

1. Hospital based surveillance for Kala-azar.

Hospital based surveillance of Kala-azar has been carried out since 2001 for establishing a Kala-azar monitoring and research support activity to identify the spectrum of Kala-azar, associated infections, therapeutic response seen at the RMRI hospital in relation to clinical, epidemiological and socio-economic characteristics.

The study revealed that maximum VL patients were in the age-group 5-14 years (38.9%) in both sex. Male cases (66.5%) were higher as compared to female (33.5%). Out of the total VL cases treated, 90.4% VL cases were from the rural areas of nearby endemic districts. Most of the cases (85.6%) hailed from endemic areas within a radius of 100 kms from the centre. 90% VL cases were newly diagnosed. About 74.3% of the cases belonged to the family whose monthly family income ranged from Rs. 1000-5000.

Nearly 56.9% of the patients were illiterate and 57.9% patients were residing in mud and thatched houses with very poor light condition inside the bedroom. More than 51.7% of the VL patients keep domestic animals in their houses, surrounded dense vegetations like banana, bamboo, small creepers and seasonal crops. Nearly 84.9% patients were living in the houses having very poor light condition and 75.4% having presence of vegetations around houses.

Clinical and laboratory characteristics were compared between two groups - VL patients less than 12 years and above 12 years of age-group. Fever $> 100^{\circ}\text{F}$ with chill and rigor was recorded in nearly 49.9% of cases in <12 years group and 37.6% in ≥ 12 years age group. There was splenomegaly (>5 cm) in 69.9% of cases in age-group <12 years and 65.6% in ≥ 12 years age-group and hepatomegaly (>5 cm) in 40.7% of <12 years and 35.3% in ≥ 12 years. Leucopenia was recorded in nearly 73.81% and 52.7% in age group <12 years and ≥ 12 years. Severe

anaemia (Hb<6.5 g/dl) in nearly 36.5% and 25.6% cases in age group <12 years and ≥12 years age group respectively. Platelet count was below normal level in 59.9% of VL cases in age-group ≥12 years as compared to 57.8% in <12 years age. SGPT and SGOT were within normal value in 85.5%, 63.8% and 73.1%, 53.9 % cases in age group <12 years and ≥12 years age group respectively. Na⁺ and K⁺ level was below normal in 40% and 26% cases respectively.

The various drugs used for treatment of VL cases were SAG, Pentamidine, Amphotericin B, Miltefosine, Amphotericin B Lipid complex (Ambisome) and Paromomycin. SAG had a cure rate of 58.5%, Pentamidine 66.7%, Amphotericin B 93.6%, Amphotericin B Lipid complex 100%, Miltefosine 97.5% and Paromomycin 93.4%. Unresponsiveness to SAG has developed, that is why its cure rate has gone down. Besides it can lead to cardio toxicity causing myocarditis. Pentamidine is not readily available and it can lead to anaphylactic shock and diabetes besides being nephrotoxic. Amphotericin B is a very good drug with a high cure rate but it is nephrotoxic and requires electrolyte monitoring most importantly potassium. Amphotericin B Lipid complex is again a very good drug but it is very costly.

Cure Rate according to Treatment Regimen			
Regimen	Treatment completed	Cured	% cured
SAG	118	69	58.5
Pentamidine	9	6	66.7
Amphotericin B	686	642	93.6
Miltefosine	161	157	97.5
Ampho. Lipid complex	2	2	100
Paromomycin	106	99	93.4

2. Study on imprint smear microscopy and PCR application on biopsy from dermal lesions for diagnosis of Post Kala-azar Dermal Leishmaniasis cases in Bihar.

This study was undertaken with an objective to compare the efficacy of PCR application in skin biopsy for diagnosis of PKDL in comparison to the conventional method of imprint smear microscopy.

Biopsy Imprint smears were collected aseptically from 16 Post Kala-azar dermal Leishmaniasis (PKDL) cases. For negative control, 3 biopsies were collected from the individuals without any skin lesions and 3 biopsies from cases with other skin diseases like fungal lesions and leprosy. Out of 16 PKDL cases, 10 had hypopigmented macular lesions and the other 6 cases had papulonodular. Past history of kala-azar was present in all the PKDL cases with duration of 1 – 9 years.

The skin biopsies were examined microscopically for *Leishmania* parasite detection and preserved in 10% buffered formaline solution for PCR study. *Leishmania* parasites, isolated from PKDL cases, were used as positive control in PCR test. In imprint smear microscopy, parasite positivity was found in 9 (56.3%) with presence of mononuclear cells (15-30/OIF): histocytes and lymphocytes in the smear; whereas in negative controls except from few mononuclear cells, no L.d. body was found.

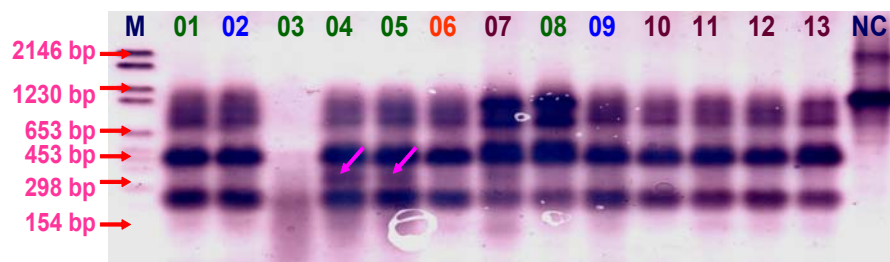
PCR result was found positive (1100 bp) in 10 (62.5%) PKDL cases. These cases were positive for *Leishmania* parasites in their imprint smear also. PCR was negative in the negative control with fungal infection and leprosy. The study is in progress.

3. Molecular characterization of SAG responsive and unresponsive Kala-azar isolates of Bihar.

The basic objective of the study was to see the variation at the molecular level in SAG responsive and unresponsive isolates of *L. donovani* in different parts of Bihar using PCR-RFLP analysis. Different clinical isolates of SAG responsive (n=5) and unresponsive (n=10) VL cases were mass cultured and harvested for DNA isolation.

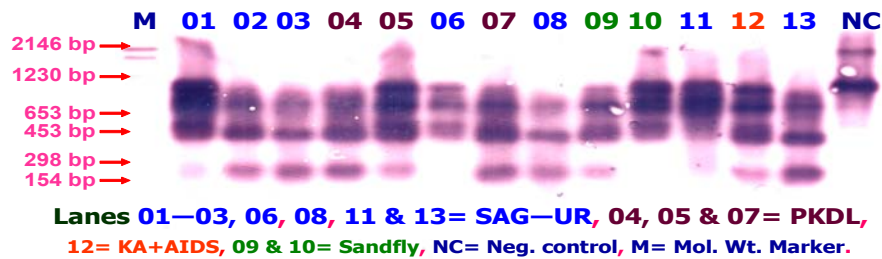
Amplified fragment (ITS region of rRNA gene) was randomly labeled with DIG 11-dUTP and amplicons were digested with various restriction enzymes such as *Taq* I, *Hha* I, *Hha* III, *Rsa* I. After restriction and electrophoresis, restricted fragments were transferred on nylon membrane by Southern blot and hybridized with DIG-labeled probe. Hybridized bands were detected with non-radioactive detection digoxigenin kit. In PCR-RFLP analysis, out of 4 restriction enzymes, *Taq* I demonstrated a band at 300 bp in SAG responsive cases but not in SAG unresponsive and PKDL strains.

Hybridization result of PCR – RFLP (*Taq* I)



Lanes 01, 03–05 & 08= SAG–R, 02 & 09= SAG–UR, 07 & 10–13= PKDL, 06= KA+AIDS, NC= Neg. control, M= Mol. Wt. Marker.

Hybridization result of PCR – RFLP (*Taq I*)



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

This study was undertaken with an objective to develop a PCR based diagnostic tool for kala-azar from blood samples and to compare the PCR results with conventional diagnostic methods. A total of 125 peripheral blood (0.2ml) and 25 Bone marrow aspirate samples were collected from suspected kala-azar patients. Imprinted smear of the aspirates were examined for the presence of amastigotes. The aspirates were cultured at 25⁰ C in the culture medium. The wet smears of cultures were examined microscopically for the presence of promastigote at the 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).

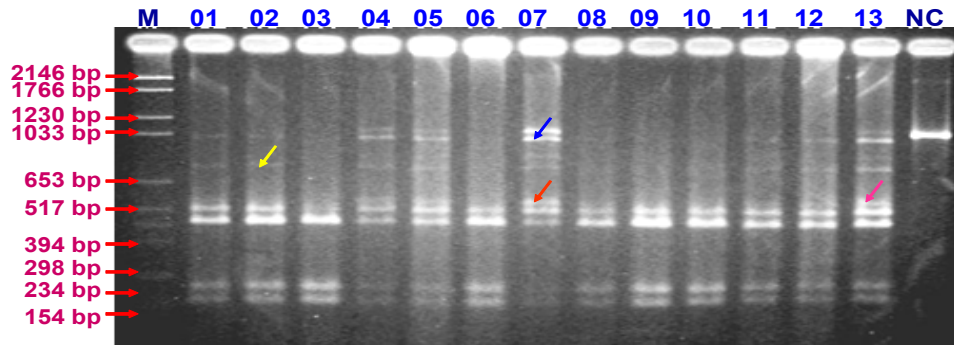
A nested PCR assay was carried out to detect parasite DNA in blood and aspirates from suspected kala-azar patients. The positive samples showed a 600bp band of leishmania specific which was not observed in negative samples. On amplification, the nested PCR primes amplified parasite DNA from a significant number of samples that were negative in

the primary reaction. Comparison of PCR (Blood and aspirate) results with that of conventional diagnostic methods is under progress.

5. Genetic Heterogeneity of ribosomal internal transcribed spacer (ITS) in clinical isolates of *Leishmania donovani* by DNA Polymorphism

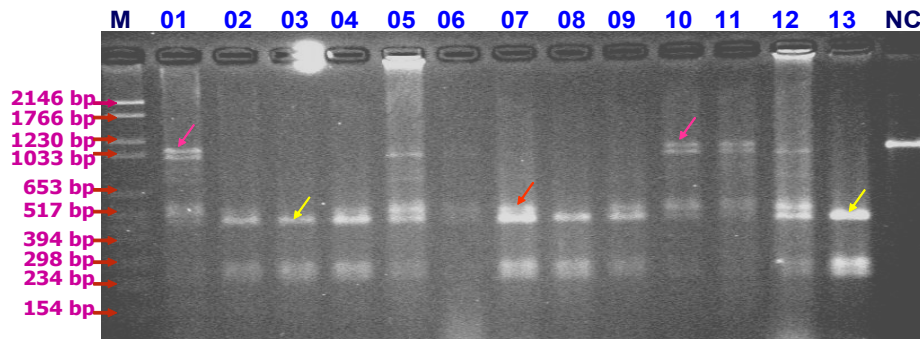
The main objective of the study was to investigate intra-specific variation among different clinical isolates of *Leishmania donovani* from different geographical region of Bihar with different level of endemicity. DNA was extracted from mass cultures of 33 isolates using SDS and Proteinase K with cetyl trimethyl ammonium bromide (CTAB); followed by PCR testing of the same isolates for NTS / ITS region of rRNA sequence. It was observed that PCR amplified bands were similar in size and digested with five restriction enzymes in different clinical isolates. PCR-RFLP patterns were observed with some intra-specific variations in ITS fragment produced by restriction enzyme (*Taq* I) digestion among the different isolates of *L. donovani* from different endemicity and geographical regions of Bihar. The five types of clusters found in the study is an encouraging finding to correlate these different clusters with different degrees of endemicity in various geographical regions of Bihar.

Result of PCR – RFLP (*Taq I*)



Lanes 01–13= Different clinical isolates of *L.donovani*
NC= Neg. control, M= Mol. Wt. Marker.

Result of PCR – RFLP (*Taq I*)



Lanes 01–13= Different clinical isolates of *L.donovani*
NC= Neg. control, M= Mol. Wt. Marker.

6. *In vitro*, role of *Leishmania* isolates of responsive and unresponsive patients in IFN- γ & IL-4 production by similar sets of T-cells.

The objective of this study was to evaluate the protective cytokine (IFN- γ) and the disease promoter cytokine (IL-4) production in two similar sets of T-cells (collected from SAG unresponsive patients and healthy controls) stimulated with SAG responsive and unresponsive strains of *Leishmania donovani*.

The mononuclear cells collected from 34 VL patients were stimulated with SAG responsive / unresponsive *L. donovani* isolates and

stained with anti-human PE- labeled CD₄ monoclonal antibodies. The cells accumulated cytokines i.e. IFN- γ & IL-4 were detected with anti-human FITC- labeled IFN- γ monoclonal antibodies and anti-human APC labeled IL-4 monoclonal antibodies respectively. The images were acquired by Flow cytometer and data were analyzed by BD Cell Quest software.

There was significantly longer duration of illness ($P < 0.01$), larger spleen size ($P < 0.05$) and less hemoglobin level ($P < 0.05$) in non-responder patients than those of responder patients. Significant up regulated IFN- γ production ($P < 0.01$) was observed in T-cells of unresponsive patients when stimulated *in vitro* with isolated responsive parasites of SAG while down regulated IFN- γ production ($P > 0.05$) was found in T-cells of unresponsive patients when stimulated with unresponsive parasites.

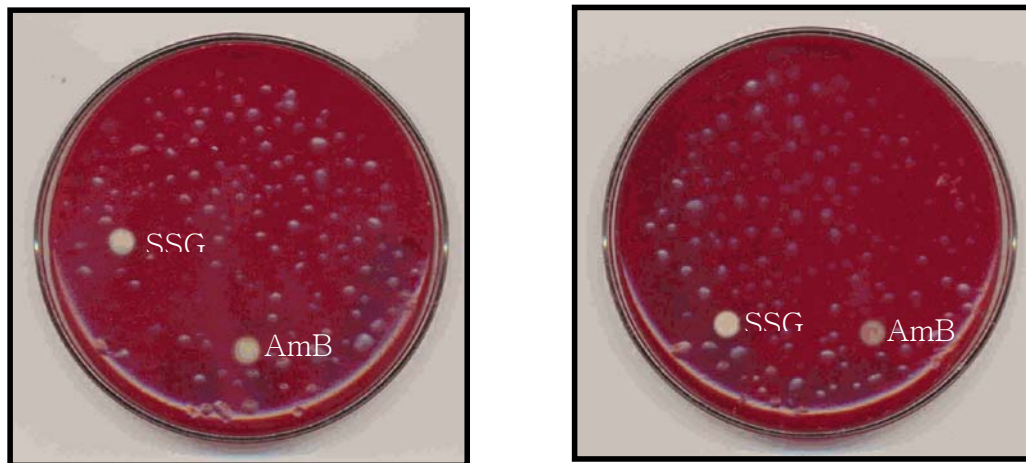
Non-significant down regulation of IL-4 production ($P > 0.05$) in T-cells of unresponsive patients, stimulated by SAG responsive parasites and non-significant up regulation of IL-4 production ($P > 0.05$) by unresponsive parasites was observed. Cytokine (IFN- γ) modulation was found significant ($P < 0.01$) in 3+ to 5+ parasite-graded unresponsive patients. There was no significance of responsive or unresponsive parasites (*in vitro*) in regulation of cytokines production ($P > 0.05$) in T-cells of healthy subjects.

7. Paradoxical drug sensitivity of *Leishmania donovani* : an *in vitro* study by Disc diffusion and Micro well plate methods

This study was undertaken to explore the possible use of disc diffusion method to determine the drug sensitivity of *Leishmania donovani*. Sodium stibogluconate (SSG) resistant (R) and sensitive (S) isolates of *Leishmania* promastigotes were inoculated on solid medium by streaking. The drugs, SSG and Amphotericin-B (AmB), which are commonly used for treatment, were impregnated in sterile paper discs at varying

concentrations and placed on the inoculated medium. In another set, the drugs were serially diluted in a 96 well plate and added with constant number of promastigotes of both isolates. Agar plates (solid medium) and micro well plates were incubated respectively for 8 and 3 days at 25°C.

The promastigotes of SSG R/S showed clear sensitivity pattern in the micro well plate. The mean value of MIC of SSG (R) isolate was >5mg SSG/ml and 0.078 µg AmB /ml and the same for the SSG (S) isolate was 0.15 mg SSG/ml and 0.078 µg AmB/ml. But the results of disc diffusion is highly contradictory, even the highest possible concentration of both drugs that can be achieved in disc, 1 mg SSG/disc and 0.1 mg AmB/disc was not sufficient to stop the growth of *Leishmania* promastigotes of both isolates on the solid medium. Much research is needed at molecular level to explain why the same isolate is giving conflicting result in solid and liquid medium.

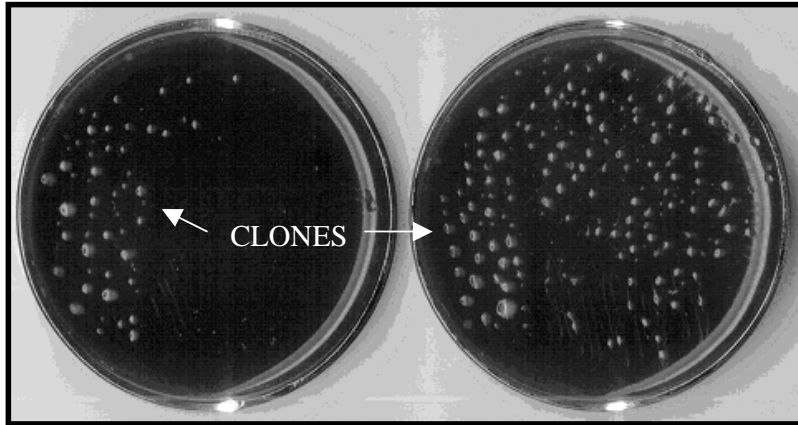


8. Quadrant streaking of *Leishmania* promastigotes culture on blood agar plate: A simple method to get clonal population

The varying clinical expression observed in Leishmaniasis depends not only on the complex interaction of genetic potential and/or immunological status of the host but also on the biodiversity of the parasite itself. The extreme biodiversity of *Leishmania* spp. appears both between species and among strains within a species. Surprisingly, the strains are themselves are not homogenous. The ability of microorganism to grow as colonies on solid medium has been enormously useful in genetic and biochemical studies, because it provides an expedient way of generating and analyzing clonal populations. For the biological, immunological, biochemical or genetic characterization of *Leishmania* parasite, it is preferable to use clonal population of cells rather than mixed one for more accurate analysis.

Despite the availability of various techniques for the isolation of clonal population of parasites, a method that is to be simpler, easy to perform, reliable and less expensive is still required. Quadrant streaking is based on the principle that streaking a small amount of culture on the surface of a semi-solid agar medium by sterile inoculation loop, gradually thin out the sample and separates the different cells spatially from each other. During streaking, a dilution gradient (decreasing concentration of cells) is established across the surface of the plate, so that while confluent growth with overlapping colonies may occur on one area of the plate, isolated, clonal colonies may develop in another region.

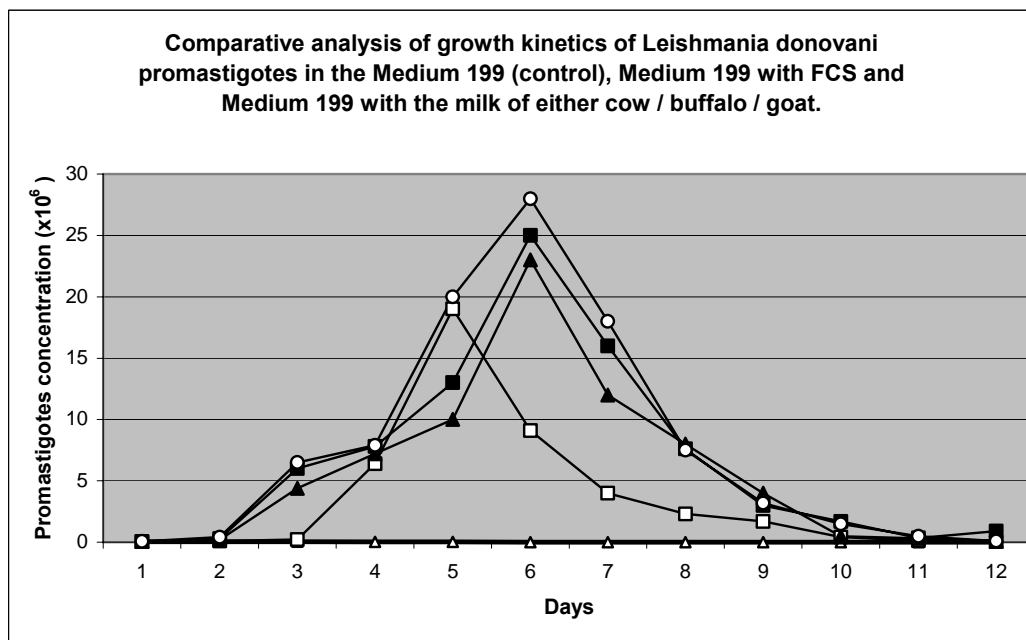
We successfully used this method to get clonal population of *Leishmania* promastigotes. The molecular and biochemical characterization of the clones are underway.

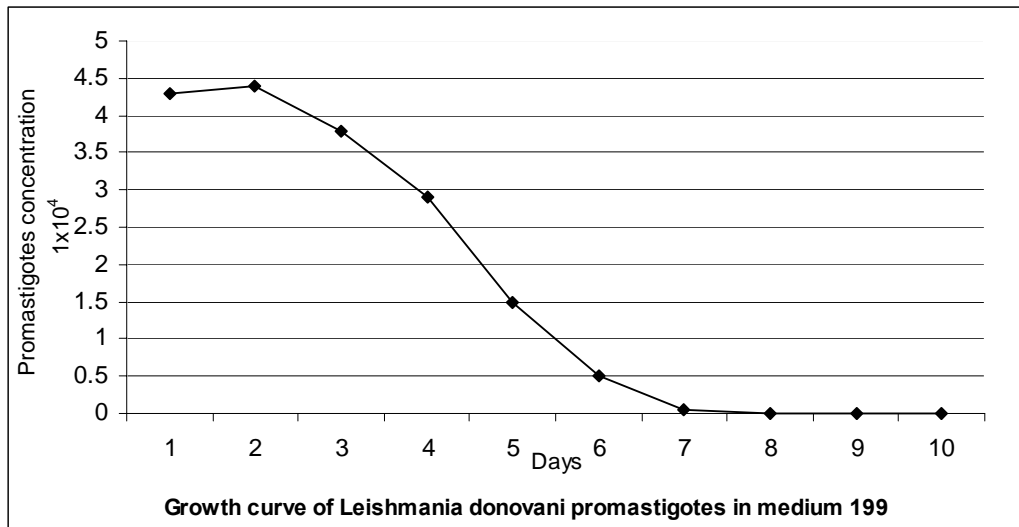


9. Milk of Cow, Buffalo and Goat: A better replacement for fetal calf serum in Media for the *in vitro* cultivation of *Leishmania donovani*

Despite the revolutionary developments in the understanding of *Leishmania*, the incidence of the diseases is still progressing. The practical advances in prevention and control and in diagnosis and proper treatment remain difficult to reach the countries where such advances are mostly needed. To reach the lowest strata of the affected countries, all the methods of diagnosis, culture, treatment etc., to be simplified and converted in to cost effective. The cultivation of *Leishmania* and other hemoflagellates has continued to be a subject of much interest due to necessity of performing biochemical and immunological studies with the isolated parasites in an effort to develop future therapeutic and preventive tools. The media for the cultivation of *Leishmania* require either fetal calf serum (FCS) or blood lysate as one of their essential ingredients (Emin 1997). Fetal calf serum is highly expensive and reliable supply is very difficult to obtain, especially in developing countries. Collection of sterile blood is quite difficult and maintenance of animal house is another expensive burden.

We investigated the use of milk from common cattle of developing countries to replace FCS in the media for the primary isolation, cultivation and long term maintenance of *L. donovani* parasites. This replacement is quite inexpensive and no sophisticated facilities required for its preparation, making it ideal for researchers working in developing countries. We also investigated a few selected biochemical parameters for FCS and milks and analysis of the differences in the protein band pattern of parasites cultured in medium with FCS and with milk supplement are in progress.





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

The objectives of this study were to understand the CD2 deficiency in Kala-Azar and its consequences on CD4 subpopulation of T-cells and to find out the possible means for modulation of this pathway as a mechanism to ensure protective cytokines in patients.

An association between deficiency in CD2 cell surface adhesion molecules with CD4 cell down regulation on surface of T-lymphocytes in VL patients was observed in previous set of experiments. The subsequent experiments also showed up regulation of PKC mediated phosphorylation, when T-cells were stimulated with CD2 monoclonal antibody and this resulted in up-liftment in IFN- γ production. Since CD2 was involved in reinforcing the signal and induced positive activation in T-cells, it was speculated that in vitro stimulation with anti CD2 might influence T-cell proliferation status to Leishmanial antigen.

Cytometric analysis of DNA content of T-cells showed that after CD2 antibody stimulation, VL patients showed significant bias of their T-cells with G2/M content. The contribution of CD2 to influence IFN- γ , IL4

and IL10 responsiveness was also studied in the absence of APC to assess if CD2 can activate T-cells without any support of costimulation provided by APC. A higher frequency of intracellular CD4 cells produced IFN- γ but there was reduction in the ability of CD4 cells to produce IL4 after stimulation. CD2, however, did not revert IL10 pattern, which remained high even after stimulation. Collectively these results suggest that CD2 can serve as an important component of Th1 development but may not direct a particular T-cell subset polarity.

In order to ascertain the Immuno therapeutical value of CD2 antigen, CD2+ T cells from VL patients were sorted on FACS Calibur and were used to stimulate the T cells from the same patients. This again showed that CD2 antigen from Patients also had potent effect, like anti CD2 antibody, in the augmentation of IFN-gamma responsiveness of CD4 cells; as 2.87% CD4 cells had produced IFN gamma compared to 1.26% produced in absence of it. A contrast feature of this investigation was that unlike anti- CD2 antibody, CD2 antigen significantly down regulated IL4 from 11.67% to 9.25%.

Fig. 1

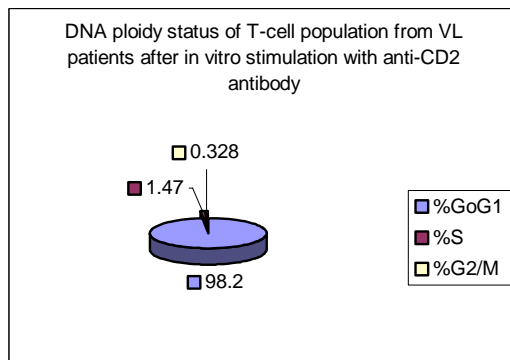


Fig. 2

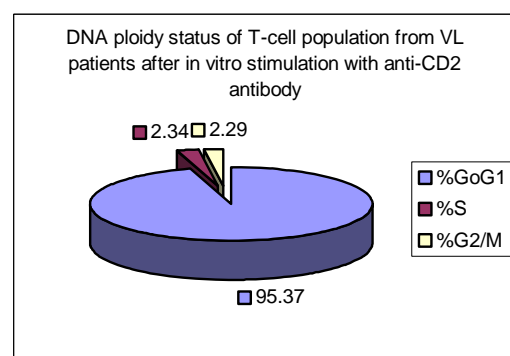
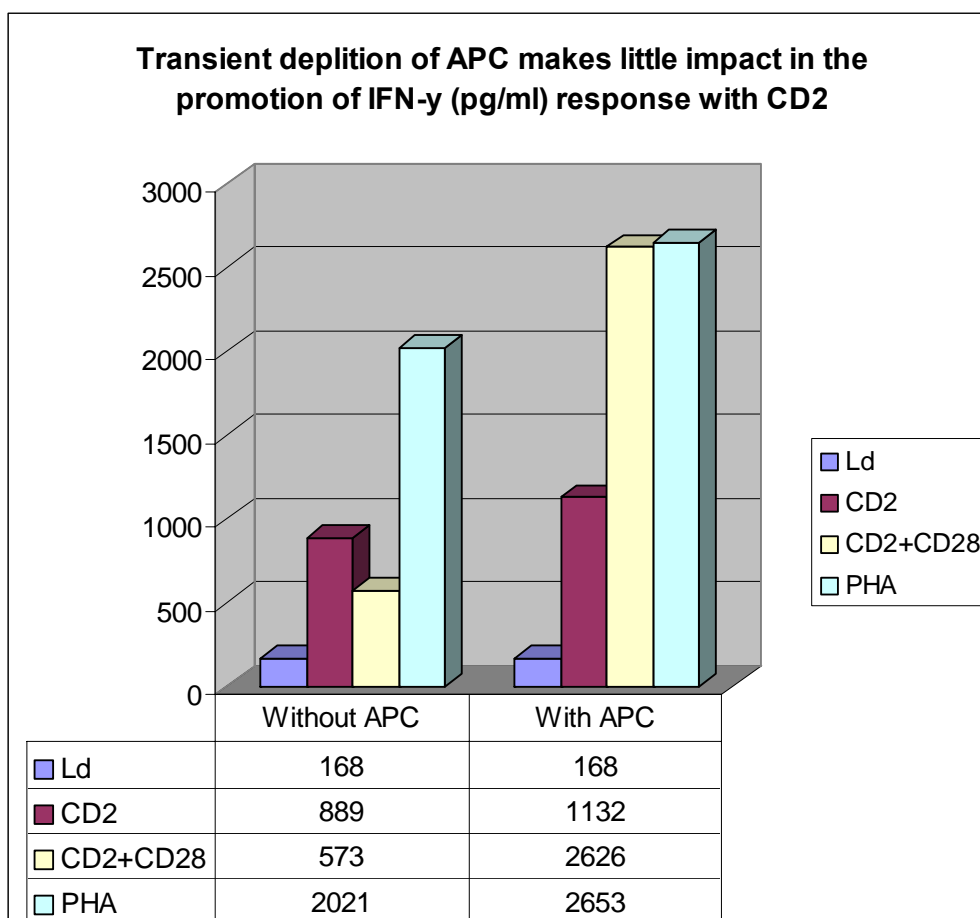


Table: Contribution of CD2 in antigen recognition process during TCR contact on APC: influence on IFN- γ response compared to IL4 and IL10.

Cytokines produced by CD4 ⁺	MNC culture with		
	L. donovani	CD2	PHA
IFN- γ	5.21 \pm 3.39	12.39 \pm 8.28	11.96 \pm 6.97
IL4	13.89 \pm 5.05	9.9 \pm 7.18	15.56 \pm 7.77
IL10	14.59 \pm 10.21	13.41 \pm 9.00	16.30 \pm 10.67



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

This study was carried out with objectives to know the parameters associated with oxygen intermediate metabolism in order to assess the effector mechanism of macrophage in VL patients and to study molecular mechanism of oxygen intermediated pathogenesis that may help administering suitable antioxidant therapy in VL patients.

Twenty kala-azar patients were investigated for non- enzymatic proteinaceous antioxidants and non- enzymatic small molecular antioxidants; both before and after treatment in order to reach the final conclusion. A few small molecular antioxidants like plasma albumin, iron, glucose, uric acid, and bilirubin were investigated both before (B/T) and after successful chemotherapy (A/T). Down regulation of uric acid indicates the possible defect in chelation of metal ions and of albumin observed in B/T group indicates further possible defect in metal binding capacity. Other non-enzymatic and small molecular anti-oxidants like glucose and iron also showed down regulation in B/T group compared to A/T, suggesting pro-oxidant mechanism more active within the body and may contribute to the pathogenesis of the disease. While other non-enzymatic anti-oxidant, Bilirubin showed high value in B/T group compared to A/T. The shift in the balance from pro-oxidant to anti-oxidant was observed in general in A/T.

Table: Small Molecular Anti-Oxidants Parameters in VL

Group	B/T (mean)	A/T (mean)
Albumin (g/dl)	2.27	3.81
Uric acid (mg/dl)	2.02	3.83
Iron (µg/ml)	47.41	87.05
Glucose® (mg/dl)	76.98	96.22
Bilirubin (mg/dl)	0.20	0.22

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

The objective of the study was to determine the parasite antigen in the gut of *P. argentipes* by immuno dot-blot and determine the polypeptides of *L. donovani* promastigotes present in sand fly gut possibly take part in the kala-azar disease infection. After successful completion of work on dot blot to identify natural infection in *P. argentipes*, experiment for SDS-PAGE analysis of proteins of wild caught and lab. bread *P. argentipes* was standardized. Further, 540 female *P. argentipes* in a pool of 25 sand flies were analyzed for antigenic study. Relative molecular weight of sand fly protein fraction was determined. The protein profile obtained from SDS-PAGE showed several major proteins with molecular weight values ranging from 29 to 160 kDa.

The prominent response was obtained with antigens having molecular weight of 63 kDa that showed 100% response in the Kala-azar cases. However, no immunoreactive band was present in the lysates of *P. argentipes* collected from non-endemic areas and laboratory reared uninfected female *P. argentipes*.

13. Evaluation of the impact of DDT and malathion indoor residual spraying being used in kala-azar control programme on the disease prevalence.

Even after the DDT spraying by the state Govt. in north Bihar, the incidence of kala-azar is increasing. Therefore, with the objective to evaluate the efficacy of DDT indoor spraying under kala-azar control programme, this study was undertaken in Bochaha (Muzaffarpur district)

and Patepur (Vaishali district). As per the official record, DDT spraying (@1 gm/m²) was done in Patepur whereas no spraying was conducted in Patepur. But even after one month of spraying, resurgence of sandflies was noticed in Patepur and the pattern of man-hour density of sprayed and unsprayed villages was found almost same.

Bioassay test conducted in sprayed village showed only 30% mortality of sandfly after one month and 20% after 4 month of spray., Further, to study the effectiveness of the sprayed surface, parity rate was studied which was 25% and 33.3% after 3 and 4 months of spray respectively. These findings revealed that even after spraying neither the case nor the vector population came down. Insecticide susceptibility test was carried out four villages of four districts (Table) and the data is suggestive for development of tolerance against DDT in *P.argentipes*.

Table: Bioassay test

Post spray duration	Sandfly tested	Knock down (%) after 1 hr.	Knock down (%) after 24 hrs.	Control mortality (%)
One week	20	45	60	10
One month	20	0	30	10
Four months	20	0	20	20

Table: Insecticide susceptibility test by WHO Tube method for the year 2004

Test species : *P.argentipes* Insecticide : 4% DDT
 Relative Humidity : 75-85% Temp. : 28-30.5°C

District (Village)	Date of experiment	Replicates No.	Exposed (n)	Knocked down in 1 hr.	Dead after 24 hrs.	Mortality %	Corrected mortality % for 24 hrs.
Muzaffarpur (Bochaha)		R-1	10	0	6	60.00	50.00
		R-2	10	2	7	70.00	62.50
		Control	10	0	2	20.00	
Vaishali (Mahua)		R-1	12	2	8	66.67	62.96
		R-2	10	3	7	70.00	66.67
		Control	10	0	1	10.00	
Samastipur (Bande)		R-1	12	0	8	66.67	58.34
		R-2	10	0	7	70.00	62.50
		Control	15	0	3	20.00	
Samastipur (Runisaidpur)		R-1	12	0	10	83.33	79.16
		R-2	10	2	8	80.00	75.00
		Control	10	0	2	20.00	

14. Application of remote sensing and GIS in identifying and mapping sandfly distribution in endemic and non-endemic Kala-azar foci in Bihar.

The objectives of this study were to correlate sandfly (*P.argentipes*) distribution in relation to visceral leishmaniasis and various land use features like vegetation, soil and sub-soil water, human settlements etc. in epidemic and non-endemic foci using Remote sensing and GIS technology and thus to define macro-ecosystem of sandfly and VL as well as to ascertain its role as an “Epidemic predictor”.

The study was conducted in 18 endemic villages in Patepur block of Vaishali district and 12 non-endemic villages of Lohardagga block. The study sites were digitized over the LISS III satellite images, loaded with Bands 3,2,1 (RGB) to produce a FCC. Six models were derived to find out correlation of geographical distribution of vector and disease by comparing the collected land use data and FCC. Out of these, the best

model was derived from the environmental variables and land cover features i.e. temperature, humidity, dry fallow, minimum NDVI may be better used to map vector distribution.

Table: Correlation of vector density with different land cover and environmental variables.

<i>Environmental variables</i>	<i>Endemic sites</i>	<i>Non-endemic sites</i>	<i>Pearson's coefficient of correlation</i>	<i>P-Value</i>
Temp. (°C)	23.83 (7.33)	25.71 (8.51)	0.532	<0.01
Humidity (%)	65.22 (6.11)	56.00 (9.19)	0.567	<0.01
Water body (area)	1.81 (3.95)	2.04 (1.54)	-0.18	0.17
Orchard/ Settlement	16.44 (13.88)	4.11 (3.21)	0.734	<0.01
Crop	43.01 (13.74)	15.91 (10.52)	0.537	<0.01
Dry fallow	9.04 (7.00)	23.56 (16.01)	-0.433	<0.01
Moist fallow	24.00 (12.00)	34.45 (7.87)	-0.657	<0.01
Mean NDVI	0.57 (0.025)	0.107 (0.091)	-0.253	0.051
Max. NDVI	0.41 (0.085)	0.49 (0.088)	-0.210	0.108
Min. NDVI	-0.21 (0.075)	-0.32 (0.13)	0.439	<0.01
S.D. of NDVI	0.084 (0.02)	0.108 (0.063)	-0.265	0.041

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

The project was initiated with the objective to identify the symbiotic bacteria present in the gut of *Phlebotomus argentipes* and to develop shuttle plasmid and transformation system with genetically modified symbionts to make sandfly refractory for the development of *Leishmania* parasite inside the gut.

Sandflies were collected from different Kala-azar endemic villages like Mohanpur of Vaishali district, Wajitpur Majhauri of Muzaffarpur and Gulmehiyabagh of Patna district for identification of microbial fauna

present inside the gut of the established vector *P.argentipes* to find out the symbiotic bacteria. Sandflies were dissected in completely aseptic condition under laminar airflow. Twenty morphologically different colonies were isolated in previous study. One bacterial isolate was shown to have symbiotic relationship with the development of parasite. The replication of the experiment was made with similar procedure to ensure that the colonies are similar or different. The results resembled with the previous and no new colonies were found. Further characterization of bacterial fauna is in progress.

16. Sentinel Sero-surveillance for HIV infection among STD, ANC, FSW & MSM cases in Bihar.

As a part of National AIDS Control Strategy under Annual Sentinel Surveillance Programme, this study was conducted with objectives to see the trend of HIV infection in STD and ANC cases / attendees of hospitals and in FSW & MSM in Bihar over a period of time and place; and to know the sex, age, literacy status, occupation, migration status, type of STDs and locale distribution of seropositive cases.

A total of 5300 sera sample (2800 STD, 2000 ANC, 250 FSW and 250 MSM) of 17 different sites (8 STD, 7 ANC, 1FSW & 1MSM) were tested for HIV antibodies. Overall percentage of positivity of HIV in STD clinic attendees, ANC attendees, FSW & MSM were 2.4%, 0.1%, 4.8% and 1.6%, respectively and for VDRL was 10.1, 7.2, 4.4, & 4.8, respectively.

Among STD clinic attendees –

The two sites, one near the border of Nepal & Bangladesh and another at the border of Nepal have envisaged maximum number of sero-positivity, i.e, 9.2% & 7.6%, respectively. Sero-positivity was more in age

group 30 – 44 yrs (3 %; 5 % male & 1.2% female) followed by 20–29 years (2.4%, 2.8% male and 1.9% female) and in below 20 (1.6%; 2.3 male, 0% female). Total positivity in male & female was 3.4% & 1.4%, respectively. Percentage of positivity was more in urban areas (3.1%, 3.8% male & 2% female) than rural areas (1.9 %, 2.9% male & 1% female) and positivity were also higher in both sexes. Positivity in migrated person (4.4%, 6.3% male & 1.9% female) was more than double of non – migrated (2%, 2.7% male & 1.3% female) indicating its major role. Positivity in illiterate, literate and till 5th, till 12th and graduate & above were 1.8%, 2.4%, 3.2% & 2.2%, respectively, are indicating less awareness/knowledge about AIDS in educated people also. Maximum positivity in unemployed (6.7%; male 6.9% & female 0.0%) followed by truck/ taxi/ auto drivers /cleaners (5.4%), hotel staff (5.3%), industrial / factory workers (4.4%), agricultural / unskilled worker & service class (2%). Maximum sero-positivity was found in male having urethral discharge (5%) & genital warts (4.2%) where as in female having genital ulcers (4.2%) & cervical discharge (1.3%).

Percent positivity for VDRL was 10.1% (9.1% male & 11.4% female), which was little more in rural area (11%) than urban area (9.1%) and was approximately double (16.1%) in persons having history of migration. VDRL was more positive in age group of more than 20 years (10.4%). Insignificant differences in percentage of positivity in different literacy status (13% graduate & above, 12% literate & till 5th, 9.3% illiterate) indicated a little awareness / knowledge about these infection. Maximum positivity for VDRL (22.8%) was found in hotel staff & followed by agriculturer and unskilled worker (12.7%) industrial and factory workers (11%) and truck/auto/taxi drivers/cleaners (8.1%). VDRL positivity was much more in STD cases having symptoms of genital warts (20%) followed by ulcer and discharge (17.7%).

Among ANC attendees –

Maximum HIV sero-positivity (0.4%) was observed in below 20 yrs age group followed by 20–29 yrs (0.1%). Maximum positivity (0.5%) was in Patna, might be due to presence of more medical facilities for which cases are coming more from neighboring districts followed by Begusarai (0.25%). Higher positivity (0.2%) was found in urban area than rural (0.1%). All positive were non-migrated women (0.1%) as migration of women is not much common in Bihar and might be acquired through their husband. Positivity in literate till 5th (0.1%) and also in illiterate (0.1%) indicate less awareness / knowledge about AIDS. Maximum positive was in truck/ taxi/auto drivers/cleaners & industrial factory workers (0.8%) followed by agriculturer / unskilled workers (0.1%).

Percentage of positivity for VDRL was 7.2% which was little more in age groups <20years(8%) and in rural areas (7.6%) than urban areas (6.5%). Maximum VDRL positivity (9.3%) was found in graduate and above, indicating unawareness of disease. Highest positivity (9.2%) for VDRL was found in industrial and factory workers followed by agriculturer/unskilled worker (6.8%) and truck/taxi/auto drivers/ cleaners (6.7%). Difference was not observed in VDRL positivity having history of migration or not i.e., 7.3% and 7.2%.

Among FSW & MSM –

Marginal difference in HIV seropositive was observed in FSW and MSM i.e., 4.4% & 4.8%, respectively. VDRL positivity was three times more in FSW (4.8%) than MSM (1.6%). Tabulated data had been sent to NACO, New Delhi & BSACS, Patna for appropriate planning in control strategy.

This project was running under NACO, New Delhi & BSACS, Patna. At present, this project has been decentralized.

17. HIV screening at Voluntary Counseling and Testing center (VCTC – under Bihar State AIDS Control Society).

The Bihar State AIDS Control Society set up a Voluntary Counseling and Testing Centre (VCTC) at this Institute on 1st April 2001. The objective of this center was to assess the prevalence of HIV infection among the individuals who were at different risk behaviors.

Till May 2005, 6725 individuals (Male 4427, Female 2298) were counseled under VCTC. A total of 6487 individuals (Male 4271, Female 2216) were tested for HIV, out of which 1471 samples were found positive for HIV (22.7%). The samples of positive sera were retested to confirm the positivity. The HIV positivity in males (23.1%) was a little bit higher as compared to females (21.9%). Most interestingly, an increasing trend of HIV positivity was observed since 2001.

Sero-positivity was observed more in individuals hailing from rural areas (71.8%) as compared to that from urban areas (28.2%). Based on the marital status, maximum positivity was observed among married (70%), followed by unmarried (20.1%). The analysis of data regarding referring agent reveals that most of the cases visited VCTC themselves (56.8%) which indicates good awareness amongst the individuals regarding the Government's endeavour for voluntary counseling and testing.

18. Study on intervention programme on Sickle cell anaemia and other Haemoglobinopathies among the general population of Bihar. (In collaboration with Institute of Immunohaematology, Mumbai)

This study was undertaken with objectives to identify the carriers of β -thalassaemia and other abnormal haemoglobin in the women attending

antenatal clinics during their first antenatal checkup. Under this study, 211 samples from female patients, attending antenatal clinics of different Govt. and private hospitals/ centers of Patna, were collected after taking their written consent. The details were filled up in the pre-designed questionnaire. All the 211 samples were subjected to Grouping/ Rh factor test, NESTROF test, complete haemogram, ZPP for iron estimation, Hb electrophoresis, HbF and peripheral blood smear examination for abnormal cells. None of the subjects showed abnormal value for the assessed parameters. All samples, along with the filled questionnaires, were sent to IHH, Mumabi for variant testing and data interpretation.