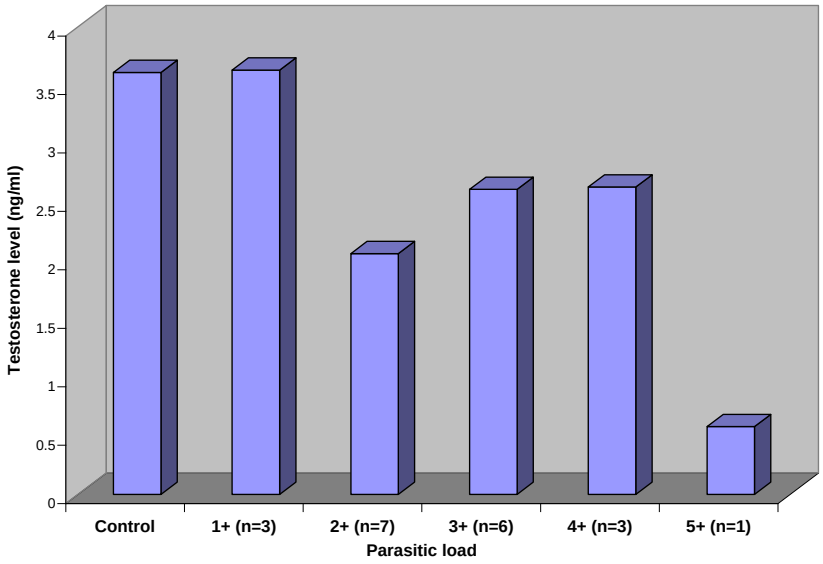
It was observed that testosterone level in control and VL cases with 1+ parasitic load was almost the same, but in VL cases with 5+ parasitic loads it was sharply decreased as compared to control. Preliminary results showed that the level of testosterone in cases and controls do not differ significantly (p-value > 0.05). However, further studies with more number of samples as well as incorporation of cytokine profile are required to arrive at any definite conclusion.

Table: Testosterone level (mean ± S.D.) in VL patients compared to healthy control

Parameters (ng/ml)	VL cases (n=20)	Control (n=20)	p-value
Testosterone	2.475 ± 1.8	3.610 ± 2.55	>0.05

*Normal range: 1.8 – 9.0 ng/ml in male

Figure: Testosterone level in VL cases and its relation to parasitic load



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

Objective:

- To develop a PCR based diagnosis of kala-azar from blood samples and compare the PCR results with conventional diagnostic methods.

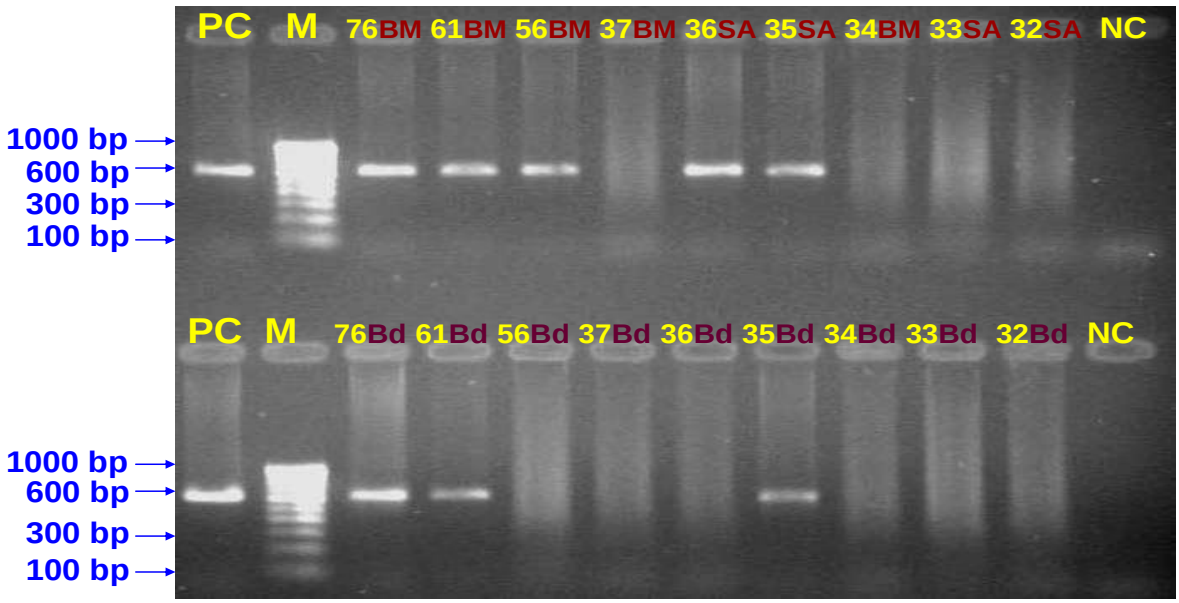
Progress:

0.2 ml peripheral blood (n=125) and bone marrow (n=25) aspirates samples were collected from suspected kala-azar patients, attending OPD for diagnosis and treatment in the indoor ward of the Institute. Imprinted smear of the aspirates were examined after Giemsa staining for the presence of amastigotes. The aspirates were inoculated in culture medium and incubated at ambient temperature. The wet smears of cultures were examined microscopically for the presence of promastigotes at an interval of 2-3 days up to at least 4 weeks before

considering the samples as negative. DNA was isolated from all the blood and aspirate samples using a QIAamp DNA blood minikit (QIAgen).

A nested PCR assay to detect parasite DNA in blood and aspirates from suspected kala-azar patients was done. Nested PCR primers encompassing an approximately 600bp fragment internal to the ITS region of rRNA sequence was used. Use of the nested PCR was found helpful from a significant number of samples that were negative in the primary reaction. All samples were tested; results of the test are being under evaluation. Comparisons of the results of the PCR (Blood) with the results of conventional diagnostic methods are under progress.

PCR results of Blood & BM / SA



Lane NC= Negative control, Lanes 32 – 76 =Blood & BM / SA samples, Lane PC= Positive control, Lane M= Mol. wt. marker.

5. Study of Imprint smear microscopy and PCR application on biopsy from dermal lesions for diagnosis of Post kala azar dermal leishmaniasis cases in Bihar.

Objectives:

- To apply the PCR for diagnosis of PKDL and compare it with the conventional microscopy of skin biopsy imprint smear.
- PCR application in PKDL cases after treatment and during follow up.

Progress:

PKDL cases (n=38) were selected from RMRIMS outdoor and nearby endemic villages of Bihar. They were asked about any past history of Kala-azar, treatment and duration of skin lesions and examined for the type of the lesions (i.e. macular, papulo-nodular and erythematous), site on the body, coalescence and any loss of sensation.

Biopsies were collected aseptically from different skin lesions of the PKDL cases after obtaining informed consent from the patients. Imprint smears on clean glass slides were prepared and stained with Leishman /Giemsa stain for detection of leishmania parasites under microscope. Blood sample were collected for haematological tests. Biopsy samples were collected in the Tris buffer solution for PCR study. PCR was carried out to detect the sequence specific to whole ITS region of the ribosomal RNA (rRNA) gene of *L.* donovani.

Tissues were grinded in Tris EDTA buffer solution (pH 8.0); washed twice with T.E. buffer and DNA was isolated by commercially available 'Qiagen' kit. The amplified PCR products reactions were visualized on 1.5 % agarose gel, using a DNA marker. Samples were scored as positive when a PCR product of 600 bp could be detected as in the positive controls. The results obtained by PCR were compared with microscopy of the imprint smear.

Six biopsies from cases with fungal skin lesions and leprosy were used as negative controls for PCR test, whereas leishmania parasites culture isolates from PKDL cases were used as positive control. Out of 38 PKDL cases, 19 had only hypopigmented macular lesions and the other 19 cases had mixed lesions of papulonodular-erythematous with or without macular lesions. Past history of kala azar was present in all the cases with duration of 1 to 16 years.

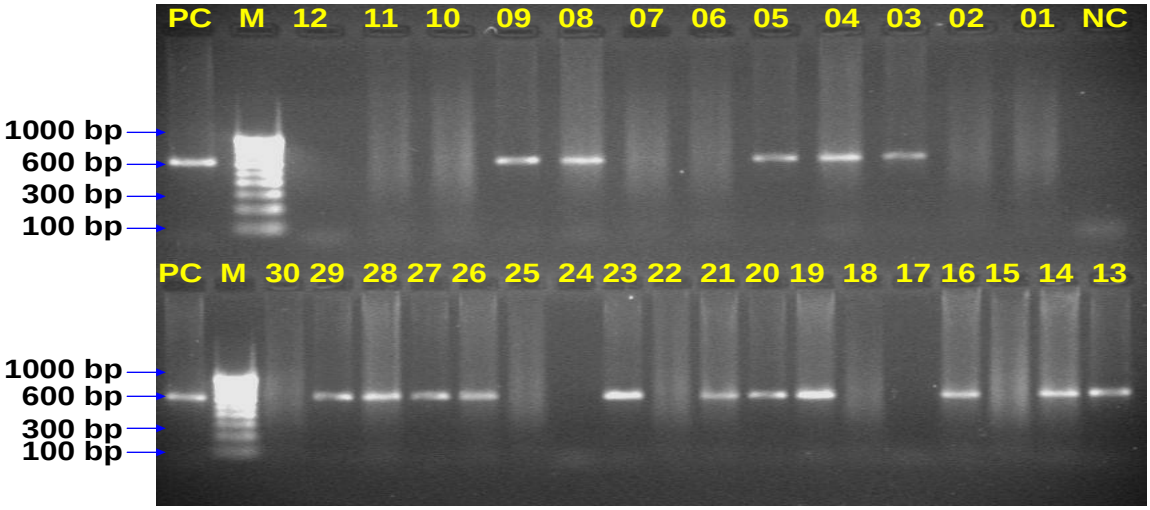
In imprint smear microscopy, parasite positivity was found in 17 (89.47%) papulonodular cases and only in 7 (36.8%) macular cases, with presence of mononuclear cells, histiocytes and lymphocytes in the smear. The biopsy smear from skin lesions of fungal infection and leprosy cases were observed negative for the Leishmania parasite.

PCR showed positivity in 17 (89.47%) papulonodular cases of PKDL. PCR positivity was significantly higher in the hypopigmented macular cases of PKDL, where it was positive in 16 (84.21%) cases, however the microscopy could detect only 36.8% positivity in the same group. Both PCR and imprint smear were negative in the negative control subjects. The study is still in progress to include 50 fresh cases of PKDL for final interpretation.

Table : **Compare of microscopy and PCR for Leishmania positivity in different types of lesions of PKDL cases.**

PKDL Lesions	N	Microscopy of Biopsy Imprint smear	PCR of Skin Biopsy
Papulo-nodular lesions	19	17 (89.47%)	17 (89.47%)
Hypopigmented macular lesions	19	7 (36.8%)	16 (84.21%)
Total PKDL cases	38	24 (63.15%)	33 (86.84%)
Negative controls	6	0	0

Nested PCR of skin biopsy samples



Lane NC= Negative Control, Lanes PL 01—30= Skin biopsy samples, Lane PC=Positive Control, Lane M= Mol.wt. Marker.

6. Establishment of national repository for Leishmania parasites and sera bank.

Objectives:

1. To isolate and maintain Leishmania parasites from different clinical materials as well as from the vector of Kala-azar, *P. argentipes.*

2. To cryopreserve different isolates of Leishmania of different geographical areas and WHO referral centers.
3. To characterize the various isolates of Leishmania.
4. To preserve sera samples of Kala-azar and PKDL cases; other diseases; and healthy controls.

Progress:

Leishmania parasites from splenic/ bone marrow aspirates and dermal lesions of kala-azar and PKDL cases respectively, hailed from different endemic areas, have been isolated and maintained *in vitro* in biphasic media. Out of 30 cropreserved isolates, 8 were revived and 20 new isolates (Kala-azar 19; PKDL 1) are being maintained for its cryopreservation in liquid nitrogen at -20^{o}C. The Amphotericin B unresponsive (N=2) and PKDL (N=1) isolates have been adapted in culture for inoculation in Balb/c mice for *in-vivo* maintenance. The study is in progress.

7. Molecular characterization of SAG responsive and unresponsive kala-azar isolates of Bihar.

Objective:

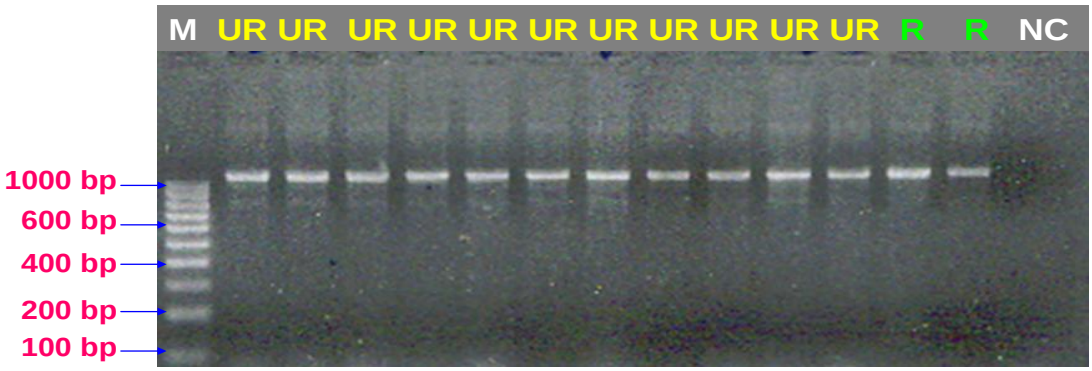
- To demonstrate, if any variation exists in SAG responsive & unresponsive isolates of kala-azar cases of Bihar using molecular tools.

Progress:

After primary isolation & culture adaptation, mass cultures of different clinical isolates of SAG responsive (n=2) & unresponsive (n=10) strains were carried out in monophasic media. DNA was isolated from these isolates by chemical method (i.e. proteinase K, SDS and CTAB/NaCl) and ITS region of the rRNA gene was amplified from all isolates. PCR products were analyzed in 1.5% agarose gel and a band of 1100bp (approx.) was found in all.
In previously experiments four restriction endonucleases i.e. *Hha* I, *Rsa* I, *Hae* III and *Taq* I, were used, out of which only *Taq* I demonstrated the differentiation in banding patterns among the SAG (R) and SAG (UR) isolates. This time amplicons were digested with *Mae* II (*Tai* I), *Hpy* F1OVI (*Mwo* I), *Tru*1 I (*Mse* I), and *Tas* I (*TspE* I) restriction endonucleases. PCR–RFLP patterns showed 3 restriction cutting sites (i.e. 140, 250, & 561bp) with *Mae* II

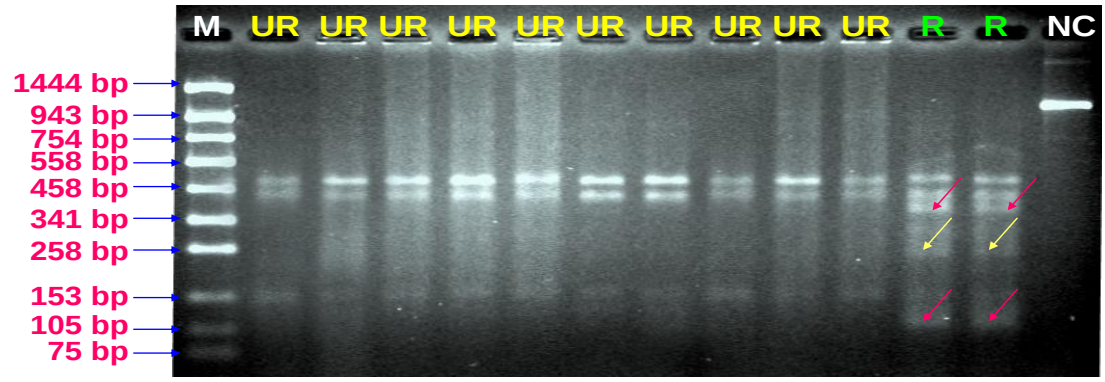
(*Tai* I), 2 restriction cutting sites (i.e. 360 & 647bp) with *Hpy* F1OVI (*Mwo* I) and 2 restriction cutting sites (i.e. 769 & 820bp) with *Tru*1 I (*Mse* I) in all (SAG-responsive/ unresponsive) isolates. But differences were observed among the SAG (R) and SAG (UR) isolates restricted with *Tas* I (*TspE* I) restriction endonucleases. A band of 400bp (approx.) was observed in SAG (R) isolates but not in SAG (UR) isolates restricted with *Tas* I (*TspE* I).

Amplification of ITS region



Lane NC =Negative control, Lanes R =Responsive isolates (SAG), Lanes UR =Un responsive isolates (SAG), Lane M =Mol. wt. marker.

Result of PCR—RFLP *Tsp* EI (*Tas* I)



Lane NC =Un digested, Lanes R =Responsive isolates (SAG), Lanes UR =Un Responsive isolates (SAG), Lane M =Mol. wt. marker.

8. Malnutrition as a risk factor in the severity of visceral leishmaniasis.

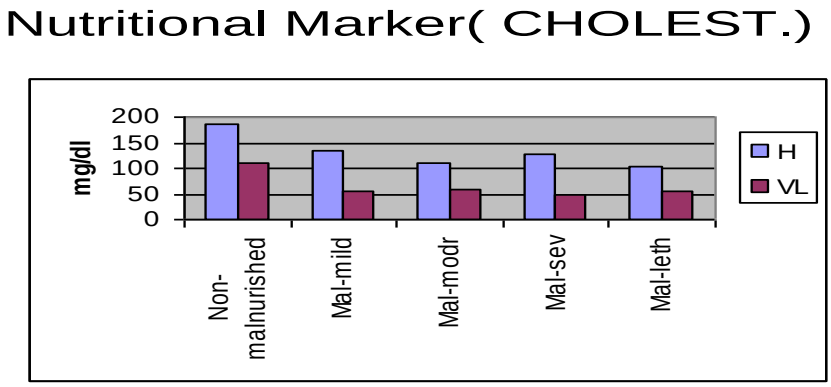
Objectives:

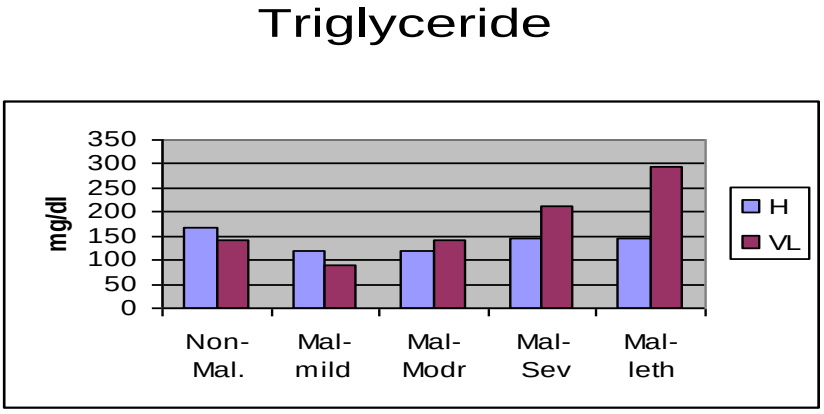
- To identify and assess the nutritional markers/factors in the malnourished VL patients
- To evaluate the correlation between malnutrition factors and VL

- To assess the nutritional factors predisposing to severity in VL.

Progress:

Malnourished VL subjects were categorized into different sub-groups, i.e. mild, moderate and severe according to their Body Mass Index (BMI) as well as by clinical assessment. Various nutritional biochemical laboratory tests for malnutrition based on BMI and their correlation with the severity of the disease were studied. The nutritional parameters studied were albumin, Cholesterol, Triglyceride, LDL, HDL, apolipoproteins, copper, zinc, magnesium and iron. Severe down regulation of Cholesterol was observed in all the malnourished VL cases, which suggest that leishmania parasite might be playing an important role in the down-regulation of cholesterol in order to establish the infection. Hypertriglyceridemia and down regulation of Iron and Zinc were the other parameters observed in VL malnourished patients. The study is in progress.





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

Objectives:

- To understand the role of CD2 deficiency in VL and its consequences on CD4 subpopulation of T-cells
- To find out the possible means for modulation of this pathway as a mechanism to ensure protective cytokines in patients.

Progress:

The VL patients were observed with less CD2 expressed on the cell surface of T-lymphocytes and human CD2 optimized T-cell response to proliferate in response to *Leishmania* antigen. Further, it has been observed that the T-cells of VL patients reverted the gross impairment in the Protein Kinase-C mediated signaling in the presence of anti CD2 antibodies. The above changes also enabled T-cells to alter their behavior for cytokine production. The major impact noticed was on CD4 lymphocytes blast gate population, which accounted for the majority of the Interferon-gamma (IFN-γ) producing cells after anti-CD2 triggering. In a separate experimental, it was demonstrated that activation of CD2 antigen by anti-CD2 antibody uplifts the Th1 associated CD4 cell response as IFN-γ production by T-cells occurred even in absence of APCs. CD2 pathway of T-cell activation was further studied in terms of its impact on Th2 associated CD4 response. It was observed that CD2 on T-cells once activated it downregulates IL-4 production, which remained unaltered even though recombinant IL-4 was added to promote CD4 cell differentiation into Th2 during culture. Thus a critical role of CD2 in transducing a negative signal for IL-4 is identified in Visceral Leishmaniasis, which once decreases, brings a shift in Interferon-gamma production.

Figure 1: **Immunophenotyping of CD2, CD4 and CD8 + T-cells in VL patients and control.**

A. **B.**

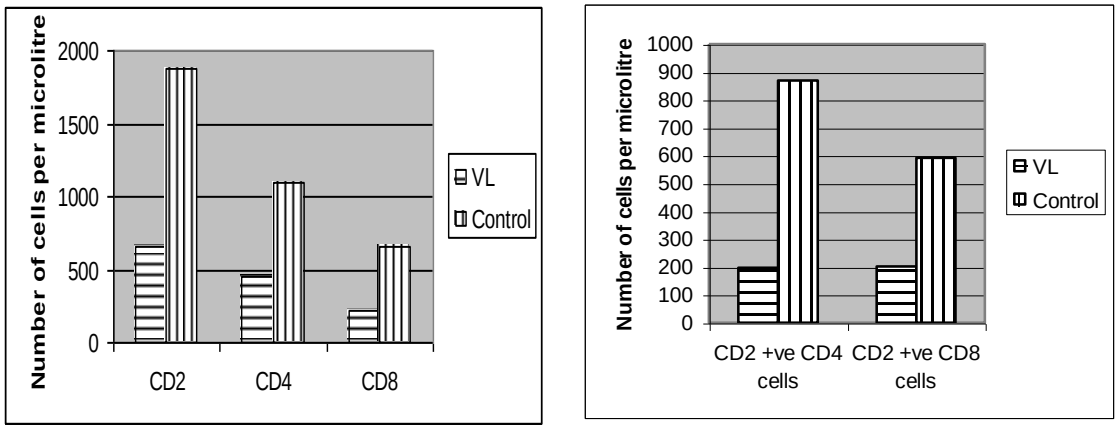


Figure 2: Flow cytometry analysis on reversal of T-cell activation pattern in response to *L. donovani* antigen upon activation of CD2 antigen in VL patients.

A. B.

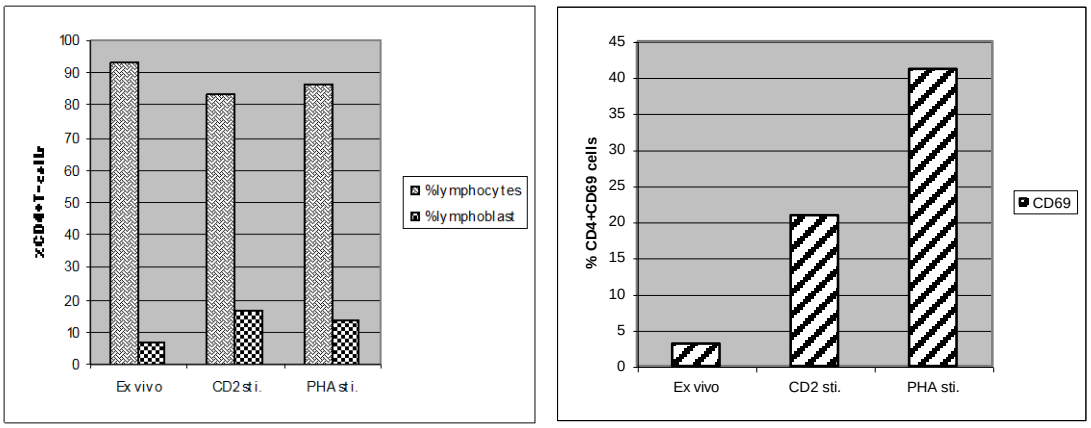
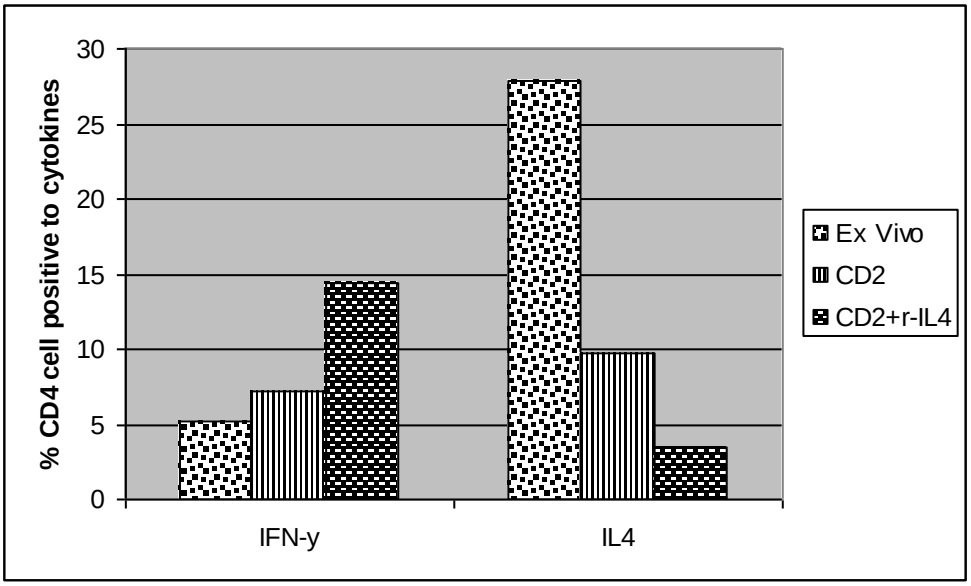


Figure 3: CD2 activation in VL patients influences IFN-γ responsiveness in T-lymphocytes by down regulating IL4 production.



10. Role of proteophosphoglycan in protection of Visceral leishmaniasis.

Objectives:

- To isolate, purify and characterize proteins of *L. donovani* promastigotes with and without Glyco-phospholipid anchors.

- To assess whether *L. donovani* surface proteins with and without GPI-anchor may be potential targets for anti-host cellular immune response, such as TGF-β and Interleukin-10.
- To assess a correlation of *L. donovani* proteins with and without GPI-anchor with multi-drug resistance {ATP-binding cassette (ABC) transporters- pgp-1}.

Progress:

We investigated the prospect of Glycoinositolphospholipid (GPI)-anchors on proteins of *L. donovani* promastigotes as a potential target of cellular immunity particularly those that govern regulatory mechanism to add in therapeutic unresponsiveness in VL patients. *L. donovani* promasigotes (10^8) were equilibrated with 2 ml TBS and 2ml Triton-X 114. Following incubation, suspension was pelletted and supernatant suspended in ice-cold PBS was subject to 37^{o}C water bath. The centrifuged material containing soluble proteins (upper phase) and trans-membrane protein anchored by Glycol-phosho-lipid structure (lower phase) were collected. NCP-blotted Polypeptides of these proteins were probed with mouse anti-human pgp-1 and MRP-1 and on the basis of reactive bands in a western blot, relevance of these proteins with drug resistance was established. In a separate experimental set-up, Ficoll separated PBMC (2x $10^{6/}$ ml) suspenstion from VL patients (n=5) were re-stimulated in 96 well round bottom micro-titre plate in presence and absence of these proteins for 48-72 hrs. The TGF-β and IL-10 level in cells in the presence or absence of the Leishmania proteins were measured using cytokine based ELISA.

Both GPI-anchored and GPI-non-anchored surface proteins were shown to contain polypeptide fraction that phosphorylated significantly the pgp-1. The 36kDa protein with and without GPI-anchor significantly phosphorylated pgp-1 reactivity. Efficient replication of *Leishmania* parasite is initiated with cellular immune activation of Th2 (IL-10 and IL-4) associated CD4 cell response during which possibly the protective immunity (Th1) is abrogated by Th2+CD4cells with TGF-β support. Thus, we next examined whether these *Leishmania* associated proteins were associated with activation of anti-host cellular response to facilitate *Leishmania* replication and promote resistance in patients. To address this, we used antigen-induced analyses of the induction of TGF-β and IL-10 secretion. VL patients produced a 5-fold more TGF-β compared to control. An increase in induction of TGF-β of 2.10-fold and 2.12-fold in response to GPI-anchored *Leishmania* protein and PHA, respectively, was noted in Patients. Overall, though there was no significant difference in the

induction of IL-10 ex-vivo, after LD and GPI-protein induced stimulation in patients; we observed that there was significant increase in IL-10 by cells stimulated with GPI-proteins compared to control. Further studies are in progress.

Figure 1: A. Antigenic profile soluble protein (S2) and GPI anchored protein.
B. Immunobloting of the antigen with anti pgp-1 antigen.

A. **B.**

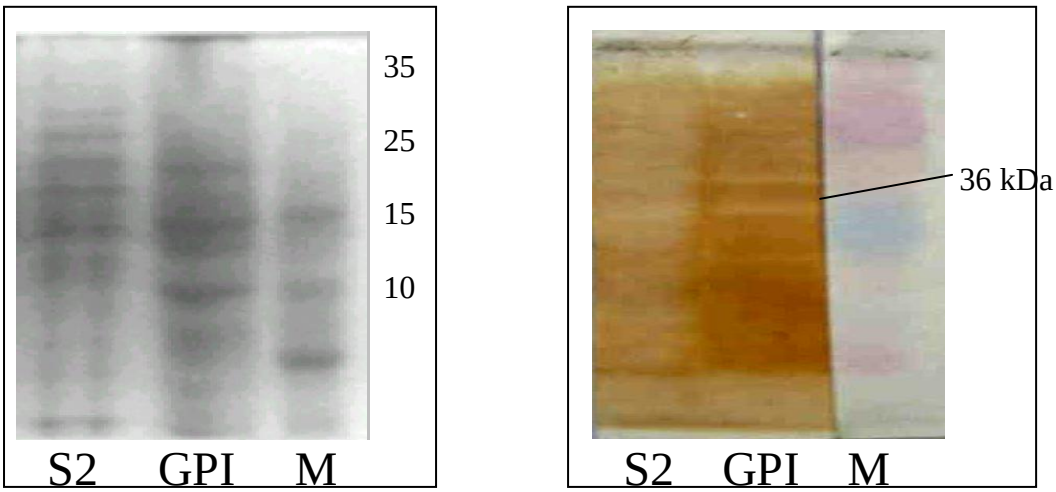
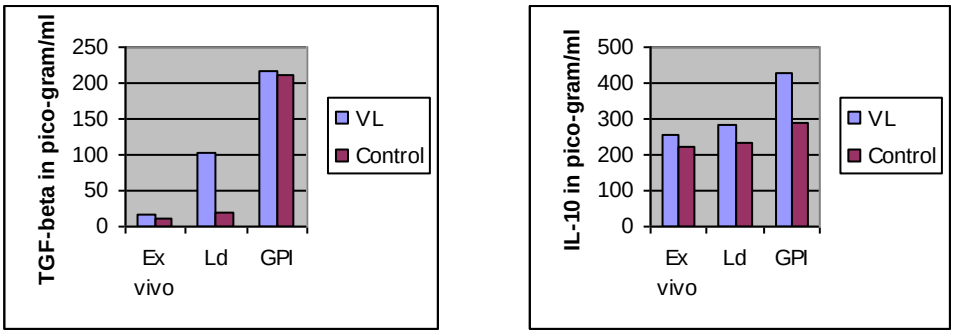


Figure 2: A. *Leishmania donovani:* GPI-anchored protein activates TGF-β
B. IL-10 associated cellular mechanism during VL infection.

A. TGF-β **B. IL-10.**



11. In vitro, role of antigen of *Leishmania* isolates of SAG responder and non-responder patients in IFN-γ & IL-4 production by similar sets of T-cells.

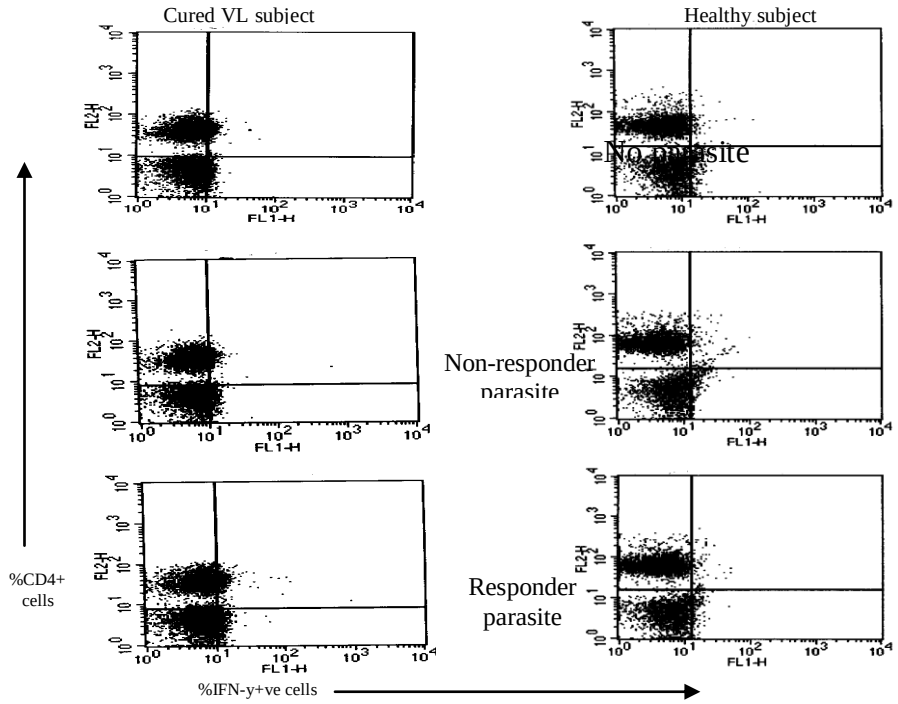
Objective:

To evaluate cytokines (IFN-γ & IL-4) production in two similar sets of T-cells collected from visceral leishmaniasis cured subjects and stimulated with whole antigen of SAG responder and non-responder isolates of *L.donovani*.

Progress:

The mononuclear cells from collected samples were stimulated with whole antigen of SAG responder and non-responder isolates and stained with anti-human CD4PE monoclonal antibodies. The cell accumulated IFN-γ was detected with anti-human IFN-γ labeled FITC monoclonal antibodies. The images of six samples were acquired by Flow Cytometer and data were analyzed by BD cell quest software. The work on IL-4 detection in T-cells of VL cured subjects stimulated with whole antigen of SAG responder and non-responder is in progress.

The data of IFN-γ production suggested approximately two fold increased production of IFN-γ in responder than non-responder parasites against T-cell of cured patients. Further work is under progress.



12. Study on Immunopathology of Post Kala azar Dermal Leishmaniasis (PKDL): T-cell subsets.

Objective:

To observe the changes in T cell subsets in PKDL lesions and in circulation in relation to VL cases and to understand its role in the Pathogenesis of PKDL

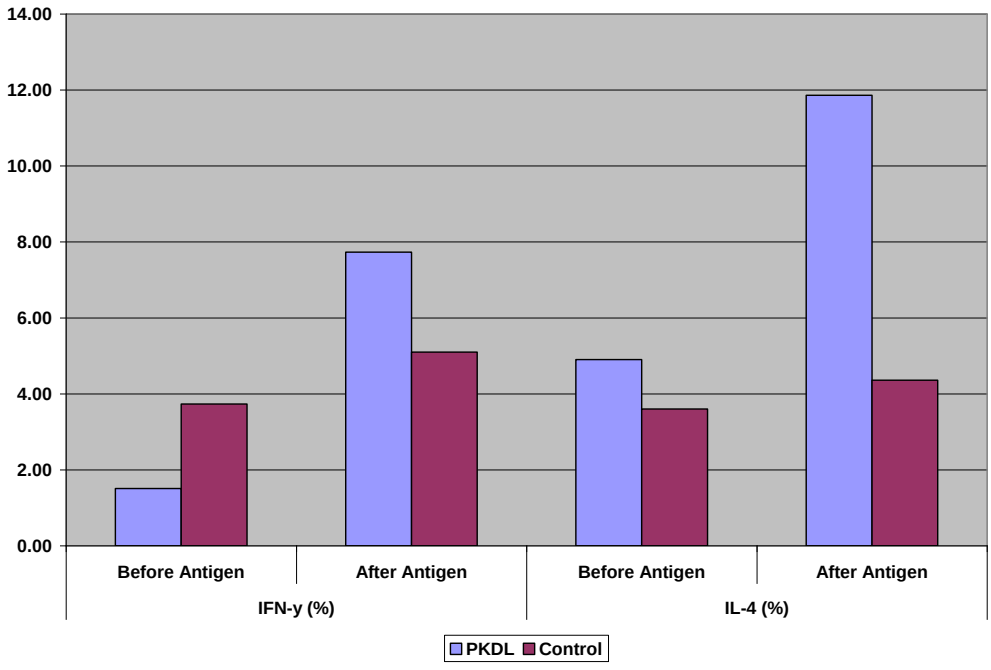
Specified Objectives:

- To determine the level of T helper and T suppressor cell in skin lesions and in the peripheral circulation of PKDL cases..
- To measure the levels of cytokine (IL-2, IFN-ϒ, IL-4 and IL-10) in PKDL cases and compare it with VL and control subjects.

Progress:

The Cytokine study has been conducted in 6 PKDL cases and 5 normal controls. It was found that cytokine response pattern in PBMNC of PKDL cases does not show much difference from kala azar cases as there is downregulation in IFN-y producing abilities of CD-4 cells with an almost 2-fold rise in the frequency of CD-4 cells positive for IL-4.

Intracellular IFN-γ and IL4 production by CD4 cells in PKDL cases compared to control



13. Crucial role of plants' extract in propagation of *Leishmania donovani* promastigotes.

Objective:

- To explore the possibilities of some plants' extract
  - as a source for replacement of blood/blood products/FCS/serum in routine culture of *L. donovani* promastigotes.
  - as a source of antileishmanial compound, if show lethal effect.

Progress:

Based on initial suggestive observations, fresh lot of plants' extract of some plants' (n=13) that belong to different families (n=10) having different characteristics such as habit, habitat, occurrence, flowering season, duration of plants etc. were prepared in distilled water. Three different culture media (2 commercially available i.e. RPMI-1640, pH 7.4 and Schneider's insect medium, pH 7.2; and another one LGPY, pH 7.4) were supplemented with 20% plant's extract, sterilized by Millipore filter. The plain medium was taken as negative control and medium with 10% FCS was taken as positive control. Equal numbers of washed log phase promastigotes were inoculated in experimental as well as control medium and incubated at 24°C, cultures were sub-passaged in the respective fresh medium at every 5^{th} days. Non-existence of alive cell / lethal effect on promastigotes was confirmed by sub-culture in LGPY medium having 10% FCS. In RPMI-1640, lethal effect of 2 plants' extract was also observed even after addition of 10% FCS. Adequate proliferation was exhibited by 9 plants' extract as supplement in LGPY medium for 74 sub-passages, which is quite enough to ensure its use for long term maintenance of promastigotes.

To find out more choice in selection for getting plants' extract easily & economically in different seasons and places, 19 new plants' extract were also screened in LGPY medium, out of which 8 exhibited adequate proliferations of promastigotes in continuous sub passaging over 24 successive sub- passages. One plant's extract showed lethal effect on promastigotes in LGPY medium, and in RPMI-1640 even after addition of 10% FCS. (Table 1).

Out of total 21 plants' extract, 2 exhibited thermal stability as they supported promastigotes proliferation in long-term successive sub passaging over 22 sub passages when used as supplement after autoclaving at 121°C for 20 minutes. Plants' extract kept at -20°C for 3 years and at 4°C for 1 year exhibited luxuriant growth indicating long storage stability/ self life. Three different plants' extract (20%) were supplemented to 1% agar base in LGPY medium and inoculated with 3 cells/ plate. Whitish mucoid colonies (size 3-4 mm approx.)

were observed on 10th day in each plate indicating the capability of these plants' extract to promote cell growth from single cell.

As a pilot experiment to demonstrate whether plant extract can be used in primary isolation of *L. donovani*, LGPY medium, supplemented with FCS (10 & 20%) and different plants' extract (n=6), were inoculated with splenic/bone-marrow aspirate. Except one, all the five plants' extract supported primary isolation of parasite within 4-5 days. More sets of experiments is in process to authenticate the observations.

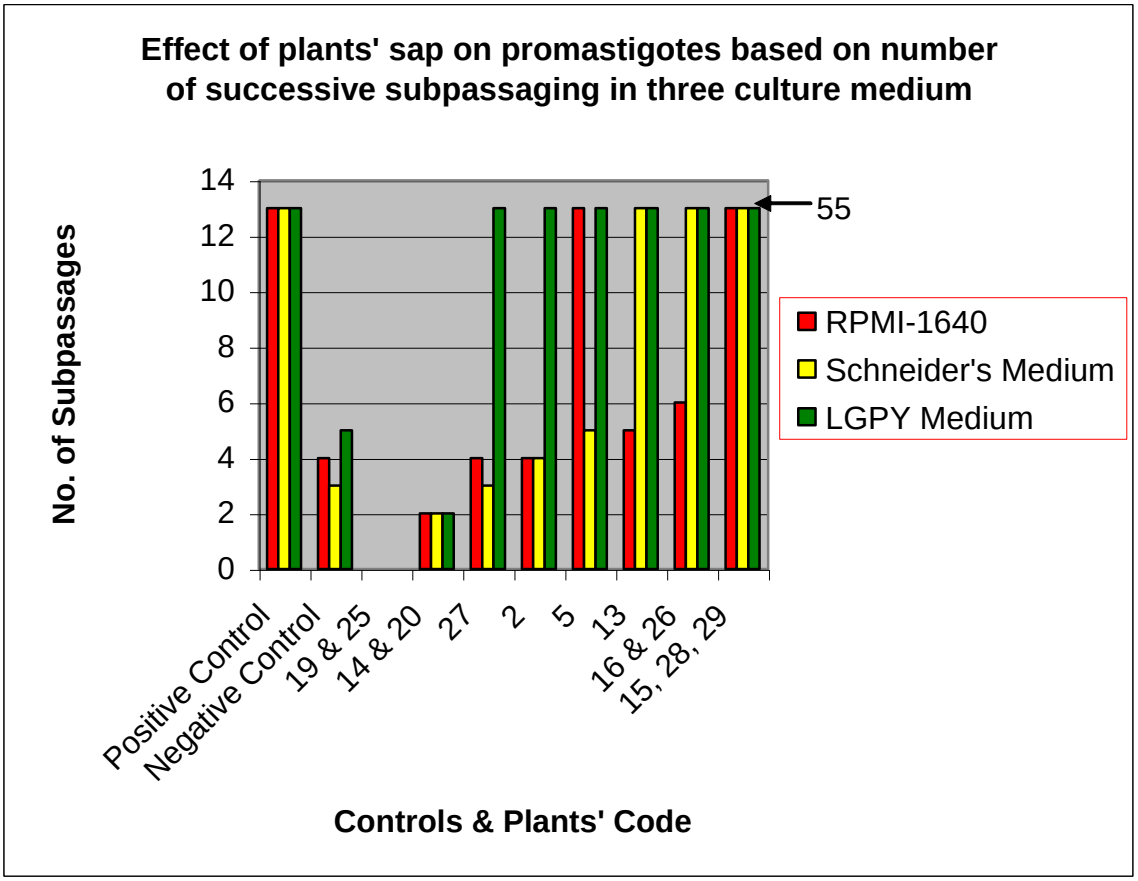


Table 1: Effect of plants' extract on promastigotes based on number of successive sub-passages.

Effect on promastigotes	Description of effect	Survival in No. of sub-passages	PE code/Total No.
Lethal	Non-motile / deformed cells	0	32 /1
Harmful	Cells sustainment < control	1 to 4	33, 34, 36, 45, 46, 49/6
Short-term survival	Cells sustainment = control	5	35, 37, 38, 39 /4
Long-term survival	Cells sustainment > control (continued)	24 cont.	31, 40, 41, 42, 43, 44, 47, 48 /8
Positive control	Cells sustainment > control (continued)	24cont.	
Negative control		5	

14. Identification of sibling species of *Phlebotomus argentipes* population in Bihar

Objectives:

- To identify the sibling species of *Phlebotomus argentipes* in Bihar
- To find out the relevance of sibling species vis-à-vis Kala-azar endemicity

Progress:

The work was initiated with the scrutiny of different forms of *P. argentipes* based on relative length of ascoid and 4th antennal segment. The sandflies were collected from Gulmehiyabagh village of Patna district. Individual sandflies were confined separately in rearing pot. After egg laying the dead sandflies were mounted in Hoyer's medium and the head was observed minutely under microscope for the measurement of ascoids and 4th antennal segment and based on the observation, all pots were marked as 0.2, 0.3, 0.4, 0.5 and 0.6. For each type a bigger rearing pot was taken and approximately 100 eggs of each type were collected and observed for further developmental stages like all four instars of larvae and pupae, morphologically.

No significance difference was observed during comparison of different developmental stages. The adults (male and females) of each type were separated just after emergence and cross-mated among different forms for reproductive isolation. No mating was observed

through naked eye during 12 hours of close observation from emergence. The study is under progress for more set of experiments. Isoenzyme and molecular assay will be followed to find out the genetic variability in different forms of *P.argentipes* from different geographical regions.

15. Exploratory investigations to detect the existence of chemical communication between male and female *Phlebotomus argentipes* (Old-world Sandfly), Indian vector of Kala-azar for mate and host location. (Sponsor: DBT)

Objectives:

- To develop improved laboratory culturing methods for larger scale fly output of *P. argentipes.*
- To design simple laboratory experiments to detect the existence of semiochemical mediated communication for behavioral responses between male & female *P. argentipes.*
- To isolate semiochemicals released by male by *P. argentipes* by solvent extraction or by volatile entrainment technique.
- To study behavioral aspects for validation of the chemical communication between male and female *P. argentipes* using male extracts in Y-tube olfactometer.

Specific objectives:

- To establish the critical parameters for improving *P.argentipes* colony with increased fly output
- To assess experimental evidence for the role of chemical communication in *P.argentipes* for host and mate location.
- To setup cross mating experiments to establish species homogeneity in *P.argentipes.*

Progress:

Wild sandflies were collected from highly endemic Muzaffarpur and Vaishali districts for establishment of colony in the insectorium for this study. So far, 65 sandflies were collected in two field visits. Out of 65 sandflies, 23 were fed and were confined for egg laying. The fly

out put from these confinements was 160. Further improvement in sandfly rearing on larger scale is under progress.

Table 1: Collection of sandflies from endemic districts

Date	Name of the District	No. of sandflies collected			Total	Temp. ^{0} C	RH %
		No. of ♂	No. of ♀				
			Fed	Un fed			
09.02.07	Muzaffarpur	20	9	6	35	16	95
14.02.07	Vaishali	10	14	6	30	19	100

Table 2: Number sandflies emerged from wild caught flies

Date of confinement	No. of ♀ confined	Total no. of eggs laid	No. of eggs/female	No. of eggs hatched	% hatching	No. of adults emerged	
						♂	♀
09.02.07	9	100	11	70	70	33	37
14.02.07	14	130	9	104	80	48	42

16. Control of Indian Kala-azar by genetic changing of the symbiotic bacteria of the vector, *Phlebotomus argentipes*.

Objectives:

- To identify the symbiotic bacteria from the gut of *Phlebotomus argentipes*
- To transform the bacteria genetically and ensure the paratransgenic transmission of GM bacteria
- To ensure development/nondevelopment of *Leishmania donovani* in the presence of modified bacteria

Progress:

In the previous year, 20 morphologically different bacterial colonies were identified from the gut of *P.argentipes* collected from different endemic villages of Bihar like Wajitpur Majhauli (Muzaffarpur), Mahnar (Vaishali) and Mohanpur (Samastipur). The identified colonies were mostly gramnegative *Pseudomonas*. To assess the symbiotic association of all colonies with *L. donovani* viability test was conducted with metabolites of all colonies which were serially

diluted in RPMI with 20% FCS in 96 well plate. The wells were charged with promatigotes (2 x10^5/ ml) and incubated for 3 days at 25^0C. The viability was tested under haemocytometer resulted good growth with one metabolites than control. Further work of paratransgenic bacteria is under progress.

17. Vector Biology in Control trial (KALANET Project; European Commission)

Objective:

- To demonstrate whether the blanket use of LLINs in a community provides any mass effect, which would provide protection to those in the community who fail to use LLIN for any reason, i.e. due to a reduction in the Entomological Inoculation Rate (EIR) by
    - reduction in sandfly abundance and/or
    - reduction in the sandfly infection prevalence.

    Both parameters were estimated monthly in 3 villages with LLIN and 3 villages without LLIN.

Progress:

The efficacy of long-lasting impregnated bednets (LLIN) on VL prevention is being assessed as a community based intervention trial in Bihar. The study aims to demonstrate a 50% reduction in *L.donovani* incidence rates in the intervention group compared to control. Six clusters of households were randomly allocated to the intervention and the control arm. Complementary to the trial, a study to monitor the entomological impact of the intervention is being conducted.

In each cluster 25 households were given temporary number (T1 to T25) after search for vector by two insect collectors for 10 minutes in up to two rooms. The houses positive for vector were selected and recorded during the demographic survey. Out of 25 households, 10 households and 10 cattle sheds (CS) in 6 clusters were selected randomly and followed for one night per month from September to November 2006.

In every month, light traps were set up in 10 selected households and untreated bed nets were provided to the household members for use. Sandflies were collected by light trap method**.** After light traps collection, the treated bed nets were returned and aspiration was done in two

rooms per house and cattle sheds. The collected sandflies and mosquitoes were preserved for further study.

Installation of CDC light trap for collection of sandflies

18. Efficacy, acceptability and cost-effectiveness of long lasting insecticidal nets in the prevention of Kala-azar (KALANET: European Commission)

Objectives:

- To evaluate the efficacy of long lasting insectidal bednets (LLIN) in the prevention of VL in the endemic focus of Nepal and India
- To estimate the cost and cost effectiveness
- To examine the acceptability of LLIN
- To evaluate the efficacy of alternative control technology

Specific Objectives:

Under Work Package (WP)-7, the specific objectives are as follows:

- To measure and compare efficacy of two types of Long lasting insecticidal bed nets
- To measure and compare efficacy of other community based vector control intervention methods

Progress:

In the previous pilot study to evaluate the efficacy of different collection methods of sandflies in Indian context, various indoor collection methods viz. flash light and aspirator; sticky traps; CDC-light traps and CDC-VU traps were adopted. Flash light and aspirator were used for collection from resting walls in the morning where as sticky traps, CDC-light traps and CDC-VU traps for moving sandflies over night. CDC light trap was found more effective in collecting moving sandflies during night.

During first year of the study, three types of bed nets were used namely a) OLYSET: wide mesh (4mm x 4mm), polyethylene, blended with permethrin 2%; b) PermaNet 2.0 (second generation): with small mesh (156 holes/inch^2) and c) Traditional net. A five-arm study including Permanet, Permanet control, Olyset, Olyset control and Traditional untreated nets, was set up in three highly endemic hamlets of Gulmehiya bagh (Patna, district), Rasoolpur and majlishpur (Vaishali, district).

Per hamlet 10 mix and 10 normal houses were selected for their high vector density. Labeled nets were distributed during the week after the first survey. The entomological efficacy was estimated on sandfly abundance during one sandfly season (April-June 2006). Collection of sandflies was conducted using CDC- light trap during night time followed by morning resting collection with aspirator before the intervention (week 0), 3, 6 and 9 weeks after treatment. The bed nets were utilized properly under constant supervision. The data is under compilation.

Impregnated bednets in the study area

19. Cost Effective Integrated Vector Management as a Contribution to the Visceral Leishmaniasis Elimination Initiative in the Indian Sub-continent: a multi-centre study. (Sponsor: WHO/TDR)

Objective:

- To contribute evidence for more effective vector management in support of the VL elimination initiative.

Specific objectives:

- To evaluate the safety and effectiveness of IRS/ITN/Eco-environmental management in reducing vector density & transmission of VL.
- To evaluate cost of IRS/ITN/eco-environmental management.
- To identify most cost-effective intervention amongst IRS/ITM/eco-environmental management.
- To evaluate acceptability of the three strategies.

Progress:

Six hamlets were selected based on high incidence of VL cases in Vaishali district of Bihar for IRS and Muzaffarpur district for LLIN. Altogether 24 clusters were selected for the study. In each hamlet, based upon the types of interventions, clusters were selected for comparing 3 interventions namely IRS, LLINs, Eco-environmental and one cluster was selected as control. From each clusters, 5 households were randomly selected after stratification into mixed houses (N=2) or human houses (N = 3).

Baseline estimates: Vector density was measured in the month of November and December and the relative density of the sandflies were estimated for each house with 2 overnight CDC light traps collections. Based upon the average vector density, 6 groups of 4 clusters/villages were identified. The clusters in each group were listed 1 to 4 and randomly allocated for interventions.

Arm A LLINs

In every month, light traps were set up in 5 selected houses before and after intervention. Untreated bed nets were provided to the household members for use and sandflies were collected by light trap in night. After light trap collection, the treated bed nets were provided

and aspiration was done in two rooms per house and in the cattle sheds. The collected sandflies and mosquitoes were preserved for further study.

Arm B IRS

IRS was carried out in coordination with district public health centre (PHC) as per Government of India (GOI) policy. Prior to spraying, the head of households were informed of the procedure and date of DDT spray and asked to sign a consent form. An entomological technician was deputed for the insecticidal spray, particularly in targeting the cracks and crevices in the households and the peridomestic sites. The work is in progress.

Bioassay test to ascertain the efficacy of LLIN

Preparation of DDT solution for IRS

20. Evaluation of impact of DDT indoor residual spraying being used in Kala-azar control programme on the disease prevalence.

Objective:

- To assess the susceptibility of *P.argentipes* against DDT.

Progress:

DDT Sensitivity test reveals the development of tolerance in *P. argentipes* in all the four districts i.e. Bochaha (Muzaffarpur District), Patepur (Vaishali district), Bande (Samastipur district) and Runisaidpur (Sitamarhi district). However *P. argentipes* of Bahapur (Patna district), which has no history of spraying since last ten years, showed 100% sensitivity against 4% DDT.

In order to study the epidemiological impact of DDT and vector resurgence, an endemic village, Belladam (Patepur PHC) in Vaishali district, was selected having maximum number of cases (8 cases) in the year 2004–05. The population of this village was 1448 in 221

households. DDT spraying was carried out under our supervision and all parameters like pre and post evaluation of vector density, bioassay test was carried out. Simultaneously, pre post sand fly density was monitored in 11 districts the result in the following tables.

Table 1: Pre spray vector density observation

District	Date	Present status of sandfly		Total sandflies collected	MHD
Muzaffarpur, PHC- Bochaha	07.04.06	*P.argentipes*	63	79	13.1
		P.papatasi	06		
		Sergentomyia spp	10		
Sithamarhi, PHC-Runisaidpur	18.04.06	*P.argentipes*	48	62	10.3
		P.papatasi	14		
		Sergentomyia spp	00		
Nalanda, PHC- Chandi	19.04.06	*P.argentipes*	60	66	8.25
		P.papatasi	02		
		Sergentomyia spp	04		
Samastipur, PHC- Tajpur	20.04.06	*P.argentipes*	22	22	11.0
		P.papatasi	00		
		Sergentomyia spp	00		
Dharbhanga, PHC- Hayaghat	20.04.06	*P.argentipes*	08	18	9.0
		P.papatasi	10		
		Sergentomyia spp	00		
Madhubani, PHC- Sakari	21.04.06	*P.argentipes*	38	56	9.3
		P.papatasi	11		
		Sergentomyia spp	07		
Saran, PHC- Garkha	22.04.06	*P.argentipes*	79	79	11.0
		P.papatasi	00		
		Sergentomyia spp	00		
Vaishali, PHC- Patepur	10 & 11.05.06	*P.argentipes*	52	69	11.5
		P.papatasi	08		
		Sergentomyia spp	09		
Patna, PHC- Bakhatiapur	31.05.06	*P.argentipes*	50	50	12.5
		P.papatasi	00		
		Sergentomyia spp	00		
Arah, PHC- Arah	24.04.06	*P.argentipes*	26	43	7.1
		P.papatasi	10		
		Sergentomyia spp	07		
Araria, PHC- Araria	26.06.06	*P.argentipes*	20	20	10.0
		P.papatasi	00		
		Sergentomyia spp	00		
Kishanganj, PHC- Bhadurpur	27.06.06	*P.argentipes*	38	38	9.5
		P.papatasi	00		
		Sergentomyia spp	00		
Supaul PHC – Pipra, Supaul	01 to 03.07.06	*P.argentipes*	19	19	6.2
		P.papatasi	00		
		Sergentomyia spp	00		

Table 2: Post spray vector density observation

District	Date	Present status of sandfly		Total sandflies collected	MHD
Vaishali PHC – Patepur	02.06.06	*P.argentipes*	00	00	00
		P.papatasi	00		
		Sergentomyia spp	00		
Araria PHC – Araria	26.06.06	*P.argentipes*	17	17	8.5
		P.papatasi	00		
		Sergentomyia spp	00		
Kishanganj PHC - Bhadurpur	27.06.06	*P.argentipes*	28	28	7.0
		P.papatasi	00		
		Sergentomyia spp	00		
Supaul PHC – Pipra, Supaul	01 to 03.07.06	*P.argentipes*	01		0.5
		P.papatasi	00		
		Sergentomyia spp	00		

21. Monitoring and supervision of DDT 50% spray operation in Kala-azar endemic districts.

Objective:

- Eradication programme of kala-azar initiated by Bihar Govt.

Progress:

Study of pre spray density

As per the assignment by NVBDCP, Govt. of India, this institute initiated monitoring of DDT spray and pre and post entomological surveillance in various districts endemic for Kala-azar viz. Muzaffarpur, Vaishali, Sithamarhi, Nalanda, Samastipur, Dharbhanga, Madubani, Saran, Patna, and Arah. Later on three more districts namely Araria, Kishanganj and Supaul were included. In Vaishali district (village, Belladam & Bella) spaying was conducted under our supervision.

After consultation and discussion with respective CS/DMOs, the team visited the villages where spraying operation was going on for monitoring. Sandfly density was measured. During the visit it was found that the spraying was not satisfactory. In many houses, only irregular patches of DDT were found on the sprayed wall. In some areas, no staff from the respective PHC was found available at the spot for supervision of the operation. The villagers were cooperative and some of them reported about unsatisfactory spray in their households.

- Total No. of villages visited : 113
- Total No. of households searched : 2414
- Total No. of Sandflies collected : 275
    - Total No. of *P.argentipes* : 253
    - Total No. of Sergentomyia : 22

DDT spray monitoring in an endemic village of Vaishali district

22. Validation of sandfly distribution and Kala-azar disease prevalence through Remote Sensing & GIS in endemic and non endemic foci of Kala-azar to reaffirm the earlier out come and its applicability for the entire Kala-azar endemic region of Bihar. (Sponsor: ICMR)

Objective:

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- To correlate geographical distribution of sandfly (*P.argentipes*) in relation to visceral leishmaniasis and the satellite data obtained in respect of macro and micro system and

other ground truth in the endemic and non-endemic areas evaluate its applicability in entire Kala-azar endemic area and its role as "**epidemic predictor**".

Progress:

Under the ICMR task force project "Application of Remote Sensing in identifying and mapping sandfly distribution in endemic and non –endemic Kala-azar foci in Bihar", it was observed that temperature, humidity, settlement/orchards, land areas of crop plantations, dry fallow, moist fallow and minimum normalized-difference vegetation index and standard deviation of NDVI are the main environmental variables independently associated with the vector density or presence of vector in endemic sites.

To further validate the outcomes at macro level, ground survey is being carried out at two levels i.e. district boundary level and village level. Boundary lines of each and every selected districts are being surveyed and sketched indicating the various important features viz. river, ponds, vegetation, forests, hills and information on various socio-economic parameters like total population, sex ratio, literacy, areas of water bodies, crop, settlement, peridomestic vegetations, trees, plants, cattle sheds are been collected and updated at village level.

Site preparation for Remote sensing

23. Early identification of asymptomatic cases of Kala-azar in endemic foci of Bihar, India: An epidemiological and socio-behavioral study. (Sponsor: WHO/TDR)

Objective:

- To determine the asymptomatic cases and their conversion into disease stage.
- To study the socio-behavioral aspect of VL in the community

Progress:

This study was conducted in two villages namely Banthu and Haribanshpur of Vaishali district of Bihar on the basis of high endemicity of the VL cases as per the State Govt. data. Out of 1525 population (in 282 households) enumerated, 1017 were subjected for clinical examination, rk-39 dipstick test and PCR. The current and past history of case, clinical findings, diagnosis and treatment details were recorded.

Out of 1017 screened individuals, 97 were found positive by rK-39 and 31 were PCR positive in 345 blood samples tested so far. A total of 36 individuals were found for past history of kala-azar. All rk39 strip test / PCR positive cases are being followed on first week of every month. After 3-months follow up, out of 61 rk-39 positive (excluding past history of kala-azar), 21 developed as full blown kala-azar case and remaining 40 are still asymptomatic. The follow up has to be extended for one year to assess the proportion of asymptomatic cases and their conversion rate into disease stage.

A separate pre-tested semi structured questionnaire was canvassed to all the head of the households to elicit the information on socio-behavioral aspect in relation to disease, transmission, diagnosis, prevention and treatment seeking behavior. Data analysis on socio-behavioural aspect is under progress.

24. Magnitude of under-reporting of Visceral leishmaniasis (VL) cases in Bihar, India.

Objective:

- To determine the proportion of under-reporting of VL cases
- To estimate the actual annual incidence of VL cases

Progress:

Three highly endemic districts for kala-azar namely; Vaishali, Muzaffarpur and Samastipur have been selected based on State Govt. of Bihar data. Three most highly endemic PHCs from each district have been selected. The first round information on details of the VL patients viz. name, age, sex, village, treatment etc. from each PHC is in process. Simultaneously, 10 villages from Lalganj PHC of Vaishali districts have been identified wherefrom VL cases are reported at the PHC. Door-to-door survey in one village namely, Banthu, has been initiated. The work is at preliminary stage that requires extensive door-to-door survey in the study area for cross-matching the cases recorded at the PHCs and actually found during survey; as well as assessment of the proportion of the under-reporting of the VL cases.

25. Implementation Strategies for visceral Leishmaniasis treatment in India (Sponsor: WHO/TDR)

General Objective:

To develop improved implementation strategies for early case detection and treatment of all VL patients.

Specific objectives:

- To determine the knowledge of, and the importance given to, leishmaniasis and its control by different sections of society
- To describe what public and private health services are available for VL care and to analyze their current practices for VL diagnosis & treatment
- To study people's health seeking behavior with respect to VL treatment and its determinants
- To determine the proportion of patients served by different health care providers and the proportion of patients never reported

Progress:

The survey covered a population 28343 in 5443 households spread over in 13 villages of an endemic Block in District Vaishali. On a sub sample basis, the total of 445 household interviews were conducted to elicit the information on Knowledge, Aptitude & Practice (KAP). During the survey, 61 fever cases (>2weeks) were subjected to rk-39 test and 31 were

found positive. Patient interviews were conducted, and were referred to PHC/RMRI. Apart from this, a total of 69 Health Care Providers were interviewed in the study area. (Table 1)

Table-1: Important indicators

Total study population	28343
Total No. of Villages	13
Total No of households screened	5443
Total No of households interviewed	445
Total No. of current and past KA cases	56
Total No of fever cases of more than 15 days	61
Total No of fever cases positive for rk39	31
Proportion of rK39 positive cases positive for LD bodies	51%
One yr period prevalence of KA	2.0/1000 pop
Health Care Providers interviewed	69

Screening Schedule

A total of 5443 households were covered for screening wherein head of households were interviewed through pre-tested questionnaires. Age-sex distribution of the population screened shows that average age was 24% and mean SD was 18.9%. Proportion below 15 years of age was 40.4%, while 43% in the age group 15-45 years. The proportion of male was 53% and 43% in female. It was found that 40% of the individuals having past history of Kala-azar preferred Qualified Pvt. Doctors followed by Govt. Doctors (35%), Chemist (10%). Total number of fever history more than 15 days were 61 and all were subjected to rk39 test, out of which 31were found positive.

Household Schedule

Head of the household (N = 445) for every 13th household during the survey and houses having past KA case or current KA case were interviewed for detailed Household schedule. The analysis shows that the mean age of the head of the household was 42.1% with SD 18.9. Among the total respondents 81% were male and 19% were female. Majority of the respondents were illiterate (44.8%) followed by middle or high school (26.87%), primary level (20.8%), and intermediate & above (7.6%). More then half were engaged in agricultural activities 33% as agricultural labour while 19% reported awn agriculture, 15% were engaged in their own petty business and 11% were skilled labour.Mean number of living rooms were found to be 2.24 in the study area. As far as the house material is concerned 73.6% were hatched / Tati, 12.1% were mud plastered and 14.3 were brick un-plastered.

Kala-azar awareness

A very high Proportion 98.4% were aware of Kala-azar, but regarding symptoms the most common were fever 93%, chill/rigor 72%, blacking of Skin 9% and enlargement of abdomen 4%.Curable nature of disease was reported by 98.9%. Regarding transmission of the disease 80.2% reported by mosquito to bite, 10.4% reported through exterminated water and 13% had no knowledge. Knowledge regarding protection seems to be encouraging as 74% reported use of bed nets, 39% use of smoke, 26.9% repellents and 12% use of insecticide, around three fourth (73.6%) household reported having bed nets.

General health seeking behavior

For general health seeking behavior local unqualified doctor was the first preference with 82.9% of responses followed by private qualified doctor 56.9%, local chemist 41.2% and Govt. doctor 31.0%. Time required to reach the provider was 52 minutes for private qualified doctor and Govt. doctor and for the rest it is less then 15 minutes. The cost for reaching the private qualified doctor/Govt. doctor is around 50-55 rupees. As far as priority Health Care Provider is concerned the 1st priority for provider was local unqualified doctor with 49.7% respondents followed by private qualified doctor 39% and Govt. doctor 10% where as in the second priority it is the private unqualified doctors (60.6%) and Govt. doctor (36.6%).

The main reason for selection of Health Care Provider in the first priority was proximity to the house (41.8%) and faith (37.9%) whereas in the second priority it is faith (60%) and cheaper (28.2%). Specifically in case of Kala-azar, the choice is private qualified doctor (44.7%) followed by Govt. doctor (33%) and unqualified doctor (21%). Here again faith (61.5%), cheaper (37.4%) and proximity to the house (18.7%) were the main consideration for selection of Health Care Provider.

Table-2: Availability of various health care providers with average time taken and expenditure in transportation

Characteristics	Percentage	Average time taken to travel (in mts)	Average money spent in reaching (in Rs)
Indigenous healer	3.9	11.4	0.10
Local chemist	41.2	14.5	0.17
Local unqualified doctor	82.9	13.7	1.30
Private qualified doctor	56.9	52.2	51.2
Govt. doctor	31.0	50.9	55.2

KA patient interviews

Duration between falling ill and seeking health care was reported by 2 weeks by 50% and more then 4 weeks 50%.Duration between going to health care provider and receiving the diagnosis was reported 3 weeks by 50% and more then 4 weeks by the rest of 50%.Duration between receiving the diagnosis and starting of treatment was reported <1 week by 25%, 3 weeks 25%, and more then 4 weeks by 50%.Paramedical were reported as the first contact by 50% of the cases while 25% contacted private medical doctor but for the second contact all have gone to private doctor. It was found that 75% cases were diagnosed by Aldehyde test and rest by bone marrow/spleenic aspiration.

Table-3: Treatment seeking behavior and practices

Variables	Percentage
i. Interval between illness and seeking care	
< =1 week	0.0
2 weeks	50.0
3 weeks	0.0
=> 4weeks	50.0
ii. Interval between seeking care and diagnosis	
< =1 week	0.0
2 weeks	0.0
3 weeks	50.0
=> 4weeks	50.0
iii. Interval between diagnosis and starting of treatment	
< =1 week	25.0
2 weeks	0.0
3 weeks	25.0
=> 4weeks	50.0
iv. Diagnostic test as basis of treatment	
Bone marrow/spleenic aspiration	25.0
Aldehyde test	75.0

26. In-depth review of current Kala-azar Programme. (Sponsored: NVBDCP, Govt. of India and World Bank)

General Objective:

To review the implementation status of current Kala-azar control Programme.

Specific objectives:

- To review the disease surveillance.
- To review the access of early diagnosis and treatment.
- To review vector control strategies.
- To review IEC activities.

Observations:

Households Survey

Quantitative data analysis reveal that a total of 8 districts and 12885 households covering 76942 population comprising 40565 male and 36377 female were covered for IDR Survey. Good proportion (45.87%) of the respondents was found illiterate and 55.09% houses were found Kuchha/Thatched. Among the surveyed household, 48.14% of houses got the cattle-shed inside or nearby the houses and 84.14% houses were having no toilet facility inside their houses.

Maximum surveyed population (27255) was from the age group 0-14 Yrs and 1380 (76942) were found Kala-azar cases, out of which 832 (60.29%) were male and 548 (39.71%) were female. Among the cases 121 (0.16%) were current cases and rest 1259 (1.6%) were past cases of Kala-azar. Thus the Prevalence and Incidence was observed as 17.93 and 1.57 respectively per thousand in the study area. Pregnancy was observed in 47 (0.20%) cases out of reproductive female age population (23172). There were 115 (9.13%) deaths reported out of 1259 past Kala-azar cases. As regard to diagnostic practices adopted for Kala-azar still diagnosis was mainly based on Aldehyde test /rk-39 test. Good proportion 622 (45.07%) of the Kala-azar cases prefer their treatment at Private Set-up. (Qualified Private Doctors, MBBS) As regard to IRS status only 5247(40.72%) households were sprayed with DDT. Two rounds IRS were reported by 1237 (23.58%) and out of 2nd round spray only 289(23.36%) households sprayed as per the schedule i.e. in the month of July- August.

Health Facility Survey

It was found that a total of 229 Health Facility Providers including medical officers, qualified private doctors, un-qualified private Doctors, District Malaria Officers, Malaria Inspectors/Supervisors, health workers/ANM/MPW/BHV/LHV/BEE etc were interviewed. Among all the categories at Govt. set-up only 16.59% had attended some kind of KA training in last one year and all most all need further training/ orientation of Kala-azar. Generally the diagnostic practices adopted for the treatment of Kala-azar were rK-39 (58.89%) followed by Aldehyde test (33.33%) at Govt. Set-up as well as Private Set-up. Presently majority (23.33%) of the health facility providers at Govt. set-up start the treatment of Kala-azar on the basis of Aldehyde test followed by 15.55% on rK-39. It has been concluded from the findings that presently no diagnostic facilities were available at Govt. set-up except Aldehyde test. SAG was available at majority (77.55%) of the centers under Govt. Set-up followed by Amphotericin B (22.44%) for the treatment of Kala-azar. Among the Govt. health functionaries 26.53% fails to maintained buffer stock throughout the year.

Table 1: Distribution of Current and Past Kala-azar cases

State	District	HH Surveyed	Pop. Surveyed	Current KA Cases	%	Past KA Cases	%	Kala-azar cases	%
Bihar	East Champaran	1597	11405	23	0.20	197	1.73	220	1.93
	Muzaffarpur	1623	10961	18	0.16	135	1.23	153	1.39
	Saran	1672	12243	5	0.04	93	0.76	98	0.80
	Vaishali	1672	10105	11	0.11	150	1.48	161	1.59
Sub Total		***6564***	***44714***	***57***	***0.13***	***575***	***1.29***	***632***	***1.41***
U.P.	Ballia	1601	9725	7	0.07	103	1.06	110	1.13
Jharkhand	Godda	1378	6797	48	0.71	284	4.18	332	4.88
W.B.	24 Pargana (S)	1679	7551	1	0.01	243	3.22	244	3.23
	Murshidabad	1663	8155	8	0.10	54	0.66	62	0.76
Sub Total		***3342***	***15706***	***9***	***0.06***	***297***	***1.89***	***306***	***1.95***
Total		**12885**	**76942**	**121**	**0.16**	**1259**	**1.64**	**1380**	**1.79**

Table 2: IRS during current year and as per the scheduled month

State	Name of the district	No. of Household	No. of Household Sprayed	1st Round	2nd Round	IRS during scheduled month	
						Mar - Apr	July - Aug
Bihar	East Champaran	1597	303	281	22	0	1
	Muzaffarpur	1623	817	802	15	0	0
	Saran	1672	601	593	8	0	0
	Vaishali	1672	1213	756	457	20	62
Sub Total		***6564***	***2934***	***2432***	***502***	***20***	***63***
U.P.	Ballia	1601	25	23	2	0	0
Jharkhand	Godda	1378	1194	613	581	67	162
W.B.	24 Pargana (S)	1679	1003	851	152	28	64
	Murshidabad	1663	91	91	0	0	0
Sub Total		***3342***	***1094***	***942***	***152***	***28***	***64***
Total		**12885**	**5247**	**4010**	**1237**	**125**	**289**

Honours and Awards received by scientists

1. Dr. Das, Director received Dr. C.K.Singh Oration award, Dept. of Zoology, Prof. V.K.Singh University, Arrah, Bihar.
2. Dr. Das, Director received M.M.Chakraborty Memorial Oration award, Zoological Society, Kolkata.
3. Dr. Das, Director as Programme Advisor, National Consultative Workshop, Vector Borne Disease Control Programme, Delhi.
4. Dr. Das, Director as Chairman, Joint Monitoring Mission (JMM) team for Kala-azar of National Vector Borne Disease Control Programme.
5. Dr. Das, Director as member of Scientific Advisory Group.
6. Dr. C.S.Lal, Sr. Research Officer as an external examiner for M.Sc. (Biochemistry), Patna University.
7. Dr. C.S.Lal, Sr. Research Officer as referee of International Journal *Memorias do Instituto Oswaldo Cruz.*
8. Dr. S.Bimal, Sr. Research Officer as an external examiner for M.Sc. (Biochemistry), Patna University.
9. Dr. S.Bimal and Dr. C.S.Lal, Sr. Research Officers were awarded Advanced WHO/TDR Refresher course fellowship on Immunology, Vaccinology and Biotechnology applied to Infectious Diseases.

Meetings/ Seminars/ Trainings organized

1. German Doctors' scientific meeting and training program held on 9th April, 2005.
2. Miltefosine Phase IV meeting, sponsored by WHO/ICMR/TDR/Zentaris, held on 3rd July 2005.
3. A group meeting was organized by Mr. Chip Bernett, Technical Advisor, iOWH, USA on 13th May 2005 to discuss the protocol of the study entitled "Incidence of kala-azar in a selected district of the State of Bihar, India: A study assessing the need and strategies for use of paromomycin".
4. German Doctors' scientific meeting and training program held on 27th Nov., 2005.

5. A scientific deliberation on "Neutrophil granulocytes – Trojan horses for *leishmania* major and other intracellular microbes" was given by Professor Tamaslaskay, University of Lubeck, Germany on 2nd Dec., 2005.

6. Deshratna Dr. Rajendra Prasad Memorial oration lecture on "environment and Health", delivered by Prof. Shelley Bhattacharya, Vishva Bharati University, Shanti Niketan on 3rd Dec., 2005.

7. KICK OFF Meeting for the project "Implementation Strategies for Visceral Leishmaniasis treatment in India (WHO/TDR Collaborative Project)" was organized from 3rd -6th March 2006. The meeting was attended by various participating teams i.e. BHU, Varanasi, Balaji Uthan Sansthan Patna, Nepal, Bangladesh and TDR/ WHO.

8. Theoretical training on "Confocal microscope & its application in medical science" was organized on 7th April, 2006.

9. GCP training, sponsored by Quintalis, held on 11th – 12th April 2006.

10. Training imparted to the 40 National talent scholarship students, sponsored by NCERT, on the initiation of Ministry of Science & Technology, Govt. of India on 13th April 2006.

11. GCP training, sponsored by Glaxo SmithKline (GSK), UK, held on 25th – 26th April 2006.

12. GLP training, sponsored by Institute of OneWorld Health (iOWH), held on 03rd May 2006.

13. 2nd International meeting on KALANET consortium, sponsored by European Union on 23rd – 25th May, 2006.

14. GCP Workshop on "The Informed Consent Process" and "Patient Screening Process", sponsored by Institute of One World health (iOWH), USA, held on 30th May 2006.

15. Meeting for In-Depth review of Kala-azar Programme with Dr Vijay Kumar, World Bank Consultant to finalize the tools for IDR from 9th -10th Sept., 2006.

16. Theoretical training on "Real Time PCR" was organized by Roche Diagnostic India Pvt. Ltd. on 21st – 22nd Sept., 2006.

17. Theoretical training on "Confocal microscope & its application in medical science" was organized on 1st Nov. 2006.

18. Scientific meeting with Dr. A.Jeyaram, RRSSC, Kharagpur for ICMR Task force project on Remote sensing held on 2nd – 3rd Nov., 2006.

19. German Doctors' scientific meeting and training program held on 18th Nov., 2006.

20. A Workshop on "Microarray" was organized by Roche Diagnostics from 29th Nov – 1st Dec, 2006.

21. Deshratna Dr. Rajendra Prasad Memorial oration lecture delivered by Prof. Sandeep Basu, Emeritus scientist, IICB, Delhi on 20th Dec., 2006.

22. In-Depth review of Kala-azar Programme meeting with Joint Monitoring Mission (JMM) team from 30th Jan-5th Feb, 2007.

23. Scientific oration on "Paratransgenic approach to vector borne diseases", delivered by Prof. R.V. Durvasula, Prof. & Chief of Medicine, University of New Mexico, School of Medicine, USA on 7th March, 2007.

24. Training program on "Good Clinical Practices & Clinical Trial" was organized by Odyssey Research India Pvt. Ltd on 22nd Feb., 14th March and 22nd March, 2007.

25. Investigators' meeting and training workshop for Paromomycin IM injection Phase IV protocol – Module 1 –Pharmacovigilance, sponsored by iOWH, held on 26th – 27th March 2007.
26. Two months training programme for dissertation course to the students of B.Sc. Biotechnology of various universities.

27. Three months training programme for dissertation course to the students of M.Sc. Biotechnology/ Biochemistry of various universities.

28. Training imparted to the Medical Officers and Supervisors on the different aspects of Bionomics of sand fly and DDT spraying operations.

Meetings/ Seminars/ Conferences/ Training attended

Dr. P.Das, Director

1. Participated in the "1st National Conference of AIDS Society of India (ASICON 2005)" held in New Delhi from 2nd - 4th April, 2005 and delivered a talk on "Opportunistic enteric parasite in AIDS".

2. Delivered a lecture on "Studies on factors Post trarnscriptional modification of rRNA processing in *Giardia lamblia*" in the Department of Molecular Medicine, Jawaharlal Nehru University, New Delhi on 4th April 2005.

3. Delivered a lecture on "Recent Developments in Molecular typing of Parasitic Diseases", organized by Hindustan Lever Ltd held at Cochin on 20th -22nd April, 2005.

4. Attended the Paromomycin meeting at London, U.K organized by the Institute of One World Health and WHO/TDR/PDT from 13th -17th June, 2005.

5. Attended the Principal Investigators meeting of Clinical trial entitled "A double blind, randomized, multicentre, three-arm study to access the safety and efficacy of

Paromomycine administered intra-muscularly at three different dosing regimens", organized by iOWH, SanFrancisco, USA, at new Delhi from 18th -19th Aug,2005.

6. "The first Oration lecture on Dr. C.K. Singh" at Zoology Department, Prof. V. K. Singh University, Arrah, Bihar on 20th August, 2005.

7. Participated as the Principal Investigator form the Indian side in the Indo-US workshop on "Diarrhea and enteric Parasites: New Challenge in the Era of HIV/AIDS" held at Calcutta, India from 3rd - 5th October, 2005.

8. Attended the "INSERM – ICMR clinical research workshop 2005" at Kolkata on 20th -21st Oct., 2005.

9. Attended Task Force Project Committee meeting on "GIS and Remote Sensing", held at New Delhi on 8th Nov., 2005.

10. Attended the Principal Investigators meet on "Safety and efficacy of oral Miltefosine in patients with Post Kala-azar Dermal Leishmaniasis (PKDL) – dose finding study comparing 8 and 12 week treatment", held on 27th Nov., 2005 at Patna.

11. Delivered a lecture on the "Role of fibrillarin and small nuclear RNAs in post transcriptional modification of rRNA in *Giardia lamblia*" at the 75th Annual Session of the National Academy of Sciences, India, held at Pondicherry University from 8th - 9th Dec, 2005.

12. Attended the 54th Annual meeting of American Society of tropical medicine and Hygiene in Washington DC, USA from 11th -15th Dec., 2005.

13. Attended the Workshop on Water, Sanitation & Environmental Health in India, organized by ICMR and CDC&ASTDR at Kolkata, India from 21st-22nd Feb., 2006.

14. Attended the workshop on proposal writing on "Cost effective integrated vector management as a contribution to the VL Elimination initiative in the Indian Subcontinent" held at Kolkata from 28th Feb – 2nd March, 2006.

15. Delivered a talk on " 29kDa Thiol Dependent peroxidase : a key factor for survival of *Entamoeba histolytica* during oxidative stress" in the 11th Asian Conference on Diarrhoeal Diseases and Nutrition at Bangkok, Thailand from 7th -10th March, 2006.

16. Attended "National Consultative Workshop on Vector Borne Disease Control Programme" as Co-Chairman and Programme Advisor for Kala-azar elimination, held at Delhi on 21st March, 2006.

17. Delivered a lecture on "Is 29kDa thiol dependent peroxidase responsible for survival of Entamoeba histolytica during oxidative stress?" at the National Symposium on Recent Advavancement on Parasitology Research, organized by Dept of Zoology, University of Calcutta, from 25th - 26th March, 2006.

18. Attended the 1st meeting of the Advisory group on Public Health as member organized by West Bengal State Council of Science and Technology, on 18th May, 2006.

19. Attended ICMR Task Force meeting on "GIS and Remote Sensing", held at New Delhi on 14th Sept., 2006.

20. Attended the National Parasitology Congress 2006 as member of the local organization committee at IICB, Kolkata from 22nd – 24th Nov, 2006.

21. Attended World Bank Pre-appraisal Mission for the proposed India – Vector Borne Disease Control Programme, held at Delhi from 14th -22nd Dec., 2006.

22. Participated as special guest in one day Sensitization Meeting under Kala-azar Elimination Project, held at Bihar State Health Society, Patna on 18th Dec., 2006.

23. Attended ICMR Director's meet on 23rd - 24th Dec, 2006 at Jodhpur, Rajasthan.
24. Attended as Chairman, JMM (Joint Monitoring Mission) team for Kala-azar of National Vector Borne Disease Control Programme, held at NVDCP, Delhi from 29th Jan – 9th Feb., 2007.

25. Attended In-Depth review of Kala azar Programme meeting, held at NVBDCP, Delhi from 7th – 9th Feb, 2007.

26. Participated as a resource parson for ToT course of NVBDC for faculty of Medical Colleges of Bihar, from 19th - 24th March, 2007 at SIHFW, Patna.

Dr. P.K. Sinha, Dy. Director

1. Attended Third World Congress on Leishmaniasis, held in Palermo-Terrasini, Sicily, Italy from 10-15 April 2005 and delivered a lecture on "A Phase III multicenter, Randomized, Controlled, Clinical trial to assess the safety and efficacy of injectable paromomycin in patients with visceral leishmaniasis: Paediatric subset analysis and preliminary final results".

2. Attended the Expert Committee meeting to review strategies for "Kala-azar elimination from India", under the Chairmanship of Director General of Health Services, Govt. of India, held at Nirman Bhawan, New Delhi on 21st April 2005.

3. Attended Principal Investigator meeting of Paromomycin drug trial, organized by Institute for OneWorld Health (iOWH), San Francisco, California, USA held on June 12 – 18, 2005 in London.

4. Attended Principal Investigators meeting of Clinical trial entitled "A double- blind, randomized, multicentre, three-arm study to assess the safety and efficacy of Paromomycin administered intra-muscularly at three different dosing regimens", organized by iOWH, San Francisco, USA at New Delhi from 18th – 19th Aug, 2005.

5. Attended Principal Investigators meet on "Safety and efficacy of oral Miltefosine in patients with Post Kala-azar Dermal Leishmaniasis (PKDL) – dose finding study comparing 8 and 12 week treatment" held on 27th Nov., 2005 at Patna.

6. Attended Principal Investigators meeting of Paromomycin (Shorter duration) drug trial, organized by iOWH, , San Francisco, USA and The 54th ASTMH meeting in Washington DC, 10-15 Dec, 2005.

7. Attended Task Force Meeting on Miltefosine Phase IV at ICMR Hqr., New Delhi on 10th Feb. 2006.

8. Attended Second Cycle of RATNEI-IHO, module III on "HIV-AIDS Prophylaxis, Care, Treatment and Support", held at Hotel Chanakya on 6th March 2006, Patna as Guest faculty and delivered a talk on "History of HIV/AIDS, Virology, immunology and Pathogenesis".

9. Attended VII Sir Dorabji Tata Symposium on "HIV/AIDS – Research Issues", held at National Institute of Advanced Studies (NIAS), IIS, Banglore from 10th – 11th March 2006 and presented a paper entitled "HIV - Visceral Leishmaniasis co-infection – A new challenge".

10. Attended Principal Investigators meeting of Paromomycin Phase IV drug trial, organized by iOWH, San Francisco, USA and The 55th ASTMH meeting in Atlanta, 12-15 Nov, 2006.

11. Attended PDT meeting of WHO/TDR on Miltefosine phase II drug trial in PKDL and Combination therapy, 15th Nov'2006 at Atlanta, GA, USA.

12. Attended Third Cycle of RATNEI-IHO, Module III on "HIV-AIDS Prophylaxis, Care, Treatment and Support", held at SCADA Business Centre, Patna on 19th March 2007 as Guest faculty and delivered a talk on "History of HIV/AIDS, Virology, immunology and Pathogenesis".

Mr. Narendra Kumar, Assistant Director

1. Attended Third World Congress on Leishmaniasis, held at Palermo – Terrasini Sicily, Italy from 10th -15th April, 2005 and presented a paper (oral) entitled "Study of Grass-Root level functionaries of Kala-azar in Bihar".

2. Attended workshop on Population, Health and Development for Panchyatraj Leaders, organized by Population Foundation of India, New Delhi and A.N.Sinha Institute of Social Sciences, Patna held on 30th Sept & 1st Oct 2005.

3. Attended meeting on In-Depth review of Kala-azar Programme at NVBDCP, Delhi to discuss the protocol from 3rd-4th Aug, 2006.

4. Attended In-Depth review of Kala-azar Programme meeting, held at NVBDCP, Delhi from 7th -9th Feb., 2007 and presented the IDR findings.

5. Attended WHO/TDR meeting on Intervention strategies for the control of Kala-azar held at Varanasi from 26th March - 3rd April 2007 and presented the findings of the project.

Dr. Neena Verma, Assistant Director

1. Attended training programme on "Drug Sensitivity testing (in vitro) & culture of Leishmania donovani isolates" at London School of Hygiene and Tropical Medicine, London, U.K from 22nd July to 30th September, 2005.

2. Attended XV Annual Conference of Association of Physicians of India, BAPICON'05 (Bihar chapter) on 4th March, 2005 at Motihari.

3. Attended 2nd Variant User Club Meeting on "Thalassaemia & Haemoglobinopathies" organized by BIORAD Laboratories India Pvt. Ltd. and held at Hotel ITC, Sonar Bangla, Kolkata on 12th March, 2005.

4. Participated in a seminar organized by Indian Academy of Clinical Medicine, at Patna from 23rd – 24th Nov. 2005.

5. Attended 61st Annual Conference of the Association of Physicians of India (APICON' 2006) at Millar High School, Patna from 30th Jan. to 2nd Feb'2006.

6. Attended 55th Annual Meeting of American Society of Tropical Medicine and Hygiene, held at Atlanta, Georgia, USA from Nov. 12th – 16th, 2006 and presented a paper entitled "Clinico-pathological changes in dermal lesions of Post Kala-azar Dermal leishmaniasis (PKDL) cases in Bihar, India.

Mr. Anil Kumar Gupta, Assistant Director

1. Participated in "Training for GCP and GLP Principles applicable to clinical laboratories", organized by the iOWH at New Delhi from 16th – 17th August, 2005.

2. Attended Principal Investigators meeting of Clinical trial entitled "A double- blind, randomized, multicentre, three-arm study to assess the safety and efficacy of Paromomycin administered intra-muscularly at three different dosing regimens", organized by iOWH, San Francisco, USA at New Delhi from 18th – 19th Aug, 2005.

3. Attended National Seminar on "Patenting in Biotechnology", organized by DBT, Govt. of India and NRDC, New Delhi at Hyderabad on 26th Oct., 2006.

4. Participated in Training Workshop on "How to Draft Specification and Prosecute Indian Patent Application", organized by National Research Development Corporation, New Delhi on 27th October 2006 at Hyderabad.

Dr. V.N.R.Das, Assistant Director

1. Participated in "Training for GCP and GLP principals applicable to clinical laboratories", organized by the iOWH, held at New Delhi on 16th – 17th August, 2005.

2. Attended Principal Investigators meeting of Clinical trial entitled "A double- blind, randomized, multicentre, three-arm study to assess the safety and efficacy of Paromomycin administered intra-muscularly at three different dosing regimens, held from 18th – 19th Aug, 2005 at New Delhi.

3. Attended WHO-NIV workshop on "Molecular Surveillance Network for Measles in India", held at Pune from 17th – 18th Oct, 2005.

4. Attended Medical Development Congress, held at New Delhi from 23rd – 24th Jan, 2006 and presented a paper entitled "Treatment and Ethical Issues in Kala-azar".

5. Attended Workshop on Bioethics, sponsored by NIH, USA and ICMR, from 11th – 13th Oct, 2006 at Kolkata.

6. Attended In-Depth review of Kala-azar Programme meeting, held at NVBDCP, Delhi from 7th -9th Feb., 2007.

Dr. Krishna Pandey, Assistant Director

1. Attended Principal Investigators meeting of Clinical trial entitled "A double- blind, randomized, multicentre, three-arm study to assess the safety and efficacy of Paromomycin administered intra-muscularly at three different dosing regimens, held from 18th – 19th Aug, 2005 at New Delhi.

2. Participated in seminar organized by Indian Academy of Clinical Medicine, held at Patna from 23rd – 24th Nov, 2005.

3. Attended Medical Development Congress, held at New Delhi from 23rd – 24th Jan, 2006 and presented a paper entitled "Miltefosine: The first ever oral drug for Visceral leishmaniasis".

4. Attended 61st Annual Conference of the Association of Physicians of India (APICON' 2006) at Millar High School, Patna from 30th Jan. to 2nd Feb'2006.

5. Attended Workshop on Bioethics, sponsored by NIH, USA and ICMR, from 11th – 13th Oct, 2006 at Kolkata.

6. Attended National CME on Infectious Diseases, held at Medical College, Kolkata on 11th – 12th Nov, 2006 and delivered a lecture on "Leishmaniasis: The current trends".

Dr. C.S. Lal, Sr. Research Officer

1. Participated in "Training for GCP and GLP Principles applicable to clinical laboratories", organized by the iOWH at New Delhi from 16th – 17th August, 2005.

2. Attended Investigators meeting on clinical trial (Study No.VLPM02), sponsored by Institute of One World Health, USA at New Delhi from 18th to 19th August, 2005.

3. Delivered lecture during UGC Refresher Course for Universities teachers, held in the Department of Zoology, Patna University, Patna on 13th Sept., 2005.

4. Attended 55th Annual meeting of ASTMH, held at Atlanta, USA from 12th – 16th Nov., 2006 and presented a paper entitled "Studies on some nutritional factors in the severity of Visceral leishmaniasis".

5. Participated in the Advanced WHO/TDR Refresher course on Immunology, Vaccinology and Biotechnology applied to Infectious Diseases at Institute of Public Health, Dhaka, Bangladesh from 29th Nov. – 12th Dec., 2006.

Dr. Sanjiva Bimal, Sr. Research Officer

1. Participated in the Advanced WHO/TDR Refresher course on Immunology, Vaccinology and Biotechnology applied to Infectious Diseases at Institute of Public Health, Dhaka, Bangladesh from 29th Nov. – 12th Dec., 2006.

Dr. Vijay Kumar, Research Officer

1. Attended workshop on proposal writing on "Cost-effective integrated vector management as a contribution to the VL Elimination Initiative in the Indian Sub-continent" held at Kolkota from 28th Feb.– 2nd March, 2006.

2. Delivered a lecture on "Vector Bionomics and its relevance to Vector control strategy", organized by NVBDCP at SIHFW, Sheikhpura, Patna on 24th May, 2006.

3. Attended training programme on "Salivary gland dissection", organized by Miss. Meredith Clements, LSHTM, UK at RMRI, Patna from 3rd-7th July, 2006.

4. Attended DBT meeting for the proposal discussion on "Study of pheromones", held at New Delhi on 1st Aug., 2006.

5. Attended meeting on "Control strategy of Kala-azar" with the team of Bill Gate & Millanda Foundation, held at New Delhi on 24th Oct., 2006.

6. Attended WHO workshop on the progress of 1st stage of IVM project and the proposal writing for the 2nd phase, held at Varanasi from 26th - 28th March, 2007.

Dr. Shyam Narayan, Research Officer

1. Attended 2nd Medical Development Congress on "Genomics & Proteomics in health and disease" held at ICMR, New Delhi on 8th - 9th Sept., 2006.

2. Attended International Symposium on "Emerging Trends in Genomics and Proteomic Sciences" held at National Institute for Research in Reproductive Health (ICMR), Mumbai, India on 15th - 18th Oct., 2006.

Dr. Nawin Kumar, Research Officer

1. Attended WHO-NIV workshop on "Molecular Surveillance Network for Measles in India", held at Pune from 17th – 18th Oct, 2005.

Mr. Dharmendra Singh, Research Officer

1. Attended "2nd Medical Development Congress with the theme as Developments in Genomic & proteomics: its implication in health & disease", organized by ICMR at New Delhi from 8th – 9th Sep., 2006.

2. Attended International symposium on "Emerging trends in Genomics and Proteomic Sciences", jointly organized by NIRRH, Mumbai; Indian society for the study of Reproduction & fertility,; and University of Mumbai at Mumbai from 15th – 18th Oct., 2006.

3. Attended International conference on "The majestic river Ganga – Health, Integrity & Management", jointly organized by Patna University and AEHMS, Canada at Patna from 13th – 15th Nov., 2006.

Dr. Srikant Kesari, Research Officer

1. Attended Task Force Project Committee meeting on "GIS and Remote Sensing", held at New Delhi on 8th Nov., 2005.

2. Attended ICMR Task Force meeting on "GIS and Remote Sensing", held at New Delhi on 14th Sept., 2006.

Dr. D.S. Dinesh, Research Officer

1. Attended scientific meting on KALANET project, sponsored by European Union, for Protocol development on "Technology assessment for work package 7", held at Institute of Tropical Medicine, Antwerpe, Belgium from 24th – 30th Jan., 2006.

2. Participated in proposal writing workshop on "Cost-effective integrated vector management as a contribution to the visceral leishmaniasis elimination initiative on the Indian sub-continent", held at Kolkata from 28th Feb. – 2nd March, 2006.

Dr. V.P. Singh, Sr. Technical Officer

1. Attended orientation training course on "Health Statistics", held at FSU, Regional Office for Health & Family Welfare, Govt. of India, Patna on 6th – 11th Nov., 2006.

2. Participated in training programme on "Epi-Info and SPSS software", held at National Institute of Medical Statistics, ICMR, New Delhi from 26th June – 1st July 2006.

Mr. R.B. Verma, Technical Officer

1. Attended Laboratory Animal supervisors' Training Course, held at NCLAS, NIN, Hyderabad from 12th Sept. – 9th Dec., 2005.

Mr. Brijnath Prasad, ALIO

1. Attended System Administrator Level training on network management, held at NICED, Kolkata from 4th – 8th April, 2005.

Mr. N.A. Siddiqui, Statistical Assistant

1. Attended In-Depth review of Kala-azar Programme meeting, held at NVBDCP, Delhi on 29th Jan., 2007 to present IDR findings before the JMM team.

2. Attended In-Depth review of Kala-azar Programme meeting, held at NVBDCP, Delhi from 7th -9th Feb., 2007.

Mr. Shubhankar Kr. Singh, Research Assistant

1. Attended 33rd Indian Immunology Society Conference on "Molecular and clinical immunology in health and disease" held at Department of Biochemistry, AIIMS, New Delhi from 28th-31st Jan., 2007.

Mr. A. Jeya Kumar, Research Assistant

1. Attended the training programme on "Indirect ELISA protocol", imparted by Miss. Meredith Clements (LSHTM) UK, held at BHU, Varanasi from 19th – 22nd July, 2006.

Mr. S.P.Sharma, Assistant

1. Attended Assured Carrier Promotion training, held at ISTM, Dept. of Personnel & Training, New Delhi on 2nd Nov., 2006.

Mr. S.K.Ghosh, UDC

1. Attended Finance Management training, held at Centre for Training & Social Research, New Delhi from 9th – 11th Nov., 2006.

Mrs. Sanju Kumari, Jr. Stenographer

1. Attended Finance Management training, held at Centre for Training & Social Research, New Delhi from 9th – 11th Nov., 2006.

Human Resource Development

1. Training Programme for students of various universities/ institutions

Name of University/ Institute	No. of Students	Subject
Patna University, Patna	35	Biochemical aspect of VL
	87	Immunological aspect of VL
	6	Application of PCR in diagnosis of VL
	17	Host parasite relationship, development of modified culture medium
	3	Diagnosis of PKDL
	1	Vector Biology and Control of VL
	7	Library & Information Science
B.N.Mandal University, Madhepura	7	Immunological aspect of VL
L.N.Mithila University, Darbhanga	1	Development of modified culture medium
B.R.A. Bihar University, Muzaffarpur	1	Immunological techniques
T.M. Bhagalpur University, Bhagalpur	1	Immunological techniques
Viswa Bharati University, Shanti Niketan	3	Development of modified culture medium
	2	Application of PCR in diagnosis of VL
	3	Immunological techniques
Dr. Zakir Hussain Institute, Patna	6	Immunological aspect of VL
Allahabad Deemed University, Allahabad	2	Immunological techniques
Veer Kunwar Singh University, Arrah	23	Immunological techniques
Magadh University, Gaya	8	Immunological aspect of VL
Kolkata University, Kolkata	8	Application of PCR in diagnosis of VL

2. Four students are under Ph.D. programme.

3. Tertiary level training programme for the Medical officers on Vector borne disease.

4. Training on Vector bionomics, disease transmission, DDT spraying operation, role of community participation and IEC to Medical officers and supervisors of 31 endemic districts of Bihar under Kala-azar elimination programme.

5. Post graduate teaching programme in different disciplines of various universities by the scientists as faculty.

Publications

1. Bera T, Lakshman K Ghanteswari D, Pal S, Sudhahar D, Islam Md. N, Bhuyan N and Das Pradeep. **(2005)**. Characterization of the redox components of trnsplasma membrane electron transport system from Leishmania donovani promastigotes. *Biochem and Biophys Acta*. 1725, 314-326.
2. Bimal S, Singh SK, Das VNR, Sinha PK, Gupta AK, Bhattacharya SK and Das P **(2005)**. *Leishmania donovani*: Effect of therapy on expression of CD2 antigen and secretion of macrophage migration inhibition factor by T-cells in patients with visceral Leishmaniasis. *Experimental Parasitology*, 111, 130-132.
3. Bimal Sanjiva, Singh Subhankar, Pandey K, Sinha Prabhat K, Das PK, Schallig H, Das P and Bhattacharya SK. **(2005)**. Comparative evaluation of aqueous and freeze dried antigen for sero-diagnosis of VL and PKDL cases by DAT. *American Journal of Immunology*, 1(2), 74-78.
4. Chakravarty D, Banerjee S, Sen A, Banerjee KK, Das Pradeep &.Roy S. **(2005)**. *L.donovani* affects antigen presentation of macrophage by disrupting lipid rags. *J. Immunol*. 175, 3214-3224.
5. Das VNR, Ranjan A, Singh VP, Siddiqui NA, Sinha PK, Pandey K, Kumar Nawin, Verma N, Bimal S & Bhattacharya SK. **(2005)**. Magnitude of unresponsiveness Sodium stibogluconate for the treatment of Visceral Leishmaniasis in Bihar". *Natl Med J India*, 18(3):131-33.
6. Debnath A, Akbar Md. Ali, Mazumdar A, Kumar S, Das Pradeep. **(2005)**. *Entamoeba histolytica*: Characterisation of human collagen type I and Ca^{2+} activated differentially expressed genes. *Exp. Parasitol*. 110, 214-219.
7. Dinesh DS, Kishore K, Singh VP and Bhattacharya SK. **(2005)**. Morphological variations in *Phlebotomus argentipes* Annandale and Brunetti (Diptera: Psychodidae). *Journal of Communicable Diseases*, 37 (1): 35 – 38.
8. Kumar V, Bimal S, Kesari S, Kumar AJ, Bagchi AK, Akbar MA, Kishore K, Bhattacharya SK and Das P. **(2005)**. Evaluation of dot-immunoblot assay for detecting leishmanial antigen in naturally infected *Phlebotomus argentipes* (Diptera: Psychodidae). *Annals of Tropical Medicine & Parasitology*, 99(4): 371-376.
9. Mandal D, Mazumdar A, Das Pradeep, Kundu M and Basu J. **(2005)**. FAS Caspase 8-and Caspase 3-dependent signaling regulate the activity of the Amino-phospholipid

translocase and phosphatidylserine externalization in human erythrocytes. *J. Biol.Chem*. 2345,

10. Sahu BR, Mohapatra AD, Majumder A, Das Pradeep and Ravindran B. **(2005)**. A flow cytometry based method for studying embryogenesis and immune reactivity to embryogenic stages in filarial parasites. *Filaria J*. 7; 4(1):11.
11. Pandey K, Sinha PK, Das VNR, Sur D, Kumar Nawin, and Bhattacharya SK.(2005) Neurocysticcercosis in a patient with Visceral Leishmanasis co-infected with HIV-A case Report. *Infectious Disease in Clinical Practice*. IDCP, May, Vol. 13(3), 1-2.
12. Pandey K, Sinha PK, Das VNR, Kumar N, Hasan SM, Verma N, Lal CS, Bimal S and Das P. HIV-1 infection, visceral leishmaniasis, Koch's chest and tuberculous meningitis in the same patient – A case report. *Ann Trop Med Parasitol*. 2005 Dec, 99(8); 807-11.
13. Ranjan A, Sur D, Singh VP, Sidiqqui NA, Manna B, Lal CS, Sinha PK, Kishore K and Bhattacharya SK. Risk factors for Indian kala-azar. *Am. J. Trop. Med. Hyg*., 2005, Vol. 73(1), 74-78.
14. Sinha PK, Pandey K and Bhattacharya SK **(2005)**. Diagnosis & management of Leishmania/HIV co-infection. *Indian J Med Res.*, Vol 121, 407-414.
15. Singh, SK, Bimal S, Dinesh DS, Gupta AK, Sinha PK, Bimal R and Das P. **(2005)**. Towards identifying immunogenic targets in visceral leishmaniasis: role of 17 kDa and 63kDa phosphoproteins. *American Journal of Immunology*, 1 (3): 94-98.
16. Banerjee S, Sen A, Das Pradeep & Saha P, **(2006)**. *L. donovani* cyclin (LdCyc1) forms comlex with cell cycle kinase subunit CRK3 and is possibly involved in S-phase related activities *FEMS Microbiol Letters*. 256(1):75-82.
17. Bera T, Nandi N, Sudhahar D, Akbar, MA, Sen A, and Das Pradeep. **(2006)**. Preliminary evidence on existence of transplasma membrane electron transport in Entamoeba histolytica trophozoites: a key mechanism for maintaining optimal redox balance. *J Bioenerg Biomembr.* 38(5-6):299-308.
18. Das Pradeep, Saha S, Roy K, Dhar Mitra, Dutta P, Bhattacharya MK, Sen A, Ganguly S, Bhattacharya SK and Lihua Xiao. **(2006)**. Molecular characterization of Cryptosporidium spp. from children in Kolkata, India *J. Clin. Microbiol*. 44 (11), 4246-49
19. Das VNR, Pandey K, Kumar N, Hassan SM, Bimal S, Lal CS, Siddiqui NA. and Bhattacharya SK. **(2006)**. Visceral leishmaniasis and tuberculosis in patients with HIV co-infection. *The Southeast Asia Journal of Tropical Medicine and Public Health.* 37(1), 18-20.

20. Das VNR, Pandey K, Kumar N, Hassan SM, and Bhattacharya SK. **(2006)**. HIV Infection, Pneumonic Patch with Tuberculosis and Hepatitis- A case Report. *Journal of communicable Diseases*. 37(2), 155-157.
21. Das VNR, Siddiqui NA, Kumar N, Verma N, Verma RB, Dinesh DS, Kar SK and Das P. **(2006)**. A pilot study on status of lymphatic filariasis in rural community of Bihar. *Journal of Communicable Dis*eases, Vol. 38 (2): 169-175.
22. Feng Y, Ortega Y, He G, Das Pradeep, Xu M, Zhang X, Fayer R, Gatei W, Cama V and Xiao L. **(2006)**. Wide geographic distribution of Cryptosporidium bovis and the deer-like genotype in bovines. *Vet Parasitol* Nov 7; 144: 1-9
23. Gatei W, Das Pradeep, Dutta P, Sen A, Cama V, Lal AA and Xiao L. **(2006)**. Multilocus sequence typing and genetic structure of Cryptosporidium hominis from children in Kolkata, India. *Infect. Genet. Evol*. 27; 7:197-205
24. Gatei W, Hart CA, Gilman RH, Bera T, Nandi N, Sudhahar D, Akbar MA, Sen A , Das Pradeep, Cama V and Xiao L. **(2006)**. Development of a Multilocus Sequence Typing Tool for Cryptosporidium hominis. *J Eukaryot Microbiol*. 53(Suppl) 1:S43-8.
25. Kishore, K., Kumar, V., Kesari, S., Dinesh, D. S., Kumar, A. J., Das, P. and Bhattacharya, S. K. **(2006)**. Vector Control in Leishmaniasis. *Indian Journal of Medical Research*, 123(3): 467 – 472.
26. Kishore K, Kumar V, Kesari S, Dinesh DS, Kumar AJ, Das P and Bhattacharya SK. **(2006)**. Correspondence concern on vector control in Kala-azar. *Indian Journal of Medical Research*, 124: 453.
27. Majumdar P,.Chattopadhyay B, Mazumdar A, Das Pradeep & Bhattacharya P. **(2006)**. Induction of apoptosis in cell expressing exogeneous Hippi, a molecular partner of huntingtin-interacting protein Hip 1. *Neurobiol of Dis*. 22(2):242-56.
28. Muniaraj M, Gupta AK, Narayan S, Singh D, Sinha PK, and Das P. **(2006)**. Long-term preservation of *Leishmania donovani* promastigotes on blood agar slants. *Annals of Tropical Medicine & Parasitology*, 100 (2), 173 – 175.
29. Pranati, Bimal Sanjiva, Pandey K, Sinha PK, Gupta AK, Singh Subhankar K, Sundaram Shanthy and Das P. **(2006)**. *Leishmania donovani*: Immunomodulatory role of 63 KDa *Leishmania* antigen in the promotion of IFN-gamma response (VL vs HIV-VL co-infection). *American Journal of Immunology*, 2(2), 52-57.
30. Saha S, Roy S, Sarkar S, Batabyal AK, Pramanik, Das Pradeep **(2006)**. Observations on the epidemiology of bovine cryptosporidiosis in India. *Vet Parasitol*. Nov 5; 141(3-4):330-3.

31. Sinha PK, Bimal S, Singh SK, Pandey K Gangopadhyay DN and Bhattacharya SK. **(2006)**. Pre and post treatment evaluation of immunological features in Indian visceral leishmaniasis patient with HIV co-infection. *Indian J Med Res.*, 123, 197-202.
32. Sinha PK, Ranjan A, Singh VP, Das VNR, Pandey K, Kumar N, Verma N., Lal C.S., Sur, D., Manna, B. and Bhattacharya S.K. (2006). Visceral leishmaniasis (kala-azar) – The Bihar (India) perspective. *Journal of Infection*, 53(1); 60-64.
33. Muniaraj M, Lal CS, Kumar S, Sinha PK and Das P. **(2007)**. Milk of Cow (*Bos tauras*), Buffalo (*Bubalus bubalis*) and Goat (*Capra hircus*); A better alternative for fetal bovine serum in media for the primary isolation, *In vitro* cultivation and maintenance of *Leishmania donovani* promastigotes. *Journal of Clinical Microbiology*, 45(4), 1353-1356.
34. Sundar S, Singh RK, Bimal SK, Gidwani K, Mishra A, Maurya R, Singh SK, Manandhar KD, Boelaert, Rai M. **(2007)**. Comparative evaluation of parasitology and serology tests in the diagnosis of visceral leishmaniasis in India: a phase III diagnostic accuracy study. *Tropical Medicine and International Health* 12(2): 284-289.

Scientific Advisory Committee

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Animal Ethical Committee

Dr. Gopal Prasad Sinha Ex- Head of the Department, Medicine, PMCH, Patna.	Chairman
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Dr. Sanjiva Bimal Sr. Research Officer, Immunology, RMRIMS	Member
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Mr. Rajendrapati Tripathi, Dept. of Geology, Patna University, Patna	Member
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Mr. R.B.Verma T.O., Animal House, RMRIMS	Member Secretary

INSTITUTIONAL COMMITTEES

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Dr. S.Bimal	Member
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Mr. N.Kumar	Chairman
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Mr. S.P.Singh, Consultant Engineer	Member

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Mr. N.A. Siddiqui	Member
Mr. S.P.Sharma	Member
Mr. R.D.Singh	Member
Mr. R.K.Singh	Member

LIST OF STAFF MEMBERS

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DR. PRADEEP DAS
M.Sc., Ph.D.

Division of Clinical Medicine

1.	Dr. P.K.Sinha, M.D.	Deputy Director
2.	Dr. V. N. R. Das, M.B.B.S.	Assistant Director
3.	Dr. K. Pandey, M.D.	Assistant Director
4.	Dr. Nawin Kumar, M.D.,D.C.H.	Research Officer
5.	Smt. Pushpa Raj	Staff Nurse
6.	Smt. Geeta Kumari	Staff Nurse
7.	Smt. Marry Shanti	Staff Nurse
8.	Smt. Raina Sinha	Staff Nurse
9.	Smt. Ajita Kujur	Staff Nurse
10.	Smt. Kalpana Kumari	Staff Nurse
11.	Mr. S.P. Singh	Technical Assistant (Rtd. on 31.01.07)
12.	Mr. N. K. Sinha	Technical Assistant
13.	Mr. S.B. Barman	Technical Assistant
14.	Mr. Umesh Kumar	Laboratory Technician

Division of Vector Biology & Control (Medical Entomology)

1.	Dr. K. Kishore, Ph.D.	Deputy Director (Rtd. on 30.04.05)
2.	Dr. V. Kumar, Ph.D.	Research Officer
3.	Dr. S. Kesari, Ph.D.	Research Officer
4.	Dr. D. S. Dinesh, Ph.D.	Research Officer
5.	Mr. A. Jeyakumar, M.Sc., M.Phil	Research Assistant
6.	Mr. N. K. Sinha	Technician
7.	Mr. M. Prasad	Technician
8.	Mr. A. K. Mandal	Insect Collector
9.	Mr. S. A. Khan	Field Assistant

Division of Microbiology

1.	Mr. A. K. Gupta, M.Sc.	Assistant Director
2.	Mr. M. Muniaraj, M.Sc.	Research Officer (Relieved on 05.04.06)
3.	Dr. Shyam Narayan, Ph.D.	Research Officer
4.	Mr. S. K. Chaturvedi	Technical Assistant
5.	Mr. S. Yadav	Laboratory Assistant (Rtd. on 31.05.06)
6.	Mr. S. K. Sinha	Laboratory Assistant
7.	Mr. Baidyanath Rai	Laboratory Attendant

Division of Pathology

1. Dr. (Mrs.) Neena Verma, M.D., D.C.P.	Assistant Director
2. Dr. Amitabh Kumar, MBBS	Senior Research Fellow
3. Mr. R.N.Sah, M.Sc.	Technical Assistant
4. Mr. Devendra Prasad	Laboratory Attendant

Division of Molecular Biology

1. Mr. Dharmendra Singh, M.Sc.	Research Officer
2. Mrs. Rakhi Kumari	Technical Assistant

Division of Biochemistry

1. Dr. C. S. Lal, Ph.D.	Senior Research Officer
2. Mr. Anil Kumar, M.Sc.	Research Assistant
3. Mr. Sanjay Kumar, M.Sc.	Research Assistant
4. Mr. S. N. Mehta	Technical Assistant
5. Smt. Manjushree Roy	Technical Assistant
6. Mr. Sudarshan Prasad	Laboratory Assistant

Division of Immunology

1. Dr. Sanjeev Bimal, Ph.D.	Senior Research Officer
2. Mr. Shubhankar Kr. Singh, M.Sc.	Research Assistant
3. Mr. Arvind Prasad	Technical Assistant

Division of Social Science

1. Mr. Narendra Kumar, M.A., Dip. In Pop.Std.	Assistant Director

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1. Dr. R.K. Topono, MBBS	Research Officer
2. Mr. Alok Ranjan, M.Sc.(Stat.), MBA, PGDSD	Research Officer
2. Dr. V. P. Singh, Ph.D.	Sr. Technical Officer
3. Dr. N. A. Siddique, Ph.D., PGDCA	Research Assistant
4. Mr. Musai Baitha	Lab. Attendant

Division of Animal House

1. Mr. R. B. Verma, M.Sc., PGDCA	Technical Officer
2. Mr. M. P.Thakur	Technical Assistant
3. Mr. R.B.Sah	Animal Attendant (Rtd. on 31.05.05)
4. Mr. K. Chowdhary	Animal Attendant
5. Smt. Geeta Devi	Animal Attendant
6. Mr. Shankar Paswan	Animal Attendant
7. Mr. Madan Sah	Animal Attendant

Library

1.	Mr. B.K.Chowdhary, M.A., B.Lib.Sc.	ALIO (Rtd. on 30.04.05)
2.	Mr. B.N.Prasad, M.A.(Eco.), B.Lib.Sc.	ALIO
3.	Shri Ragho Saran Singh	Gestetner Operator
4.	Smt. Saroj Devi	Library Attendant

General Administration

1.	Mr. B.K.P.Thakur, M.A., S.A.S.(Def.Ser.)	Admin. Officer (Rtd. on 30.06.06)
2.	Mr. Udai Kumar, M.Com, B.Ed.	Admin. Officer (F&A)
3.	Mr. S.Kumar, B.A.	Section Officer (Rtd. on 31.12.06)
4.	Mr. Naresh Kumar, B.A.	Section Officer
5.	Mr. B.K.Prasad	Section Officer
6.	Mr. M.N.Sinha, B.Sc.(Engg.)	Assistant Engineer
7.	Mr. M.Rahman	Personal Assistant
8.	Mr. M.M.Ansari	Personal Assistant
9.	Mr. Z.Abeddin	Assistant (Rtd. on 31.01.06)
10.	Mr. S. K. Verma	Assistant
11.	Mr. K.M.Prasad	Assistant
12.	Mr. Arjun Kumar	Assistant
13.	Mr. S.P.Sharma	Assistant
14.	Mrs. Anita Kumari	Assistant
15.	Mr. S.N.Rabidas	Jr. Stenographer
16.	Mrs. S.Kumari	Jr. Stenographer
17.	Mr. S.L.Marandi	Hindi Translator
18.	Mr. Ram Babu	UDC
19.	Mr. S.K.Ghosh	UDC
20.	Mr. R.D.Singh	UDC
21.	Mr. Manoj Kumar	LDC
22.	Mr. Alok Kumar	Hindi Typist
23.	Mr. B. Prasad	Daftari (Rtd. on 31.08.05)
24.	Mr. Ram Lakhan Prasad	Daftari (Rtd. on 31.05.06)
25.	Mr. Shyam Prasad	Daftari
26.	Mr. Raja Ram Yadav	Daftari
27.	Mr. Balmiki Ram	Daftari
28.	Mr. Jitan Thakur	Daftari
29.	Shri R.K. Singh	Daftari
30.	Smt. Jhalkuri Devi	Attendant (Rtd. on 31.05.05)
31.	Mr. S. N. Thakur	Attendant (Rtd. on 30.06.06)
32.	Smt. Kunti Devi	Attendant
33.	Shri Rajendra Prasad	Attendant
34.	Shri Ramchandra Prasad	Attendant
35.	Mr. S. N. Ram	Attendant
36.	Shri Yogendra Sharma	Attendant
37.	Mr. I. Haque	Attendant
38.	Mr. S. R. Sharma	Attendant
39.	Mr. Ajit Kumar	Attendant
40.	Shri Ganga Prasad	Cook (Rtd. on 31.05.05)
41.	Smt. Baso Devi	Cook Attendant
42.	Mr. Shankar Kumar	Cook Attendant

43. Mr. Ajay Kumar	Cook-cum-Guest House Attendant
44. Mr. Chandeshwar Prasad	Peon (Rtd. on 31.10.06)
45. Mr. Ram Saran Mahto	Peon
46. Mr. Ashok Kumar Singh	Peon
47. Mr. Ram Parvesh Verma	Peon
48. Mr. Sunil Kumar Hansda	Peon
49. Mr. Ashok Kumar Sah	Peon
50. Mr. Lal Bahadur Kumar Yadav	Peon
51. Smt. Madhuri Kumari	Dai
52. Mr. Rambriksha Mahto	Mali
53. Mr. Mahendra Kumar	Mali
54. Mr. Rajendra Ram I	Sweeper
55. Smt. Girija Devi	Sweepress
56. Smt. Mehar Devi	Sweepress
57. Shri Rajendra Ram II	Sweeper
58. Mr. Dina Ram	Sweeper
59. Shri Arjun Kumar	Sweeper
60. Late Ashok Kumar	Sweeper (Exp. on 12.09.06)
61. Mr. Shankar Ram	Sweeper

Transport Section

1. Mr. Rameshwar Paswan	Driver
2. Mr. A. K. Singh	Driver
3. Mr. S.Toppo	Driver
4. Mr. Nageshwar Ram	Driver
5. Mr. S. N. Sharma	Driver

Workshop Section

1. Mr. Anirudha Prasad	Technical Assistant
2. Mr. N. N. Mishra	Wireman
3. Mr. Gopal Prasad Sharma	Khalashi
4. Mr. Jawahar Prasad	Plumber
5. Mr. Suryadev Mistri	Carpenter
6. Mr. Ajit Kumar	Helper

Security Section

1. Mr. Santosh Kumar	Head Watchman
2. Mr. Anil Kumar Mahto	Watchman
3. Mr. Ranjeet Kumar	Watchman
4. Mr. B. Murmu	Watchman
5. Mr. N.K. Chowdhary	Watchman
6. Mr. V.N. Tiwari	Watchman
7. Mr. U.S. Singh	Watchman
8. Mr. Uday Shankar	Watchman
9. Mr. Parmanand Singh	Watchman

Canteen Staff

1.	Mr. Anil Kumar Prasad	Canteen Manager
2.	Mr. Baleshwar Prasad	Halwai
3.	Mr. Vijay Kumar	Bearer
4.	Mr. Kishun Mahto	Bearer

Photo Gallery

Welcome of Dr. S.K.Bhattacharya at RMRI after joining the post of Addl. Director General, ICMR

Experts at Technical Committee meeting

Hindi Divas Ceremony

Meeting with Civil Surgeon, District Malaria Officer and Medical Officers at Gopalganj

Inauguration of new lift at Institute

Finalization of IDR Survey Tools

Discussion on IDR study during JMM meeting

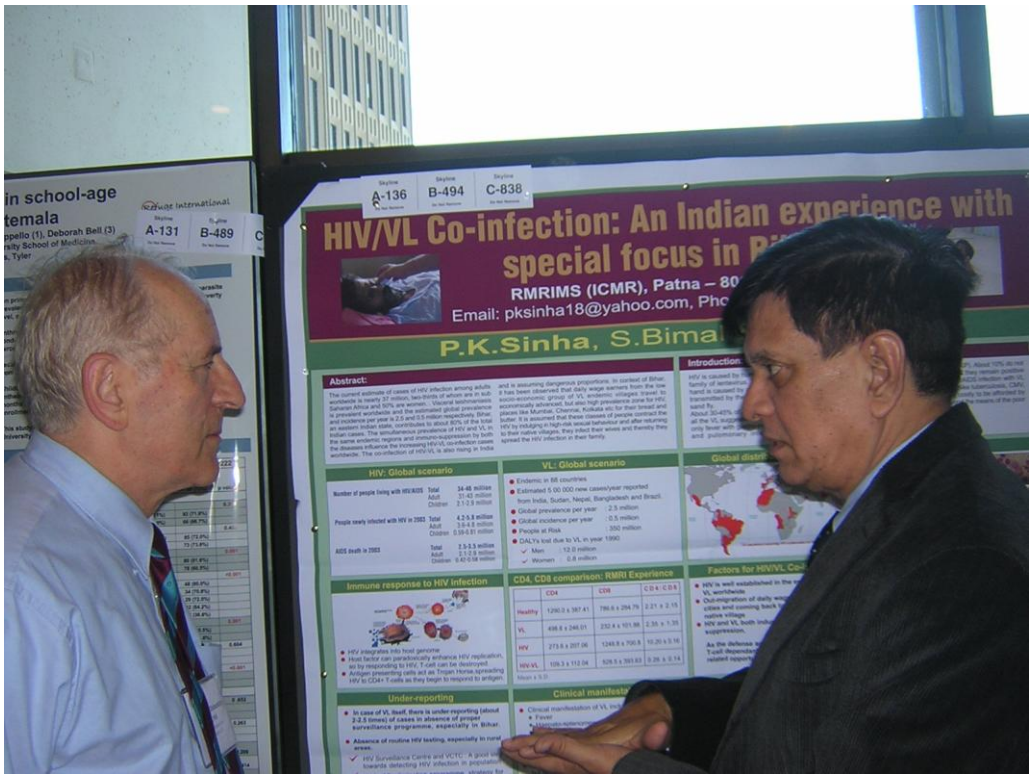
On the spot Fact verification by JMM team in IDR study area

Exhibition on HIV Awareness held at Institute

Paromomycin Phase IV Investigator's Meeting

Principal Investigators Meeting of "Miltefosine in PKDL Study"

Poster Presentation during "55th ASTMH meeting", held at Atlanta



Institutional Ethical Committee Meeting

Scientific Advisory Meeting for the period 2004-2005

Demonstration of Spraying Techniques to the Health Facility Providers by RMRI team

Staff assembly during Institute's foundation day

A view of OPD Registration Counter

A view of Library