Disease Specific Documents for XII Plan

Leishmaniasis

INDIAN COUNCIL OF MEDICAL RESEARCH

Annexure XIII

Disease Specific Documents for XII Plan

Leishmaniasis

High Power Committee to Evaluate the Performance of ICMR, 2012-13

Indian Council of Medical Research, New Delhi

Year of Publication 2014

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Published by the Division of Publication & Information on behalf of the Secretary DHR & DG, ICMR, Ministry of Health & Family Welfare, New Delhi

Designed & Printed at M/s Aravali Printers & Publishers (P) Ltd., W-30, Okhla Industrial Area, Phase-II, New Delhi-110020 Phone: 47173300, 26388830-32

Leishmaniasis

ICMR Institutions working on Leishmaniasis

1. Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna
2. National Institute of Pathology (NIOP), New Delhi
3. Regional Medical Research Centre (RMRC), Dibrugarh
4. Vector Control Research Centre (VCRC), Puducherry
5. Division of Epidemiology and communicable Diseases, ICMR, Hqrs

1. Current situation of Disease with contribution of ICMR

Leishmaniasis, a vector borne protozoan parasitic disease, is caused by leishmania parasite and transmitted by female sandflies. It occurs in three forms *viz*. visceral, cutaneous and muco-cutaneous. Visceral leishmaniasis (VL), commonly known as Kala-azar, is major health problem in Indian sub-continent mainly in India, Nepal and Bangladesh. More than 80% of the cases are reported from India alone and Bihar contributes about 90% of Indian VL cases. Other states contributing VL are West Bengal, Jharkhand, eastern Uttar Pradesh and some parts of north-eastern states. About 52 districts in the country are endemic for VL and about 165.4 million people are at risk. Estimates of disability adjusted life years (DALY) are likely to be underestimates of burden, because of weights used fail to account for secondary effects, such as stigma for PKDL, and the economic effect on households of VL, PKDL and their treatment. Based on the comparative reports on underreporting from other regions, VL underreporting magnitude was observed to be severe *i.e.* 4.0-8.0-fold based on data from India.

Cutaneous leishmaniasis (CL) is not widely distributed in India, there are sporadic reports of cutaneous leishmaniasis (CL) in some parts of Himachal Pradesh, Rajasthan and terrain region of Himalayas. The estimated annual cases of CL in India during 2004-2008 was approximately 1000 to 2000 cases

The Tripartite Memorandum of understanding (2004) between Govt. of India, Nepal and Bangladesh focused on elimination of Kala-azar from the South-east Asia Region by 2015. Govt. of India is providing 100% support to achieve the set target *i.e.* reducing the annual incidence of Kala-azar to less than one per 10,000 populations at the sub-district level in India. The major strategies include effective disease surveillance, early diagnosis and treatment, integrated vector management, social mobilization, and clinical and operational research to support elimination programme.

In context of current scenario of elimination programme, the rk39 is available at peripheral level as a rapid diagnostic test, oral miltefosine has been introduced as first line oral drug at PHC level and IRS with DDT is being continued for vector control. KTS at PHC level and VBD consultants at district level have been deployed to supervise and monitor the elimination strategies.

But in spite of available strategies, it seems that a lot of efforts need to be made to achieve the elimination goal, though independent appraisal of the elimination programme is being planned. The possible factors may be attributed to disease surveillance still lacking public-private networking for reporting system, poor reporting of PKDL cases (the known reservoir), less attention on active surveillance, unavailability of vector surveillance in programme; lack of proper supply chain management for diagnostic, treatment and other logistics; lack of strict supervision and monitoring, improper follow-up mechanism of patients under/ after treatment and other administrative issues like improper trained manpower, absence of in-time fund flow and more. Other threats to control of visceral leishmaniasis (VL) are lack of diagnostic facility for PKDL at PHC level, lack of standard treatment guidelines for PKDL, VL-HIV and co-infection with other diseases. More importantly, in endemic areas, many people have asymptomatic infection that need to be explored whether or not as possible reservoir for disease transmission. It is also not clearly known.

The role played by RMRI (ICMR), Patna in elimination programme are a) in-depth review of kala-azar control programme to assess the strength and gaps, b) under reporting of VL cases, c) estimation of disease in one whole district *i.e.* East Champaran of Bihar, d) evaluation of different approaches *viz*. Camp, index, incentive based search for active case surveillance, e) assessment of baseline survey tools for impact assessment using snowball technique, f) evaluation of RDT kits, g) development of new non-invasive diagnostic tools, h) clinical drug trials of miltefsoine and other anti-leishmanial drugs, i) development of monitoring and evaluation toolkit for IRS, j) application of CDC light trap for maximized sandfly collection, k) use of LLIN in sandfly control, l) vaccine development, m) better understanding of disease pathogenesis, n) capacity building through training to KTS, VBD consultants, MOs, DMOs and other health personnel involved in elimination programme and so more.

2. Major Achievements with leads emerged out during XI plan

1. For strengthened and effective surveillance of kala-azar, potentiality of various active surveillance tools viz. Camp approach, index approach, incentive approach, snowball techniques were compared with standard house-to-house search. Camp approach was found with higher VL case yield and it has been taken over by state Govt. on pilot level.
2. Point prevalence of asymptomatic cases in a highly endemic area was found to be 9.8%, out of which 23.1% manifested in full blown VL cases during follow up. It needs to be focussed to study its role in disease transmission.
3. Non-invasive rK39 based non-invasive diagnostic tool for VL using oral secretions was developed.
4. Nested-PCR was found more useful in diagnosis of VL and PKDL, especially in macular type.

5. ICMR funded Phase IV trial of Miltefosine, the first ever oral drug. The drug was introduced as first line drug in program mode and injectable paromomycin was registered by DCGI as anti-VL drug.
6. Combination therapy using AmBisome, Miltefosine and Paromomycin was proved to demonstrate better results than monotherapy and is under consideration for hospital and PHC level implementation through State Government.
7. Use of miltefosine for continuous 12-weeks was found safe and efficacious alternative treatment option for PKDL. In another study, it was also established that the treatment duration may be shortened with increased dose for improved compliance.
8. Monitoring and evaluation (M&E) toolkit was developed and introduced in the programme to evaluate IRS activities.
9. Use of compression pump in IRS was found cost-effective, user-friendly and more effective than the existing stirrup pump. Eventually, phase wise replacement of stirrup pump with compression pump in programme mode is under consideration.
10. Remote sensing and GIS based user-friendly database was developed for prediction of sandflygenic condition that may be used for hot-spot mapping and IRS planning.

3. Publications during XI Plan

RMRIMS, Patna	:	91
NIOP, New Delhi	:	33
VCRC	:	9
RMRC	:	1
ICMR Hqrs (extramural projects)	:	7

4. List of Patents

RMRIMS, Patna

- Plants' extract based culture medium for isolation and maintenance of leishmania parasite: The draft for patency is being prepared by the consultant. (**Under draft)**
- LeishDNAVax, an antigen-based vaccine for visceral leishmaniasis, developed European commission sponsored multi-centric study in which RMRI (ICMR) has also intellectual property (IP) right. **(Applied for patent)**

NIOP, New Delhi

- **US Patent No. 6,855,522** was awarded jointly with US-FDA in 2005 for "Species-specific PCR assay for detection of *Leishmania donovani* in clinical samples of kala-azar and post kala-azar dermal leishmaniasis (PKDL)". This assay can diagnose both KA and PKDL with high sensitivity and specificity and has the benefit of simultaneous species detection.
- Awarded **US patent no. 20060240046** in 2005 and **Indian Patent no. 243725** in 2010 jointly with US-FDA for "Live attenuated Leishmania vaccines". This is for developing centrin knockout parasites and demonstrating their vaccine potential. The vaccine potential has been demonstrated in mice model and currently studies are ongoing in hamster and monkey model.

5. Technologies developed/ transferred to the Industry

RMRIMS, Patna

- Application of rK39 RDT in samples of oral secretions as non-invasive diagnostic tool for VL
- Oral miltefosine as an oral drug for VL treatment (through multi-centric phased clinical drug trials)
- Paramomycin registered by DCGI for treatment of kala-azar in India
- Camp approach as an efficient active surveillance tool for kala-azar
- Application of CDC light trap as a tool for sandfly collection in India
- Techno-ecological approach for vector control using mud plastering with lime
- Malathion as a cost-effective alternative to DDT spray for vector control
- Monitoring & Evaluation (M&E) toolkit for IRS
- Remote sensing and GIS based database for prediction of sandflygenic condition

6. Technologies used by WHO/ Others

RMRIMS, Patna

- Miltefosine: Introduced in VL elimination programme
- Camp approach implemented in programme by Bihar State Govt. on pilot level for active surveillance
- M&E toolkit introduced in programme for IRS activities

NIOP, New Delhi

The molecular biology laboratory at National Institute of pathology has been listed as the referral lab for the speciation of Leishmania parasite in WHO handbooks on Leishmaniasis.

7. Manpower Trained

RMRIMS, Patna

- Training to the State Programme Officers of Bihar, West Bengal, Jharkhand and UP.
- Six round training to 40 District Medical Officers (DMOs) on different aspects of Kala-azar elimination programme with special emphasis on IRS and M&E toolkit
- Three-months extensive training to 23 VBD consultants, deployed at district level, to supervise and monitor activities for various vector borne diseases
- Training to 190 KTS (Kala-azar Training Supervisors), deployed at PHC level, in 8 batches.
- One-day orientation training to different batches of German doctors on clinical and therapeutic aspects of kala-azar.
- Hand-on training to a Medical officer and 2 lab Technicians of IDRB, Bangladesh on diagnostic aspect of VL with emphasis on bone-marrow/splenic aspiration technique.

NIOP, New Delhi

- 21 personnel including 13 research fellows, 8 B.Sc/M.Sc students and two lab assistants were trained on various aspects of leishmaniasis.

8. New Human Resource Generated

RMRIMS, Patna

- 484 Post graduate students of various universities did on-the Job Training/ Project Dissertation course.
- 8 research scholars were awarded Ph.D. and presently, 48 students are pursuing the course
- Mentor Institute of NIPER, Hajipur for M.S. (Pharmacy)

NIOP, New Delhi

7 PhDs were awarded during the XI plan and a few of them are continuing post doctoral studies in India/ abroad and a few of them after completion of post doctoral studies have joined as research scientist in various organizations.

Table 1. Status of Completed Research Studies undertaken during XI Plan					
Sr. No.	**Thematic Area and Title of the Study**	**Objectives**	**Completed with outcome of the study**	**If off-shoot, Refer to XII plan study**	**Institution**
Basic Research					
1	PCR-based diagnostic tool for VL using peripheral blood samples	To develop a PCR based diagnostic test for visceral leishmaniasis (VL).	PCR sensitivity 96.7% (60/62) compared to microscopic demonstration of LD bodies (88.7%, 55/62).	Followed up using same primer sets and conditions for VL diagnostic tool using urine samples. (Nationally relevant)	RMRI, Patna
2	Non-invasive diagnostic tool for VL with rK-39 strip test using oral secretion sample	To establish a non-invasive diagnostic tool for VL	The sensitivity and specificity of rK-39 strip test of VL cases in serum and sputum were 100% (365/365) & 96.6% and 96.1% (351/365) & 100% respectively.	Followed up for field-based assessment and third party analysis and application of tool in PKDL diagnosis. (Nationally relevant)	RMRI, Patna
3	Application of PCR in diagnosis of PKDL as compared to conventional microscopy method	To evaluate application of PCR in diagnosis of PKDL as compared to the conventional microscopy of skin biopsy imprint smear from PKDL lesions.	PCR was found more sensitive (91.7%) than microscopy for diagnosis of PKDL, especially in macular lesions where conventional microscopy has very low sensitivity. Ready for technology transfer to State Govt. and other stakeholders. (Nationally relevant)	-	RMRI, Patna
4	Plant extract based culture media for isolation and propagation of *L. donovani* parasite: a replacement to FBS/blood	To explore plants' extracts as replacement of FCS in routine *Leishmania* culture.	The potential plant extracts identified.	The component has been sent for third party evaluation. (Nationally relevant)	RMRI, Patna
5	Study of nutritional factor in the severity and incidence of VL	To identify any nutritional factor responsible for incidence and severity of disease.	Hypocholesterolemia and increased triglyceride was observed in VL. Cholesterol can provide an understanding of parasite burden in diseased. Decreased zinc and increased magnesium can serve as biomarker in differentiating acute and chronic VL in endemic foci. (National interest)	To study the molecular mechanism of hypocholesterolemia in VL. (Knowledge generating)	RMRI, Patna

6	Study of immunopathology of PKDL: T-cell subsets	To observe distribution of T-cell subsets and the immunopathological changes in PKDL lesions.	PKDL cases were observed with down regulated CD4+ and CD8+ T cell. Lower expression of protective cytokine IFN-γ (> 1 fold) with an elevated IL-4 and IL-10 profile in CD4+ T cells of peripheral blood of PKDL cases indicates immuno-suppression. (Knowledge generating)		RMRI, Patna
7	*Leishmania donovani* antigens and their influence on Natural T Regulatory cells in VL patients	To identify the role of natural regulatory T cells in VL	Six fold increased expression of Natural regulatory cells were found VL subjects which to produce TGF-β and IL-10 to exacerbate the disease in response to *Leishmania donovani*.	Followed up to study the cells associated with immune-suppression. (Knowledge generating)	RMRI, Patna
8	Role of CD2 antigen in modulation of signal transduction for T-cell activation	To study the effect of *Leishmania* on expression of CD2 and its role in signal transduction in VL infection	The study demonstrated conclusively that CD2 boost up PKC-α dependent protective Th1 response and also it is beneficial in enabling SAG to induce leishmanicidal molecules in macrophages. (In patients interest)		RMRI, Patna
9	Role of TGF-β in apoptosis of T- cell population in VL	To study the expression of TGF-β and its role in T- cell apoptosis, if any, in VL	This was the first study that revealed novel involvement of tyrosine phosphatases in TGF-β-induced lymphocyte apoptosis in Leishmania-infected hamsters. (Knowledge generating)		RMRI, Patna
10	Study on Fe-S clusters assembly, Fe-S proteins and thiol metabolism of *L. donovani* parasites	To study the significance of Fe-S proteins and thiol metabolism in parasite survival	Thiol metabolism was found up-regulated in drug resistant isolates of *Leishmania*.	Followed up to study new protein involved in interaction. (Knowledge generating)	RMRI, Patna

11	Identification of anemia as pathogenic factor in visceral leishmaniasis	To find out role of parasites in causing anaemia in VL	Parasites require heme from the host Hb as Leishmania parasite lower down the heme concentration by ~2 fold in erythrocyte suspension.	Followed up to study Hb trafficking up to intracellular parasites and efforts will be made to block the Hb attachment site on parasites to save the patients from severe anaemia due to parasites. (In patient's interest)	RMRI, Patna
12	Haemoglobinopathies with anemia of kala-azar cases from Bihar	To screen kala-azar cases for presence of any disorder of haemoglobin, *i.e.* β-thalassemia, Sickle cell anemia (HbS) *etc.* and to identify the carriers of β-thalassemia and other abnormal Hb. for genetic counseling.	Abnormal Hb was found in few VL cases, but it was not at significant level. (In one VL case, HbA (60.9) was markedly decreased and HBA2 (29.8) elevated with very low MCV (60.1fl) suggesting possibility of haemoglobinopathy In 2 other cases, decrease in HbA (83.3, 84.2), slight elevation of HbA2 (6.6, 5.2) was found. (Scientific breakthrough)		RMRI, Patna
13	Analysis of the *Leishmania donovani* parasite and part played by its antigen on immunological imbalances during VL	To access the advantage of the use of DCs as APC for Leishmania parasite proteins (KMP-11) for development of vaccine candidate.	KMP-11 antigen of *L.donovani* was observed with ability to produce IL-10 and TGF-β in macrophages and IL-12 in dendritic cells in VL patients. Such differences were mainly due to differences in NF-κB production after the presentation of *L.donovani* KMP-11 antigen. This may be important for the outcome of the disease	The KMP-11 will be evaluated for its capacity to help host immuno-prophylactically and ultimately to revert drug resistance. (Nationally relevant)	RMRI, Patna
14	Innate Immunity function in visceral leishmaniasis under malnutrition	To evaluate the status of innate immunity in VL subject and the impact of malnutrition on innate immune function.	Transendothelial migration ability of nutrophils and monocytes were observed highly impaired in patients with malnutrition.	Followed up to understand mechanism of disease pathogenesis. (Knowledge generating)	RMRI, Patna

15	GPI-anchored membrane proteins of *Leishmania donovani* mediated regulation of Toll-like Receptors and costimulatory molecules on antigen presenting cells and induction of cytokines	To study the association of Toll like receptors and GPI-anchored *Leishmania donovani* membrane protein with APC signalling in VL	It was observed that GPIP were able to produce enough amount of IFN-γ in-vitro in healthy controls but not in VL patients. GPIP were found capable to translocate NF-kB indicating its potential role in pro-inflammatory cytokine production. GPIP promotes DC maturation in VL patients in a TLR4 dependent but MyD88 independent manner.	This finding will be further explored to understand mechanism for disease pathogenesis using immunological approach. (Knowledge generating)	RMRI, Patna
16	Study of trypanothione metabolism and associated pathways in *Leishmania donovani:* cloning, biochemical characterization and physiological significance of trypanothione synthetase and trypanothione reductase.	To study the trypanothione metabolism and associated pathways in *Leishmania donovani* to understand physiological significance of trypanothione synthetase and trypanothione reductase.	Transcription of TryS and TryR genes was found upregulated in stationary phase and drug resistant strain. These proteins make stable complex.	Followed up to understand drug resistant mechanism. (Nationally relevant)	RMRI, Patna
17	Biochemical and functional characterization of Iron-Sulphur Cluster (ISC) assembly and cellular localization of LdIscS, LdIscU proteins in *L. donovani*.	To study biochemical and functional aspects of Iron-Sulphur Cluster (ISC) assembly and cellular localization of LdIscS, LdIscU proteins in *L. donovani*.	LdIscS and LdIscU were found to be localised in noncytoplasmic fraction of *L. d.* promastigotes and upregulated in Amphotericin B resistant strains.	Followed up to study drug resistant mechanism. (Nationally relevant)	RMRI, Patna
18	Study of immunopathology of PKDL	Study of key immune modulators/cytokines involved on PKDL pathogenesis.	Key immune modulators including cytokines, chemokines apoptotic molecules and receptors were identified using cDNA array in PKDL patient tissues. Evidence for involvement of Th17 type responses in PKDL pathogenesis was established. (Scientific breakthrough)		NIOP, New Delhi

19	Analysis of host immuno-determinants involved in the pathogenesis of Indian Cutaneous Leishmaniasis	To study host immuno-determinants involved in the pathogenesis of Indian Cutaneous Leishmaniasis exploiting cDNA microarray.	A comprehensive picture of cytokine, chemokines and receptors altered directly in human tissue lesions and implicated the involvement of JAK/STAT pathway in human CL. Treg cells were found preferentially accumulated in CL lesions during the pathogenesis. (Scientific breakthrough)		NIOP, New Delhi
20	Study of mechanism of action of the upcoming antileishmanial drugs, paromomycin and sitamaquine in *L. donovani* field isolates	To study the mechanism of action of the upcoming antileishmanial drugs in *L. donovani* field isolates.	Paromomycin, exhibiting higher efficacy against SAG-resistant parasites and having a distinct mechanism of action, appears to be a promising drug for combination therapy. (Scientific breakthrough)		NIOP, New Delhi
21	Study of parasite surface antigen (PSA-2) in antimony resistance in *L.donovani*	To study the parasite surface antigen (PSA-2) in antimony resistance in *L.donovani*	Role of parasite surface antigen (PSA-2) in antimony resistance isolates was established using genetically manipulated parasite by over-expressing PSA-2 gene in drug sensitive field isolate.	This protein will be studied for its role in parasite virulence, drug resistance and modulation of host macrophage function (Scientific breakthrough)	NIOP, New Delhi
22	Study of comparative transcriptome profiling of miltefosine resistant vs. sensitive *L. donovani*	To study the comparative transcriptome profile of miltefosine resistant vs sensitive *L. donovani*	The comparative transcriptome profiling of miltefosine resistant vs. sensitive *L. donovani* revealed the first comprehensive insight into the underlying mechanism of miltefosine resistance in *L.donovani*. (Scientific breakthrough)		NIOP, New Delhi

23	Role of Quercetin, a naturally occurring flavonoid in the alleviation of anemia in visceral leishmaniasis	To study effect of quercetin on the development of anemia and premature destruction of erythrocytes and assess the antileishmanial property of quercetin in comparison to other conventional antileishmanial drugs	The results suggest the potential of combination treatment of quercetin with anti leishmanial drug in the treatment of both anemia and infection associated with visceral leishmaniasis in future	No	Extramural funding, ICMR Hqrs
24	Determination of amplification of multi-drug resistance gene(s) in *Leishmania donovani*.	Identification of amplified gene related to MDR by using various DNA probes specific to P-glycoprotein (Pgp) related genes in *Leishmania spp*. through restriction enzyme analysis or through construction and screening of cDNA library.	Studies of the mechanistic aspects of resistance aimed at identifying certain key molecular targets for the reversal of the resistant phenotype were undertaken. However, no amplified gene could be detected in the arsenite resistant strain of *L. donovani* promastigotes	No	Extramural funding, ICMR Hqrs
25	Development of transplasma membrane electron transport inhibitor in *Leishmania donovani* as possible antileishmanial agent.	To develop a rapid primary screen that will use the clinically relevant amastigotes	The study has shown that transplasma membrane electron transport is indispensable for the survival of *Leishmania* cells, intracellularly and extracellularly	No	Extramural funding, ICMR Hqrs
26	Identification of *Leishmania donovani* reservoir(s) in various animal species of endemic areas using species specific antibodies	To detect the anti-Leishmania antibodies, using highly sensitive rK-39 antigen-antibody assay, in the blood/ serum of animals that are in contact of VL/PKDL patients and to correlate the density of kala-azar cases with density of animal reservoirs.	Developed recombinant DNA technology in the field which led to development of recombinant antigens such as rK-39 and rKE-16 for rapid and accurate diagnosis of visceral leishmaniasis. Using this antigen, animal reservoir from kala-azar endemic areas of Bihar has been identified.	No	Extramural funding, ICMR Hqrs
27	Studies on the protective efficacy of 78 KDa antigen of *Leishmania donovani* in Balb/c mice	To study the protective efficacy of 78 KDa antigen in pure form and along with different adjuvants in *L. donovani* infected Balb/c mice.	78 kDa plus MPL-A adjuvant was found to provide maximum protection against challenge with *L. donovani* followed by ALD, FCA and then 78 kDa antigen alone.	No	Extramural funding, ICMR Hqrs

28	Combinative immunoprophylactic potential of gp63 and hsp70 against *leishmania donovani* in balb/c mice.	The aim of the current project was to investigate the immune-prophylactic potential of 63 kDa and 70kDa genetic and protein vaccines against murine visceral Leishmaniasis	The T cell epitopes of gp63 and Hsp70 of *L. donovani* were mapped. The primers for the genes encoding these epitopes were designed and the genes were amplified. The gene encoding T cell epitopes of Hsp70 was cloned in pGC blue vector and sequencing was done.	No	Extramural funding, ICMR Hqrs
29	Analysis of host immuno- determinants involved in the pathogenesis of Indian cutaneous leishmaniasis exploiting cDNA microarray"	To capture global picture of cytokine gene expression in localized tissue lesions of Indian CL patients	The study provides a comprehensive picture of the cytokine, chemokines and receptors altered directly in human tissue lesions and implicated the involved of JAK/STAT pathway.	No	Extramural funding, ICMR Hqrs
30	Recombinant DNA vaccine for visceral leishmaniasis: comparison of immunogenicity and efficacy by oral and parental routes in mouse model.	The aim of the present study is to assess the immunogenicity and protective efficacy of DNA vaccine preparations using gp63 gene which encodes for the vaccine candidate Gp63, a major protein of *Leishmania donovani* in mouse model	The results achieved showed that the parenteral GP63 DNA vaccine is immunogenic and efficacious in Balb/C mouse model by both intramuscular and oral routes. More intense immune responses were seen in animals vaccinated by parenteral route, however there was not much difference in the parasite load in spleen or liver. These results suggest a possibility that the vaccine could also be administered through oral route.	No	Extramural funding, ICMR Hqrs
Clinical Research					
1	Safety and efficacy of oral miltefosine in treatment of VL	To evaluate the safety and efficacy of miltefosine for treatment of VL.	Identified as safe and effective drug. Being used in Kala-azar eradication programme.	Followed up for pharmacovigilance of miltefosine at hospital and PHC level. (Nationally relevant)	RMRI, Patna

2	Phase II clinical trial of oral Sitamaquine in VL patients	To evaluate the Sitamaquine for treatment of VL	The drug was found efficacious (initial cure 95% and final cure 85%), but due to some pharmacokinetic abnormalities, the study could not be further extended by sponsor. (Scientific breakthrough)		RMRI, Patna
3	Safety and efficacy of injectable Paromomycin in treatment of VL	To evaluate safety and efficacy of injectable Paromomycin in treatment of VL	Paromomycin was found as a safe and efficacious drug in indoor and outdoor patient setting.	Drug registered by DCGI. Followed up in combination therapy for VL treatment. (Nationally relevant)	RMRI, Patna
4	Dose-finding study of oral miltefosine in treatment of PKDL	To establish doses of oral miltefosine in treatment of PKDL	12-week treatment arm showed good results in comparison to 8-week treatment arm.	Followed up to assess its application as mono-therapy and combination therapy with other anti-VL drugs for treatment of PKDL. (Nationally relevant)	RMRI, Patna
5	Combination therapy using different combinations of miltefosine, Ambisome and paromomycin as a better alternative to mono-therapy for VL treatment	To evaluate combination therapy of miltefosine, ambisome and paromomycin as a better alternative to mono-therapy for VL treatment	Three combinations showed very good result in hospital setting.	Followed up to use this combination at PHC level. (Nationally relevant)	RMRI, Patna
6	Safety and efficacy of a combination of Amphotericin B and Miltefosine compared to Amphotericin B alone in patients with Post Kala-azar Dermal Leishmaniais (PKDL)	To evaluate the efficacy and safety of miltefosine (28 days) and amphotericin B (15 infusions) combination in PKDL treatment	The study revealed 100% initial cure in PKDL but final cure was only 60%.	Studies on new treatment modalities for PKDL (Nationally relevant)	RMRI, Patna
7	Efficacy and safety of Micafungin sodium in patients of Kala-azar (VL) (Clinical study-phase II)	To evaluate the efficacy of Mycamin, an anti-fungal drug, on *Leishmania* Parasites and to determine the efficacy and safety of an optimum Micafungin Sodium dose.	*In-vitro* study on L.d. parasite revealed no significant efficacy. (Scientific breakthrough)		RMRI, Patna

8	Study to assess the safety and efficacy of zinc supplementation in treatment of visceral leishmaniasis (VL) in Bihar	To evaluate the efficacy and safety of zinc supplementation along with the anti-leishmanial drugs in achieving a final cure rate and to evaluate immunological response as well as parasite clearance at different stages through RT-PCR	Zn supplementation was found effective in early clearance of parasite.	Followed up to explore it as treatment modalities. (Nationally relevant)	RMRI, Patna
9	Study of oral miltefosine in treatment of PKDL	To evaluate therapeutic efficacy of oral miltefosine in treatment of PKDL	Oral miltefosine was found effective in treating PKDL patients. It was established that the duration of therapy may be shortened and compliance may be improved by increasing the dose. Lesional parasites were undetectable by real time PCR at 1 month post-treatment. Treatment was safe without any relapse at 1-year follow-up	Studies on new treatment modalities for PKDL (Nationally relevant)	NIOP, New Delhi.
Epidemiological/Operational Research					
1	Assessment of active surveillance tools for kala-azar as compared to house-to-house survey	To compare active surveillance tools for Kala-azar viz. Camp, incentive and index approach with house to house survey.	Camp approach as active surveillance tool was found with higher case yield than others and undertaken by State Govt. as pilot level.	Followed up to assess its application as surveillance tool at mass level. (Nationally relevant)	RMRI, Patna
2	Prevalence of asymptomatic cases in VL endemic population	To evaluate the prevalence of asymptomatic cases in VL endemic population	Out of 9.8% (point prevalence) of asymptomatic cases identified in endemic population, 23.1% converted to VL during follow up.	Followed up to assess magnitude of asymptomatic carriers in larger population and study its role in disease transmission. (Nationally relevant)	RMRI, Patna
3	Quality of life of visceral leishmaniasis (VL) patients in Bihar, India	To assess the quality of life of visceral leishmaniasis (VL) patients	The interview with 83 VL and 83 attendants of the patient using EORTC-QOL-version 3.0 based pre-tested questionnaire revealed significant impact on quality of life measured through global quality of life scale. (Scientific breakthrough)		RMRI, Patna

4	Epidemiological investigation of Kala-zar in Assam	To study the epidemiological factors during focal epidemic in Assam.	Study revealed 80 rK39 positive subjects (70% males, 30% females; 26.3% cases <15 yrs of age). Two cases were confirmed by bone marrow biopsy. Entomological survey revealed the presence of vector sand fly *Phlebotomus argentipes*.	An exploratory study in visceral leishmaniasis endemic area of Assam will be carried out (Nationally relevant)	RMRC, Dibrugarh.
Translational Research/Technology Developed					
1	Validation of sand-fly distribution and Kala-azar disease prevalence through remote sensing and GIS in endemic and non-endemic foci of Kala-azar to re-affirm the earlier outcome and its applicability for entire Kala-azar endemic region.	To correlate the geographical distribution of sand fly (*P. argentipes*), obtained through RS and GIS, with VL prevalence and to evaluate its applicability in the entire kala-azar endemic area as 'endemic predictor'	The significant environmental variables in relation to *P. argentipes* density were determined. Based on these environmental variables (*i.e.*, temperature, relative humidity, dry fallow and minimum NDVI) a statistical model was developed and fitted with the customized GIS software for predicting sand fly density	Remote sensing and GIS based tool was found effective in sandfly abundance mapping. The developed application software is ready to be followed up as technology transfer for disease surveillance. (National interest for vector control)	RMRI, Patna
2	Evaluation of Long-lasting impregnated bed net (LLIN) on experimental basis for vector control.	To assess efficacy of the blanket use of LLINs against protection to sandfly at community level	A random effect linear regression model showed that the cluster-wide distribution of LNs significantly reduced the *P. argentipes* density/ house by 24.9% (95% CI 1.80%–42.5%)	LLIN bed net was found only 24% effective for vector control. The other formulation is being followed up as improved strategies for vector control. (National interest for vector control)	RMRI, Patna
3	Development of "Monitoring and evaluation toolkit" for indoor residual spray (IRS)	To develop a tool for systematic monitoring and evaluation of IRS activities.	The developed M&E toolkit was applied at pilot level in IRS programmes and it was found user-friendly and capable to identify programme short falls	Followed up as improved strategies for vector control. (National interest for vector control)	RMRI, Patna
4	Usefulness of compression pump as compared to stirrup pump for its application in IRS	To compare the user friendliness, efficiency and cost effectiveness of hand compression pump with conventional stirrup pump.	Compression pump was found user friendly due to light weight, easy to operate, less cost and efficient in terms of good discharge rate and better area coverage in comparison to stirrup pump.	Followed up as improved strategies for vector control (National interest for vector control)	RMRI, Patna

Table 2. Important and essential activities which need to be continued in XIIth Plan (ongoing studies)					
Sr. No.	**Thematic area and title of the study**	**Work done in XIth Plan/ Justification for continuation**	**Time frame**	**Deliverable outcome with public impact**	**Institution**
Basic Research					
1	Novel Non-invasive Method for Diagnosis of Visceral Leishmaniasis (VL) and Post Kala-azar Dermal Leishmaniasis (PKDL) by rK39 Test in Sputum (oral fluid) Samples	Field-based verification of the outcome in VL and its application in PKDL initiated. Continued to confirm the lab based outcome of sensitivity and specificity by third party and field based validation with significant number of samples.	2011-2015	Non invasive diagnostic tool for VL and PKDL at peripheral level	RMRI, Patna
2	Development of PCR based diagnosis of Visceral Leishmaniasis (VL) from Urine samples	The study initiated with application of PCR in urine samples. Continued to include significant number of samples and third party analysis followed by field based validation.	2011-2015	Non invasive PCR-based diagnostic tool at PHC level, especially for difficult to diagnose VL cases	RMRI, Patna
3	Development of non-invasive diagnostic assays for PKDL	Established Real time PCR based assay for detection an quantification of parasite load using slit aspirate samples of PKDL. Continued to include significant number of samples.	2011-2013	Development of non-invasive diagnostic tool for PKDL	NIOP, New Delhi
4	Association of HLA class I and II alleles in susceptibility to visceral leishmaniasis in endemic and non-endemic regions of Bihar	Susceptible alleles to VL have been identified with sequencing-based HLA typing. However, it needs to be validated with significant number of samples.	2010-2014	Identification of vulnerable population in endemic area and factors linked with VL	RMRI, Patna
5	Search for anti-leishmanial activity in crude plant's extract	*In-vitro* evaluation of IC50 of ethanolic extract of 4 plants exhibited good antileishmanial effect at lower concentration < 12µgm/ml. More sets of experiments are needed to confirm and further explore its potentiality for drug development.	2011-2014	There are very limited numbers of anti-leishmanial drugs. This will lead to new herbal based treatment option for VL	RMRI, Patna
6	Early detection and evaluation of Microalbuminuria and other Laboratory measurements for assessment of renal function in kala azar and PKDL cases in relation to parasite load	Increased microalbuminuria observed in VL and PKDL patients. Continued to evaluate on large number of samples and correlate with other laboratory parameters including renal function test.	2011-2014	The study will be useful in early detection of renal abnormalities before and during the treatment for timely intervention to arrest progression of renal disease.	RMRI, Patna

7	Analysis of leucocyte population in correlation with chemokines and cytokine expression in Post kala-azar dermal leishmaniasis (PKDL)	Chemokines and cytokine expression in blood and tissue samples of PKDL cases with macular lesions studied. Continued to correlate the observation with different types of PKDL lesions.	2011-2013	This will help in understanding the role of leukocyte trafficking in disease progression with increased inflammatory response from macular to nodular type.	RMRI, Patna
8	Screening cocktail Leishmania antigen (PDIS-70) along with immunomodulator for their role in Immunity and protection in Visceral leishmaniasis, Cloning, expression & functional characterization of oleate desaturase of *Leishmania donovani*	The cloned PDI gene of *L. donovani* has been identified as parasite virulent factor in promoting immuno-suppression. The inhibitor of this protein has been identified to play role in VL patients. Continued to assess potentiality of inhibitor as drug target.	2011-2015	Development of new potential drug for VL.	RMRI, Patna
9	Studies on differential proteomic responses of *Leishmania donovani* on exposure to nitrosative and oxidative stress	Sub-lethal dose with exposure time for oxidative stress, nitrosative stress and oxidative+nitrosative stress were optimized. Sub-lethal exposure for oxidative stress, nitrosative stress showed over-expression of 1653 proteins, 840 had known or predicted function. Continued for validation of functions and study the role of these proteins in stress repair.	2011-2014	The study will help in knowing how parasite overcomes the stress condition and survive inside the macrophage during infection.	RMRI, Patna
10	Purification and biochemical characterization of tryparedoxin and tryparedoxin peroxidase in *L. donovani:* a possible marker for diagnosis of visceral leishmaniasis	Recombinant proteins have been purified and antibodies produced. Continued to study cellular interaction and their localization and to assess diagnostic potential of the identified proteins with more number of human samples.	2011-2014	Identification of new antigens having diagnostic potential for VL.	RMRI, Patna
11	Analysis of Isd11 and frataxin interaction and their roles in Fe-S cluster machinery in *Leishmania donovani*	Proteins were purified and antibodies produced. To study cellular interaction and their localization	2010-2013	This study is of importance to understand drug resistance mechanism.	RMRI, Patna
12	Identification of polymorphic microsatellite loci and finding an evolutionary relationship between different *Leishmania* strains	Data annotation of 3 leishmania species, repeat information of other 2 species and incorporation of primer 3 and ePCR done. Continued to incorporate pair-wise BLAST search, length variation for polymorphic repeat search.	2011-2013	The study will be useful in knowing the origin of strains and their closeness.	RMRI, Patna

13	Whole Transcriptome Analysis of *Leishmania donovani* by Next Generation Sequencing	Samples were collected from fresh and treated VL and PKDL patients and RNA extracted. Continued to prepare libraries for sample run in Next Generation Sequencer. Analysis of short red sequences from SOLiD instrument is under progress.	2012-2017	The study will be useful in knowing the function of metabolic/ enzyme pathway and explore new potential drug target.	RMRI, Patna
14	Comparative molecular modelling of various important proteins of different Leishmania strains and ligand-protein interaction	Structure prediction of GAPDH, HGPRT and gBP21 done. Interaction with anti-leishmanial compound studied. Continued for *in-vitro* and *in-vivo* assay of best hit compounds and study the other potential targets.	2009-2015	The best hit compound will be useful in development of future drug for VL	RMRI, Patna
15	Evaluation of insecticidal effect of plant extract to sandfly in laboratory	Some plants having insecticidal effect from endemic and non-endemic areas have been identified. Continued to find out active molecules having insecticidal and repellent property in the identified plants.	2011-2015	The study will be helpful in making new repellent for sandfly and in-turn will be helpful in reducing the VL transmission.	RMRI, Patna
16	Pathogenesis of *Leishmania donovani vs* vector salivary gland homogenate (SGH), SGH from *Phlebotomus argentipes* for their role on host immune response with special emphasis to test their efficacy as vaccine for kala-azar	Optimum cell activation by SGH, SLA (soluble leishmanial antigen) and SGH+SLA has been studied. Continued for characterization of saliva protein in respect to potential target for vaccine against kala-azar.	2011-2015	The study will be helpful in identifying vaccine adjuvant.	RMRI, Patna
17	Efficacy of indoor synthetic pyrethroids spraying on sandfly population in Bihar	Cone bioassay test to compare residual effect of Deltamethrin and DDT on different types of walls using lab. bred/ wild caught fed female *P. argentipes* and optimization of deltamethrin concentration was done. Continued to validate the lab based outcome in real set up *i.e.* in field condition.	2011-2014	This study will come out as better option of IRS for vector control as well as tolerance assessment of sandfly against DDT.	RMRI, Patna
Epidemiological/Operational Research					
1	Evaluation of Parameters associated with progression of asymptomatic to symptomatic VL cases.	Household survey, population screening and 6-month follow up carried out. Continued to follow up the asymptomatic cases for more one year to know the real number of disease conversion.	2011-2013	The study will be useful to know the disease conversion rate from asymptomatic subjects and the associated factors.	RMRI, Patna

Table 3. New proposed research activities to be undertaken in 12th Plan						
Sr.No.	**Thematic area and Title of the study**	**Off-shoot/De novo/New study**	**Justification**	**Time frame**	**Deliverables**	**Institution/ Centre**
Basic Research						
1	Develop new diagnostic tool for symptomatic and asymptomatic cases of kala-azar.	Off-shoot	Field applicability of newly developed diagnostic tools and their efficacy in evaluation of symptomatic and asymptomatic cases of kala-azar needs to be studied before transfer to programme.	2013-2016	Non-invasive diagnostic tool for VL and asymptomatic carriers (Nationally relevant)	RMRI, Patna
2	Biochemical, immunological, molecular biology approaches in development of diagnostic tool for kala-azar coinfection specially HIV, tuberculosis and their treatment modalities	*de novo*/New study	New diagnostic tools need to be developed using *L. donovani* specific proteins to detect immune-suppressed cases of Kala-azar and PKDL. For that, new specific proteins need to be identified and used for the same. The same protein will be studied for possible drug targets.	2013-2017	Diagnostic tool for VL co-infected cases and their treatment modalities) (Nationally relevant)	RMRI, Patna
3	Development of Loop Mediated Isothermal Amplification (LAMP) PCR as a simplified molecular tool for diagnosis of VL	*de novo*/ New study	LAMP, a molecular diagnostic assay with potential field based application, will be developed and validated to distinguish the VL positive cases.	2013-2014	Establish a field applicable molecular test for detection of *Leishmania* infection (Nationally relevant)	NIOP, New Delhi
4	Studies on basic biology of *Leishmania donovani* using genomic and proteomic approaches to study host-parasite relationship, search new targets and molecules for drug and immunoprophylaxis.	Off-shoot	Many proteins of *Leishmania donovani* have been identified with pathogenic or immunoprophylactic potential. Evaluation of these proteins needs to be continued in respect to new drug targets. Moreover, drug modalities also need to be evaluated for new drug targets.	2013-2017	The study will provide a breakthrough for development of potential drug targets and vaccine candidates (Nationally relevant)	RMRI, Patna
5	Investigation on the mechanism of disease pathogenesis using microbiological, biochemical, immunological, structural and genomic approaches.	Off-shoot	Hypo-cholesterolemia has been found to be associated with VL. Further, pathophysiology of the cholesterol needs to be studied in search of new targets. We also observed that *L. donovani* requires haem from host haemoglobin to survive. Trafficking of haem molecule will be useful to identify new targets.	2013-2017	The study will help in identification of new pathogenic markers associated with VL. (Scientific breakthrough)	RMRI, Patna

6	Study of factors responsible for subcutaneous accumulation of *Leishmania donovani* in PKDL cases	*de novo*/New study	PKDL is a sequel of *L.d.* infection where the parasite migrates from RE system to subcutaneous region. This study will be undertaken to know why the same parasite accumulate in subcutaneous region. (Lead to new scientific break through)	2013-2017	The study will help in understanding the mechanism for visceralization and subcutaneous accumulation of Leishmania parasite (Scientific breakthrough)	RMRI, Patna
7	Study of mechanism of immuno-suppression and identification of new targets to revert immune function in diseased condition	Off-shoot	*Leishmania* involves strategies to up-regulate natural regulatory cells and other regulatory cells to induce immuno-suppression. Study will be designed to evaluate the dynamics of IL-10 producing leukocytes in visceral leishmaniasis and association of pathogenic *Leishmania* antigens. Study will be also carried out on the mechanism of *Leishmania* induced suppression of ROS in macrophages.	2013-2017	Outcome of this study may help in development of vaccine targets. (Scientific breakthrough)	RMRI, Patna
8	Studying underlying mechanism of multidrug resistance in *L. donovani* infection	Off-shoot	Resistance mechanism of some of the anti-VL drugs has been studied. Further study will be needed to carry out on the mechanism involved with emerging resistance with newly implemented drugs. It will help in reformulation of the drugs to overcome the resistance.	2013-2017	The study will help in understanding resistance mechanism and to overcome from this problem. (Scientific breakthrough)	RMRI, Patna
9	Analysis of whole genome of drug resistant and sensitive isolates of *L. donovani* by next generation sequencing.	*de novo*/New study	At present genomic and proteomic approach is based on the sequence of other *Leishmania* species. Availability of whole genome of drug resistant and sensitive isolates of *L. donovani* will help in identifying more accurately new targets for drug and knowing insights of molecular basis of drug resistance.	2013-2017	The study will help in identification of pathogen molecules for drug and vaccine designing. (Scientific breakthrough)	RMRI, Patna
10	Identification of genetic polymorphism and its correlation with host susceptibility in kala azar	*de novo*/New study	The study will be carried out to understand the mechanism involved by pathogen to encounter host defence mechanism.	2013-2017	It will help in identifying molecules involved with host susceptibility in Kala-azar. (Scientific breakthrough)	RMRI, Patna

11	Protein modelling and crystallization of key proteins to identify new ligands for vaccine/drug targeting.	Off-shoot	Various vital proteins, responsible for parasite survival, were modeled and screened with different ligand databases. Binding affinities of some key ligands able to inhibit the key receptor molecules were studied. Crystallization of the ligand protein complex and some in vivo experiments will ensure the suitability of the drug molecule	2013-2017	The study will help in identification of pathogen molecules for drug and vaccine designing. (Scientific breakthrough)	RMRI, Patna
12	Search for newer natural products to be used as anti-leishmanial agents and their mode of action	Off-shoot	Some new herbal plants have been identified. Isolation and purification of the active compounds and their characterization as suitable anti-leishmanial agent will be carried out to find new natural therapeutics.	2013-2017	This study will help in development of herbal drugs for VL (Nationally relevant)	RMRI, Patna
13	Development of new chemotherapeutics, drug modalities and establishment of effective drug delivery system for cure of unresponsive and co-infected cases of kala-azar and PKDL	*de novo*/New study	Unresponsive and co-infected VL cases and PKDL cases are difficult to treat. Study will be carried out to develop new drug modalities and delivery system from newly identified molecular and protein targets.	2013-2017	The study outcome will help in better treatment modalities for VL (Nationally relevant)	RMRI, Patna
14	Development of new live attenuated vaccine candidates for kala-azar	Off shoot	Generation of amastigote specific attenuated parasite lines which have deleted genes over expressed at the intracellular stage of the parasites. Such genes were identified earlier.	2013-2017	The mutants that are confirmed to be safe and protective in the animals will be useful as human vaccine candidates. (Nationally relevant)	NIOP, New Delhi
15	Studies on host parasite interactions using proteomic approaches	*de novo*/New study	Protein-protein interactions will be studied in order to identify definite molecular targets of host-pathogen system during invasion process.	2013-2017	The validated targets can be used for the designing entry-level inhibitors of *Leishmania* infection with potential role as therapeutic agents. (Scientific breakthrough)	NIOP, New Delhi

16	Studies on understanding the mechanism of experimental resistance to miltefosine and paromomycin in Leishmania donovani	Off shoot	Genomic and proteomic analysis in miltefosine and paromomycin resistant parasites will be carried out in order to understand the key molecules involved in development of resistance.	2013-2017	It will help to find out the markers of resistance to tackle resistance in future. (Scientific breakthrough)	NIOP, New Delhi
17	Genome wide analysis of host/parasite factors contributing to development of VL/ PKDL	Off shoot	Differences in both parasitic as well as host factors were noted earlier by using transcriptome approaches. Deep sequencing of VL/PKDL isolates will be performed and compared to determine if parasite factors are leading to development of PKDL. We will attempt to sequence the parasite genome isolated from Indian VL and PKDL patients. Studies of host genome wide SNP analysis will be carried out in samples from VL / PKDL patients.	2013-2017	Study will reveal the parasitic factors for development of VL and PKDL after treatment and relationship between VL and PKDL. (Scientific breakthrough)	NIOP, New Delhi
18	Efficacy of oral drug Miltefosine singly or in combination therapy in treatment of PKDL: Evaluation of immune status and parasite load in response to treatment	*de novo*/New study	Proteins from host and pathogen during invasion process will be identified by mass-spectrometry and validated using antibodies.	2013-2015	The validated targets can be used for the designing entry-level inhibitors of *Leishmania* infection with potential role as therapeutic agents. (Scientific breakthrough)	NIOP, New Delhi
Clinical Research						
1	Development of low cost drug/ combination therapy for treatment of kala azar and PKDL	Off-shoot	Based on earlier studies, new combination therapies and clinical trials of new investigational products will be undertaken for treatment of VL and PKDL.	2013-2017	New drug modalities for treatment of VL and PKDL (Nationally relevant)	RMRI, Patna

Epidemiological/Operational Research						
1	Studies on behaviour change communication (BCC) to make people aware of the diseases and community participation in the elimination programme	*de novo*/New study	Even after effective strategies for surveillance, diagnosis and treatment at peripheral level, community participation in program plays a vital role in its successful implementation and for that BCC is an important component. (Nationally relevant)	2014-2017	It will enhance community participation in National Kala-azar elimination programme (Nationally relevant)	RMRI, Patna
2	Establishment of sentinel sites in highly endemic districts of kala-azar with emphasis on pharmacovigilance	*de novo*/New study	To ensure adherence with national guidelines for early detection and complete treatment at the identified sites and observe its impact on disease incidence as well as pharmacovigilance of miltefosine in out-patient setting. (Nationally relevant)	2012-2017	The study will yield improved adherence to diagnostic and therapeutics strategies as well as monitoring of pharmacovigilance. (Nationally relevant)	RMRI, Patna
3	Vaccine trial for kala-azar	Off-shoot	Under the EU sponsored LeishDNAVax study, vaccine for kala-azar has been prepared. It needs to be evaluated at peripheral level through Phase I, II and III clinical trials. For this, site preparation has already been made.	2013-2017	It will help in finding a new effective vaccine for its application at peripheral level. (Nationally relevant)	RMRI, Patna
4	Improved strategies for vector control for elimination of kala-azar	Off-shoot	It is experienced that the existing biannually IRS of DDT is not being up to the mark and it involves high cost and manpower. So, there is a need of improved/ alternative vector control strategies using remote sensing tool for micro planning, LLIN, deltamethrin *etc* as alternative tool; use of compression pump in DDT spray and enforcement of M&E toolkit for quality evaluation.	2013-2017	The study will yield effective vector control measures for disease control (Nationally relevant)	RMRI, Patna
5	An exploratory study in visceral leishmaniasis endemic area of Assam	Off-shoot	Initial survey in 4 villages (population 4,973) under Kamrup Metro district revealed 8 new cases of VL and 3 PKDL cases have been found. Extensive exploratory study will be conducted in this area to understand the epidemiological parameters.	2012-2014	Disease burden of VL in Assam and associated factors (Nationally relevant)	RMRC, Dibrugarh

6	Studies on entomological and epidemiological aspects of cutaneous leishmaniasis.	Off-shoot	This proposal is based on the recent reports of incidence of cutaneous cases among the Kani tribe in Western Ghats and other regions of India. The goal is to study the epidemiology of cutaneous leishmaniasis with special reference to disease morbidity, vector incrimination and species/ strain variation of parasite in the affected area to combat the disease.	2013-2017	Disease burden of CL in the country and associated factors for outbreak/ disease transmission The epidemiology of cutaneous leishmaniasis in Western Ghats forest to eliminate the foci of transmission. (Nationally relevant)	VCRC, Puducherry in collaboration with other institutes.
7	Ecological studies on sand flies in South India.	*de-novo*/ New project	Information on receptivity will be useful in delimiting areas that require effective vector surveillance and preventive actions to eliminate indigenous transmission. Information base to delimit areas with risk of indigenous transmission of leishmaniasis / chandipura virus encephalitis.	2013-2017	Information will be helpful to delimiting areas required for elimination of indigenous transmission of leishmaniasis. (Nationally relevant)	VCRC, Puducherry
8	Implementing research to help the state Government, health workers, PHC and social worker with new technologies by transferring the modern technologies for surveillance, diagnosis & treatment of disease	Off-shoot	To transfer the research outcome in respective areas at the grass root level through state government under advocacy of NVBDCP.	2013-2017	Implementation of new technologies and strategies in elimination programme. (Nationally relevant)	RMRI, Patna
9	Task Force project on Insecticide resistance monitoring in visceral and cutaneous leishmaniasis vectors' under Vector Science Forum	New study	Resistance monitoring should be a continuous process and data needs to be generated from across the country. Besides monitoring of resistance, factors influencing resistance like operational factors, genetic factors *etc* should also be studied. Change in vector behaviour after use of LLINs and ITNs (wherever used) should also be studied	2013-2017	Data on insecticide resistance will be generated, which will be useful to decide on the national programme policy	RMRI, Patna VCRC, Puducheery DMRC, Jodhpur NCDC, Patna unit

Infrastructure & HRD						
1	Impart training to the scientists working in the field of TDR, especially to kala-azar	Off-shoot	Training to the scientists working on tropical disease will be carried out with help of super specialities working in different fields to build up man power.	2014 to continue	Manpower development (Nationally relevant)	RMRI, Patna
2	Doctors of different speciality will be trained for tropical diseases for the upcoming TDRC (an extension of RMRI) for different tropical diseases	*de novo*/New study	After inception of TDRC, the specialized training will be imparted to the doctors of different specialties.	2014 to continue	Manpower development (Nationally relevant)	RMRI, Patna
3	M.D./Ph.D. programme will be initiated in the field of epidemiology, medicine and pathology; Masters degree in specific subjects like medical biotechnology and medical microbiology	*de novo*/New study	Keeping in background the improving academic activities at the Institute such as Ph.D. programme, project dissertation for masters courses, it is planned to further extend this activity for M.D./ Ph.D. course in the field of epidemiology, medicine and pathology as well as Masters degree programme through university in specific subjects like medical biotechnology and medical microbiology.	2014 to continue	Human resource development. (Nationally relevant)	RMRI, Patna
4	Setting up of nursing training centre	*de novo*/New study	After establishment of TDRC, it is planned to utilize the facilities to train nursing staff through a separate training centre for nurses. (Nationally relevant)	2014 to continue	Human resource development. (Nationally relevant)	RMRI, Patna
5	Start a WHO reference centre for GCLP	*de novo*/New study	Various laboratories of the Institute are well equipped with state of art equipment and technical skill. Various clinical drug trails have been conducted involving the Institute's laboratories maintain GCLP. So, it is planned to mark this Institute as a WHO reference centre for GCLP. (Nationally relevant)	2014 to continue	This will help in assuring GCLP for research in this region. (Nationally relevant)	RMRI, Patna

Table 4. Status of Major Projects Funded by Other than ICMR							
Sr.No.	**Thematic Area and Title of the Study**	**Objectives**	**Completed/Ongoing with outcome of the study**	**Justification for continuation**	**Time Frame**	**Institution**	**Source of Funding**
Basic Research							
1	Quality assurance of Rapid Diagnostic Kits for diagnosis of Kala-azar.	To determine the sensitivity and specificity of RDT used in programme and other commercially available kits for VL	Ongoing; The interim comparative evaluation of KA-detect (Inbios), onsite–leishmania ab (CTK biotech), and IT-LEISH (DiaMed) revealed 100% sensitivity of all RDTs tested except for CTK biotech (94.87%). Specificity of all the RDTs tested was 100%.	The study is continued to include specified significant number of case and control samples and evaluate three other RDTs *i.e.* rk16, signal KA and crystal KA.	2012-15	RMRI, Patna	World Bank
2	Development of a DNA vaccine for Visceral leishmaniasis. (Leish DNA VAX)	To develop vaccine (LeishDNAVax) of prophylactic and therapeutic indications	Completed; ELISPOT assay revealed 31 peptides potential for IFN-γ production and responded by VL treated patients. This multicentric study came out as potential vaccine candidate suitable for phase I trial at the community level and for that site preparation has been completed.		2010-12	RMRI, Patna	European Union

3	Pre-clinical studies of a PSA based human vaccine candidate for visceral leishmaniasis	To evaluate PSA for its efficacy as a vaccine candidate in VL	Completed; PSA protein and its peptides investigated for their potential to elicit protective immune responses in human PBMCs to understand their potential as vaccine candidates.		2009-12	NIOP, New Delhi	European Commission
4	New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent	To develop new tool for screening drug resistance and treatment response	Ongoing; New markers developed for SAG resistance	The developed new markers will be tested in VL patient samples.	2009-13	NIOP, New Delhi	European Commission
Clinical Research							
1	A Prospective, Multi-centric, Randomized, Two Arm, Open-label Phase III study to Assess Efficacy and Safety of Infusion of Amphomul® (Amphotericin B Emulsion) as Compared to AmBisome in Patients of Visceral Leishmaniasis (Kala-azar)	To assess safety and efficacy of single dose of Amphomul (15 mg/kg bw) as compared to single dose of Ambisome (15 mg/kg bw) in the treatment of VL	Completed; Initial and final cure rate in test arm was 94.44%, 85.55% as compared to 100% in control arm. LFT and RFT related AE of CTC grade II and III was observed in 16 patients between Day-7 to Day-45. No SAE was observed.		2011-12	RMRI, Patna	Bharat Serums & Vaccines Ltd.
Epidemiological/Operational							
1	Enhanced VL case detection and improved case Management by the National Kala-azar Programme in Bangladeh, India and Nepal–Phase IV	To enhance VL case detection and improved case management through new established strategies under Phase III	Completed; Camp approach was found cost effective with higher case yield than index approach. Physicians and patients showed satisfaction with miltefsoine as domiciliary treatment.		2011-12	RMRI, Patna	WHO/TDR

2	Estimating the annual incidence of kala-azar in two highly endemic blocks of Bihar: A pilot study comparing Snowball and house-to-house survey	To assess potentiality of snowball tool for estimation of VL incidence	Completed; Though snowball tool was cost effective than household survey, it missed about 50-60% of cases; hence not found sensitive for estimation of VL cases		2011-12	RMRI, Patna	World Bank
3	Estimation of proportion of Kala-azar cases detected at private/Public set-up in a highly endemic district of Bihar, India	To determine the proportion of kala-azar cases treated at private and Govt. set-up in a highly endemic district of Bihar	Completed; Private practitioners were significantly higher at district level than PHC level. In current scenario, about 81% of cases were treated at Govt. set up, but 64% of cases treated at private were not found in HMIS that leads to public-private networking.		2011-12	RMRI, Patna	WHO/SEARO
4	Sentinel surveillance of Visceral leishmaniasis in endemic areas of Bihar	To generate reliable and complete information on kala-azar and evolve suitable strategy for effective surveillance in elimination programme involving public and private sectors	Ongoing; More than 750 VL cases were treated at 8 sentinel sites after RDT with rk39 and are being followed up. Treatment with miltefosine in only 50% of cases revealed unavailability of first line drug that warrants strengthened supply management of logistics in programme.	To include significant number of VL patients and post-treatment follow up to assess final treatment outcome, relapse and/ or conversion into PKDL cases.	2010-13	RMRI, Patna	World Bank
5	Pharmaco-vigilance and therapeutic effectiveness of Miltefosine for the treatment of Kala-azar in endemic areas of Bihar	To systematically document and compile both major and minor side effects among a cohort of patients treated with miltefosine	Ongoing; Out of 97 VL patients treated with miltefosine, 83 completed one-month follow up. Except one patient who developed arthralgia, none of them reported unexpected side effects with miltefosine.	To include significant number of cases to assess unexpected adverse events of miltefosine in real situation.	2010-13	RMRI, Patna	World Bank

6	HIV/Kala-azar co-infection in Bihar – a hospital based study	To estimate the prevalence of HIV/kala-azar co-infection amongst hospitalized VL patients	Completed; Out of 121 identified co-infected cases, 64 were primary VL cases. Relapse of VL encountered in about 49% of cases. The study confirms increasing prevalence of HIV-VL co infections		2011-12	RMRI, Patna	WHO/TDR – SSG
7	Point of care diagnosis of visceral leishmaniasis in India.	To compare sensitivity of the rK-39 RDT using blood vs. Serum, assess impact of ASHA involvement in patient referral, and application of rK-39 in determining risk of infection at the community level.	Ongoing; In view of application at peripheral level, almost equal sensitivity of rK39 was observed using blood and serum samples. Training of ASHA in case detection and referral has been completed.	Continued to assess post-training impact in case registration and assess potentiality of rk39 application in determining risk infection at community level.	2011-14	RMRI, Patna	Grand Challenge Canda
8	Longitudinal studies on sand fly in the three endemic states of Kala-azar of India	To study the bionomics of sandfly and importance of IRS in the control programme in the three endemic states of India	Ongoing; About 8-15% of indoor soil samples collected of Bihar, Jharkhand and W.B. were found positive for immature stages of sand fly. The highest DDT susceptibility level of vector recorded from Saharsa districts (range 80.0-83.0%) and lowest in Muzaffarpur. In Jharkhand and West Bengal, it was in the range of 33.3-46.5% and 40.0-61.5%. respectively.	Continued to study species composition, flight range and better understanding of DDT susceptibility level, seasonality and breeding habitat.	2011-13	RMRI, Patna	World Bank

9	Usefulness and application of the monitoring and evaluation toolkit for indoor residual spraying by the national vector control programme (Phase V)	To scale up the use of the newly developed M&E toolkit for identifying progress and programmatic short falls of IRS and to contribute to capacity building for effective IRS in VL elimination programme.	Completed; About 57.14% of respondents from community showed their satisfaction with IRS. Bioassay test revealed average corrected mortality after 2 weeks, 4 weeks and 5 months in the range of 44.4-55%, 36.6-40% and 22.5-26.6% respectively. Two round workshop of total 77 DMOs, VBDCs involved in IRS were conducted for capacity building.		2011-12	RMRI, Patna	WHO/TDR
10	Replacement of insecticides to control visceral leishmaniasis: Multi-centric/ Multi-countries Study.	To identify sustainable, cost effective, environmentally friendly alternative for vector control acceptable at community level.	Ongoing; Three types of intervention viz. IWFPL (Indoor Walls and floor plastering with Lime), IDWL (Indoor Durable Wall Linning) and ITN was implemented in 24 clusters of the study area. The first follow-up assessment after one-month of intervention done.	Continued to include more clusters and post-intervention follow up for comparative impact assessment as well as assess the community acceptance level.	2012-13	RMRI, Patna	UBS/ WHO/TDR