SPEAK India

Setting the Post-Elimination Agenda for Kala-azar in India

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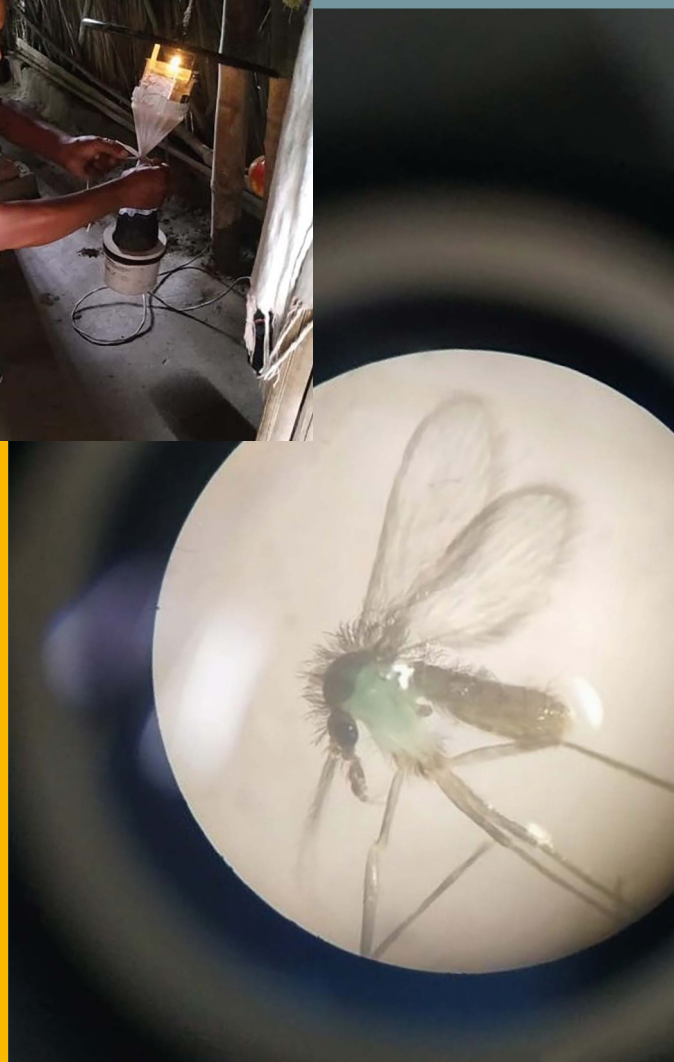
SPEAK India, funded by the Bill & Melinda Gates Foundation, is a partnership between the Indian Council of Medical Research, the National Vector Borne Disease Control Programme, and the London School of Hygiene and Tropical Medicine. It aims to bring together researchers and technical experts alike to develop a consensus on the additional knowledge required to ensure visceral leishmaniasis transmission in India is successfully interrupted and elimination is sustained.



Presentation booklet for WorldLeish7
1st – 6th August 2022
Cartagena, Colombia

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

WORLD LEISH7

NAMES AND AFFILIATIONS OF CONTRIBUTORS TO PRESENTATIONS

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Swaminathan	Subramanian	ICMR-Vector Control Research Centre (ICMR-VCRC), Puducherry, India
Shyam	Sundar	Banaras Hindu University (Varanasi, India)
Rajendran	Uma Maheswari	ICMR-Vector Control Research Centre (ICMR-VCRC), Puducherry, India

Introduction

The Setting the Post-Elimination Agenda for Kala Azar in India (SPEAK India) consortium provides a shared, accessible platform for Visceral Leishmaniasis researchers, implementers and stakeholders working in the Indian subcontinent to exchange experiences, synchronise activities, and identify synergies in their work to ensure optimal use of resources. In doing so, SPEAK India aims to identify and define the key questions, knowledge gaps and data needs that should be addressed for the VL elimination goal to be achieved, and sustained into the future.

Funded by the Bill & Melinda Gates Foundation and facilitated by the London School of Hygiene and Tropical Medicine, SPEAK India was led by the Indian Council of Medical Research (ICMR), and has strong support from India's National Center for Vector Borne Disease Control (formerly the National Vector Borne Disease Control Programme, NVBDCP), Rajendra Memorial Research Institute for Medical Sciences (RMRIMS), Vector Control Research Centre (VCRC), Banaras Hindu University, and the Institute of Public Health Bengaluru (IPH).

Since its establishment in 2016, SPEAK India has worked to identify stakeholders involved in VL elimination activities in the Indian subcontinent, and bring them together to design a research agenda for VL. Three cross-cutting themes were identified to guide the development and implementation of research:

- Surveillance
- Transmission (Molecular Xenomonitoring)
- Mathematical Modelling

Operational research projects addressing each of themes were launched in 2018, under funding from the Bill and Melinda Gates Foundation and some of the key findings are presented herein.

We are delighted that many of our investigators and consortium members are attending WorldLeish7 in Cartagena, and would like to invite you to visit our presentations listed at the end of this booklet. We would be very happy to discuss our work further with you during the meeting. The SPEAK India community wish you all a very productive and enjoyable conference.

Participants at the Launch of SPEAK India in 2016.

Surveillance Project

Project staff taking a finger prick blood sample from a consenting volunteer.

PARTNERS AND CONTRIBUTORS

This project was a collaboration between the implementing partners Banaras Hindu University (Prof. Dr. Shyam Sundar, Dr. OP Singh, Dr. Paritosh Malaviya and Dr. Sangeeta Kansal) and the Institute of Tropical Medicine (Prof. Dr. Epco Hasker, Kristien Cloots and Rian Snijders).

THE PROJECT

The aim was to explore and validate innovative approaches to prepare the surveillance system for VL in India to the specific challenges of a post-elimination context. The objectives of this project were two-fold; 1) to assess new tools to improve the current case-based surveillance system, and 2) to explore alternative approaches to monitor transmission that might be more sensitive to detect resurgence early on than case-based surveillance only.

WP1 – Improving VL surveillance at the Primary Health care Center level (This study has been published – https://doi.org/10.3390/diagnostics12030670).

Rationale. The progressive decrease in VL cases since the start of the Elimination Programme has brought new challenges. First, the accuracy of the clinical diagnostic algorithm will decrease if the prevalence of the disease is very low, potentially leading to increased proportions of false positives among the reported VL cases. Secondly, with VL cases decreasing, the relative importance of other infectious subgroups is increasing. Post-kala azar dermal leishmaniasis (PKDL) patients remain infectious while symptomatic, and can act as inter-epidemic reservoirs. Since the majority of PKDL patients occur among individuals with a history of VL, former VL patients should be followed up actively to detect PKDL timely. Similarly, VL patients co-infected with HIV can act as super-spreaders, with generally high parasite loads in their blood, while a weakened immune system complicates the clinical picture as well as the diagnostic accuracy. Nonetheless, this group is thought

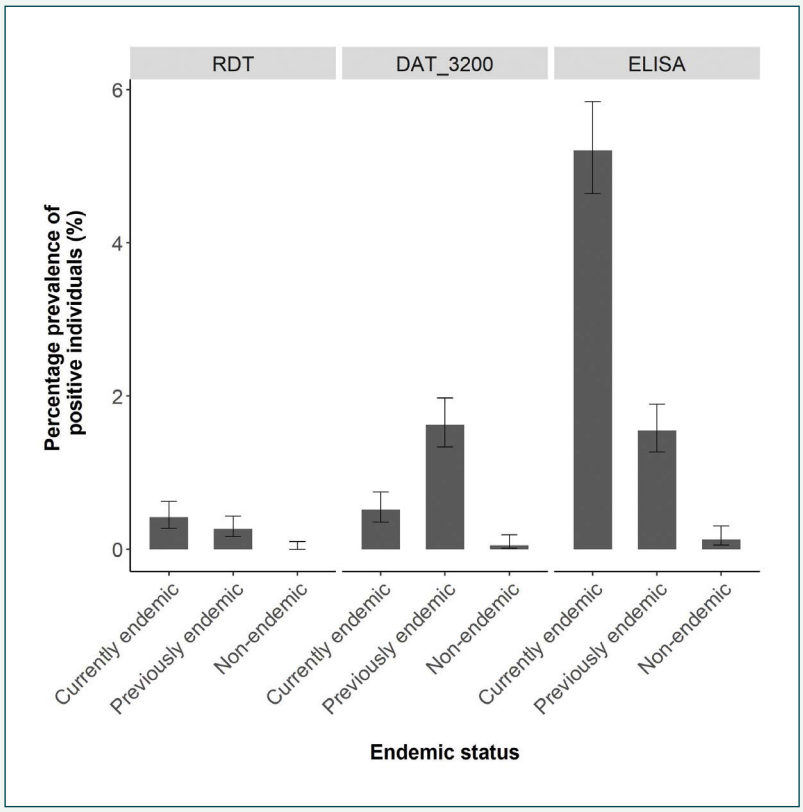


Figure 1a. Seroprevalence per endemic status of the villages included per test. RDT = rK39 RDT; DAT_3200 = Direct Agglutination Test with cut-off 1:3200; ELISA = rK39 ELISA.

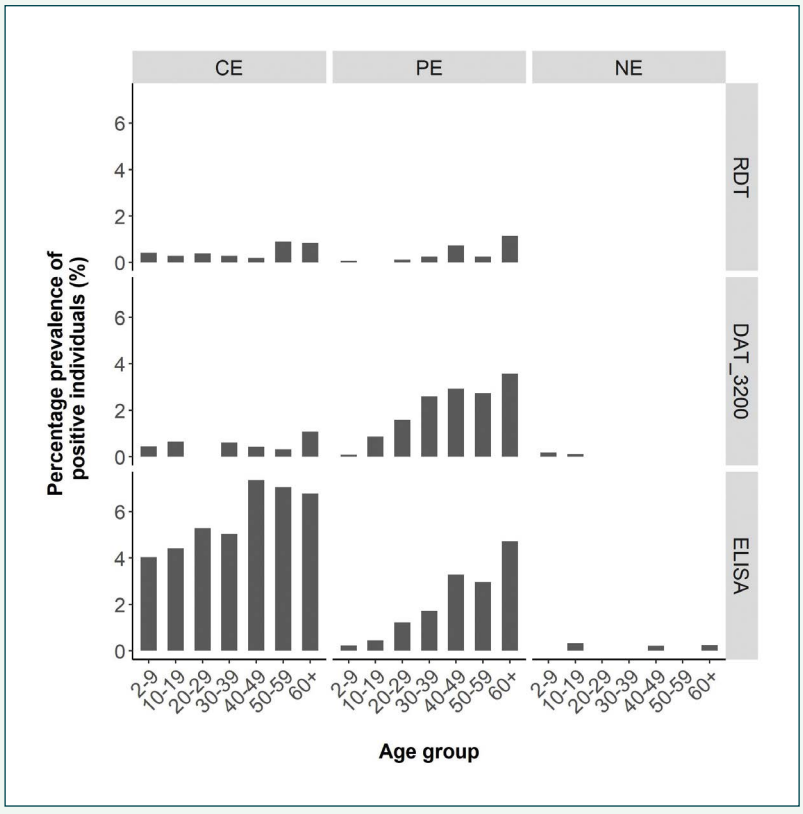


Figure 1b. Seroprevalence per test, age group and endemic status. CE = currently endemic villages; PE = previously endemic villages; NE = non-endemic villages.

to be underreported and their potential threat to a sustainable elimination underestimated.

Methods, Results & Recommendations. Data collection was done in six blocks divided between the two highly VL-endemic states of Bihar and Uttar Pradesh in India. All activities were carried out by the staff of the Primary Health care Centers (PHC) to assess feasibility and cost of the proposed activities within the existing health infrastructure. Between April 2019 and April 2021, all incident VL cases diagnosed at one of the six involved PHCs – using the standard clinical algorithm of fever since more than two weeks in combination with a positive rK39 Rapid Diagnostic Test (RDT) – were requested a venous blood sample to be tested with qPCR for the detection of parasite DNA. We found that among the 74 included patients diagnosed with VL at any of the included PHCs, 70 (95%) could be confirmed by qPCR. In addition, among 15,426 individuals without VL suggestive symptoms, only 39 tested positive with the rK39 RDT, yielding a specificity of this test of 99.7%. This illustrates that the current diagnostic algorithm for VL, still seems valid in the current low-incidence setting. However, its accuracy should continue to be monitored on a routine basis. Of the 74 VL patients diagnosed in the selected PHCs, none tested positive for HIV. Unfortunately, testing of HIV patients for VL could not be ensured within this study, and is also often not done routinely, despite being part of the National Guidelines, leading to a likely underestimation of official numbers on HIV-VL. In addition, all VL cases diagnosed 1, 3 and 5 years ago were visited at their household, and all ex-patients as well as their household members were screened for VL, PKDL, and leprosy – the most important differential diagnosis for PKDL. Among the 5618 individuals examined, belonging to the households of 1093 former VL patients, 46 new PKDL cases were identified, together with 13 PKDL cases who were already on treatment at the time of the visit, and 42 individuals with a PKDL history, illustrating the effectiveness and feasibility of this approach for the early detection of PKDL cases.

WP2 – Monitoring of transmission of *L. donovani*

Rationale. At present, the progress of the Elimination Initiative is measured by monitoring the number of VL cases. However, with VL cases decreasing, awareness of both health care workers and communities can be expected to decrease in parallel, likely leading to higher proportions of undiagnosed VL, PKDL and HIV-VL patients in the community again in the future. Therefore, monitoring of transmission rather than cases could be a more timely and sensitive approach to detect resurgence in the post-elimination phase. Although several immunological markers can be used in a clinical setting to confirm disease among symptomatic individuals, none of them is validated as a standalone marker for infection among healthy individuals.

Methods, Results & Recommendations. We conducted serological surveys in three different types of villages; Currently endemic (CE) villages which reported VL cases in each of the last three years, Previously endemic (PE) villages which stopped reporting VL cases since four years, and Non-endemic (NE) villages which had not reported VL cases for at least 15 years. All individuals above the age of 2 years provided a venous blood sample to be tested with the rK39 RDT, the rK39 ELISA, and the Direct Agglutination Test (DAT). A total of 15,426 samples were collected for serological testing.

Figure 1 illustrates the overall (Figure 1a) and age group-wise (Figure 1b) seroprevalence per endemic setting. While the rK39 RDT does not allow for a significant distinction between currently and previously endemic villages, the DAT even showed a higher seroprevalence in the previously endemic villages compared to the currently endemic villages. The rK39 ELISA on the other hand was able to clearly differentiate between all three endemic settings. Young children by definition represent relatively recent infection, and are therefore a particularly interesting target group for monitoring of transmission. Looking only at children below the age of 10y, the ELISA continued to allow significant distinction between currently and previously endemic villages. This study illustrates that serological surveys could be used to monitor transmission over time, with the rK39 ELISA showing the most potential as a marker to be used. Nonetheless, further validation is needed, and integration with serological surveys carried out for other disease control programs should be explored in order to reduce costs and ensure sustainability in the long term.

Project staff receiving training.

Mathematical Modelling

PARTNERS AND CONTRIBUTORS

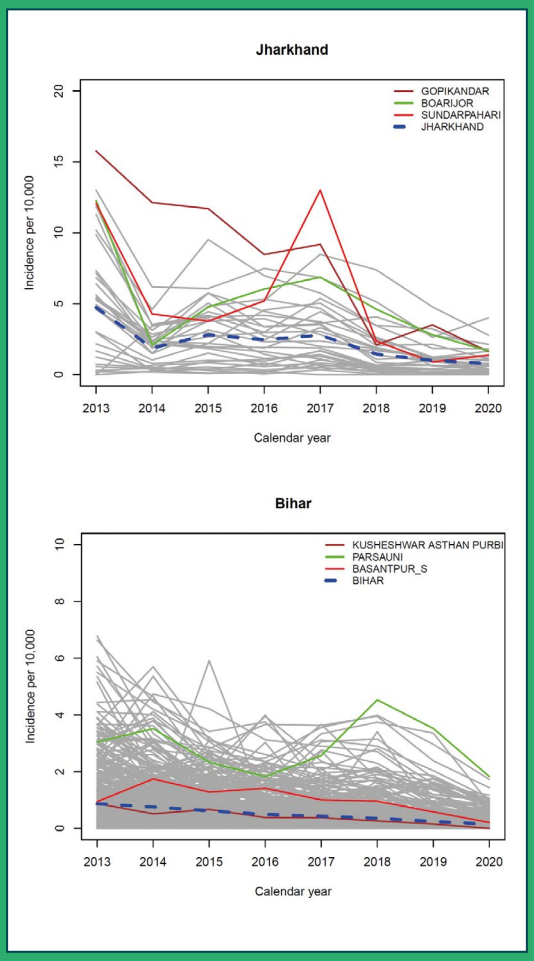
This project was a collaboration between the Vector Control Research Centre (Swaminathan Subramanian, Rajendran Uma Maheswar, Gopalakrishnan Prabavathi, Adinarayanan Srividya, Purushothaman Jambulingam) and the London School of Hygiene & Tropical Medicine (Emily S. Nightingale, Lloyd Chapman, Graham F. Medley).

THE PROJECT

Spatiotemporal models were developed to forecast the short term VL incidence at block level using the monthly block-level diagnosed cases as reported in KAMIS for the period covering Jan 2013 – Dec 2020 from the States of Bihar and Jharkhand, India.

VL incidence in Jharkhand and Bihar

- The state level incidence rates declined over the years from 2013 to 2020 in both States, but were above the elimination threshold (1 per 10,000 population) in Jharkhand and below the threshold with a steady decline over time in Bihar.
- The affected blocks of Jharkhand on average have much higher incidence than Bihar.
- Most of the blocks present incidence rates that are higher than the average for the respective states.
- Despite overall reduction in incidence across the region, there remains considerable heterogeneity in the block level burden of disease



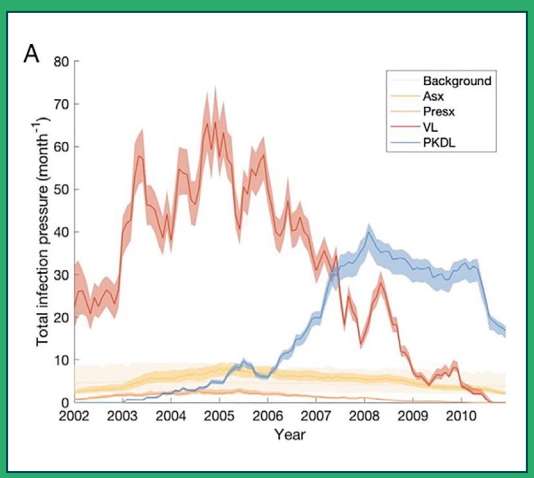
Temporal evolution of crude VL incidence rates (per 10000 persons per year) in the states (blue dashed lines) of Jharkhand and Bihar and the blocks (grey) of respective states.

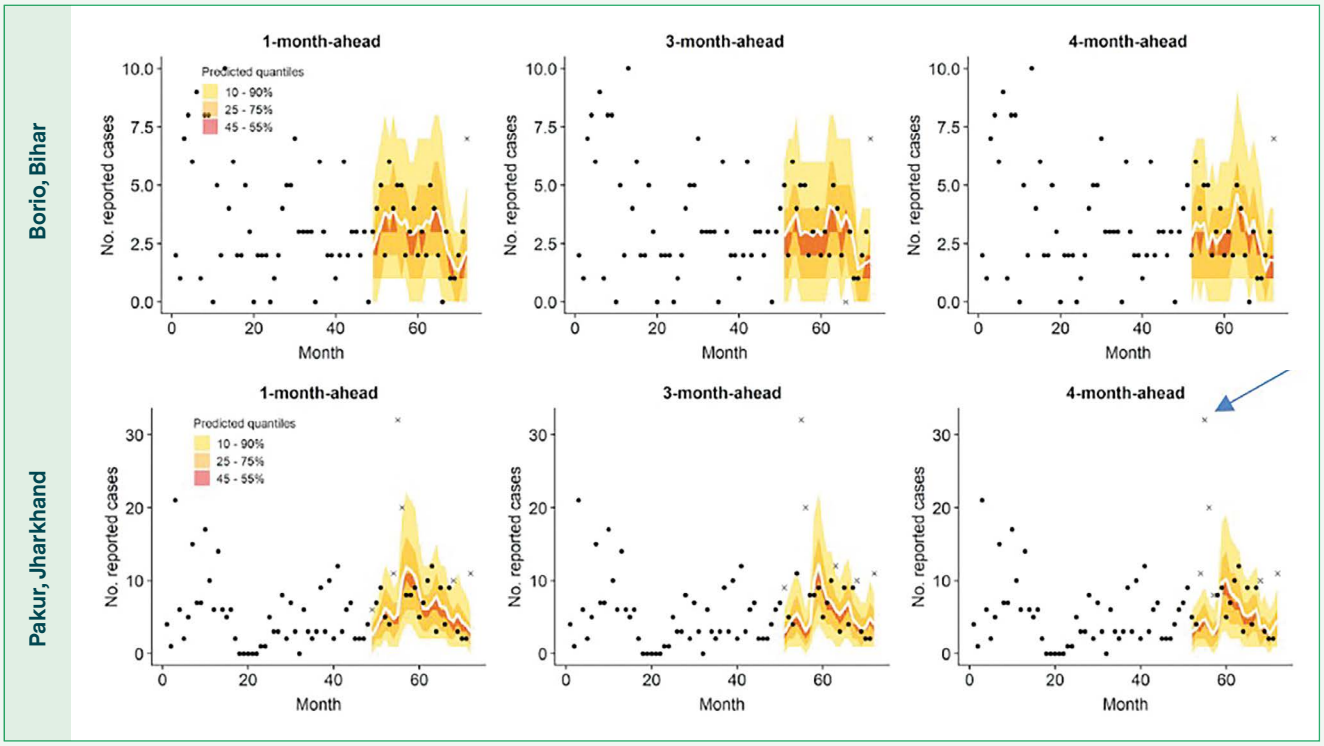
Sources of transmission during outbreaks

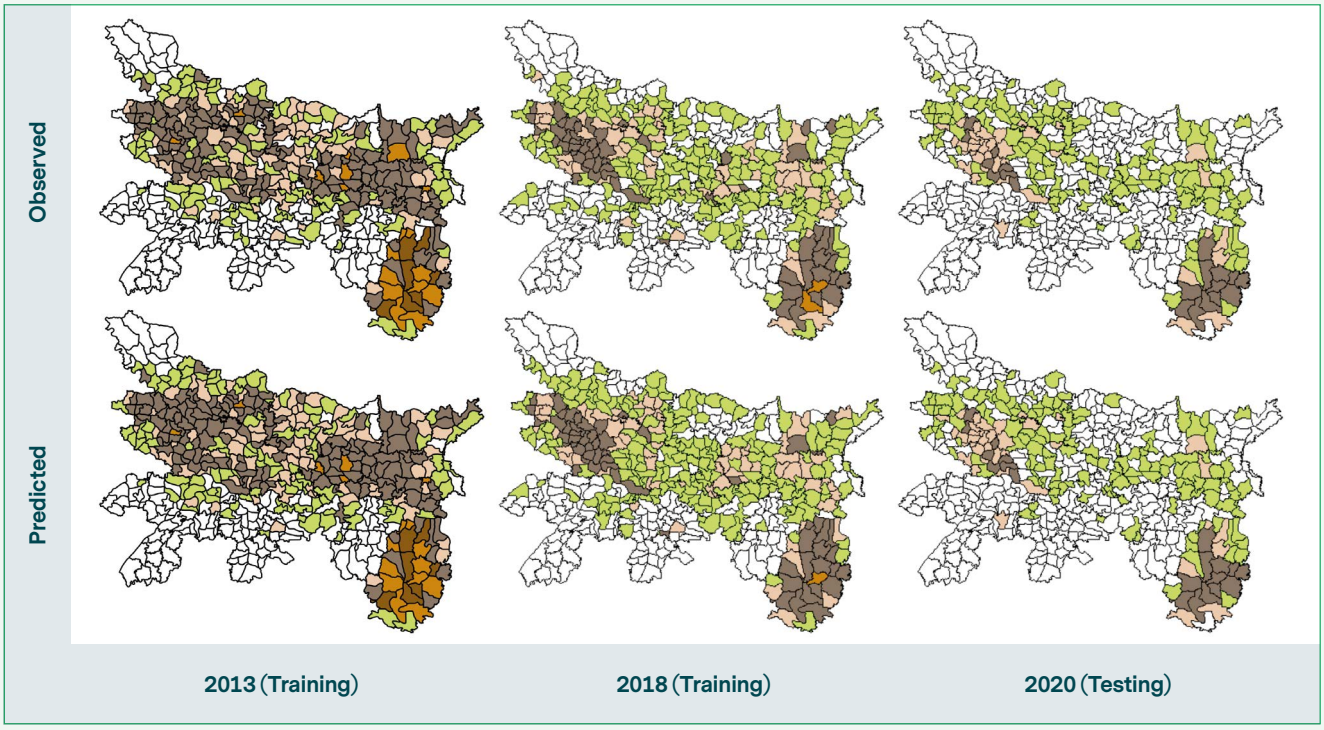
- VL cases are most infectious
- As they resolve, PKDL becomes the majority source, and substantial risk for onward transmission
- Prompt and complete diagnosis of VL and PKDL is required to reduce transmission

Modelling spatiotemporal dynamics

- Spatiotemporal modelling **without covariates** indicated predictions three or four months ahead of the training data with similar accuracy and precision as one month ahead, and may facilitate the programme to plan logistics management in advance.
- Spatiotemporal modelling framework **with environment, climatic and demographic factors** could better explain the spatiotemporal patterns in VL incidence at block level.



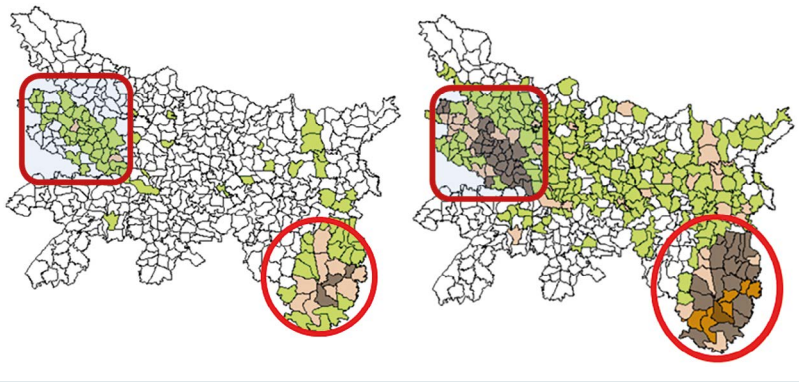




- Predicted spatiotemporal patterns of annual incidence of VL in different blocks agree with the observed declining trends in most of the blocks during the training (2013-2018), and the testing period (2019-2020).
- Predicted 95% BCI indicate that 26 blocks from 4 districts viz, Gopalganj (4 blocks), Muzzafarpur (1 block), Saran (16 blocks) and Siwan (5 blocks) in Bihar are likely to exceed the elimination threshold in 2022.
- In Jharkhand, 24 blocks from 4 districts viz, Dumka (7 blocks), Godda (4 blocks), Pakaur (6 blocks) and Sahibganj (7 blocks) are likely to exceed the elimination threshold in 2022.
- Model predictions also agree with the observed declining trends in many blocks both above and below the elimination threshold during training and testing periods.

Cases per 10,000 population

- 0.0-0.1
- 0.1-0.5
- 0.5-1.0
- 1.0-5.0
- 5.0-10.0
- 10.0-60.0



2022 (Forecasting) Mean incidence per 10,000 population

Upper 95% Bayescian Credible Interval (BCI) for predicted mean incidence

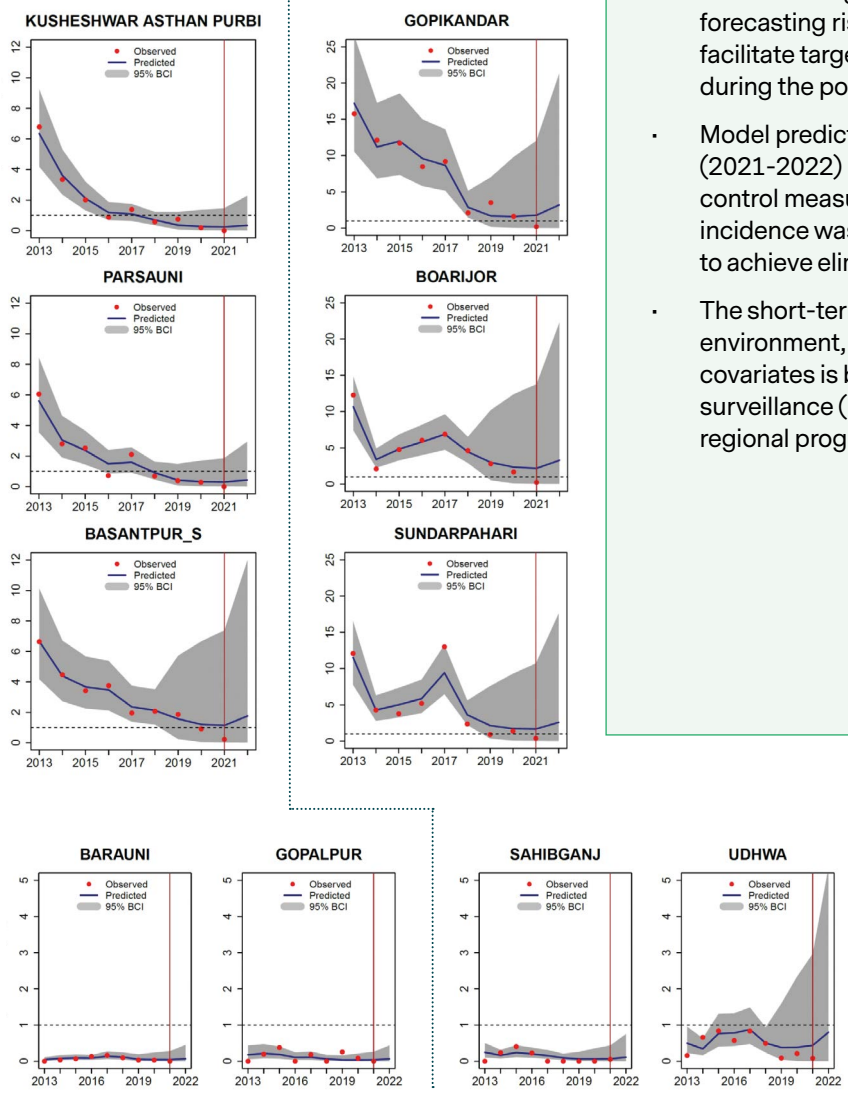
Cases per 10,000 population per block. Values in parentheses are no. of blocks

0.0-0.1 (218)	1.0-5.0 (43)
0.1-0.5 (191)	5.0-10.0 (6)
0.5-1.0 (43)	10.0-60.0 (1)

Incidence per 10,000 population

Bihar (Incidence > 6 per 10,000 population in 2013)

Jharkhand (Incidence > 12 per 10,000 population in 2013)



- Predictions beyond the period of observations (2021-2022) showed that the annual incidence is more likely to exceed the elimination threshold in the blocks where the reported VL incidence was > 6 per 10,000 population in 2013.
- The modelling framework has potential for forecasting risks and trend in incidence, which could facilitate targeting resources as endemicity drops during the post elimination settings.
- Model predictions beyond the period of observations (2021-2022) highlighted the need for targeted control measures in blocks where the annual incidence was > 6 per 10,000 population in 2013 to achieve elimination.
- The short-term prediction model without environment, bioclimatic and demographic covariates is being integrated into routine surveillance (KAMIS data) for monitoring regional progress towards elimination.

Publications

1. Nightingale ES, et al. (2020). PLoS Negl Trop Dis 14(7): e0008422. https://doi.org/10.1371/journal.pntd.0008422
2. Chapman LAC, et al. (2020). Proceedings of the National Academy of Sciences Oct 2020, 117 (41) 25742-25750; DOI: 10.1073/pnas.2002731117)

Molecular Xenomonitoring Project – Transmission: *direct measurement of sandfly infection*

PARTNERS AND CONTRIBUTORS

This project was a collaboration between the implementing partners Rajendra Memorial Research Institute for Medical Sciences (Dr. Krishna Pandey, Dr Pradeep Das, Dr. Vijay Kumar, Dr. Ashish Kumar, Dr. Vikram Pal Gandhi, Kundan Kumar, Mukesh Kumar, Ratnesh Kumar, Rahul Keshri and Santosh Kumar) and the London School of Hygiene & Tropical Medicine (Prof Mary Cameron, Prof Susana Campino, Dr. Miguella Mark-Carew, Dr. Mojca Kristan, Dr. Matthew Rogers, Shannon McIntyre and Giorgia Dalla Libera Marchiori) supported by other members of the SPEAK India 'Surveillance' and 'Mathematical Modelling' projects, as required.

BACKGROUND AND RATIONALE FOR THE PROJECT*

To meet the elimination target and move beyond elimination as a public health problem by interrupting transmission, a greater understanding of VL transmission dynamics is required. The main purpose of the project was to determine whether molecular xenomonitoring (MX) could be used to determine endpoints of visceral leishmaniasis (VL) transmission and play a role in post-elimination surveillance of VL (similar to using MX in the lymphatic filariasis elimination programme to monitor residual transmission post-MDA). To calculate these endpoints requires estimates of the *Leishmania donovani* DNA infection and infectiousness rates in *Phlebotomus argentipes* females, the only known vector of VL in the India Subcontinent (ISC), to be made and compared with VL cases.

PILOT STUDY TO COMPARE DIFFERENT *PHLEBOTOMUS ARGENTIPES* SANDFLY COLLECTION METHODS

A pilot study comparing three collection techniques was performed in two villages VL endemic villages in Saran district and two non-endemic villages in Nalanda district to develop a sampling framework for use in the main xenomonitoring study. CDC light traps (CDC-LTs) collected the highest mean density of female *P. argentipes* per trap-night (5.28) compared with the mechanical aspirator (MVA) (2.85) and Prokopack (PKP) (3.32). Therefore, CDC-LTs (see Figure 2) were the collection method selected for the main xenomonitoring study. These data further support continued operational use of CDC-LTs for sandfly surveillance by the National Kala-Azar Elimination programme.

We also investigated the frequency of mutations associated with insecticide resistance in *P. argentipes* sand flies from sprayed and unsprayed villages in Bihar. Indoor residual spraying (IRS) has been used in Bihar to support the control of VL, as part of the VL elimination

Figure 2. CDC-LT set up inside household of village (field collector wearing PPE during covid-19 pandemic).

programme. If vector control is to succeed, systematic entomological surveillance is required, and part of that is insecticide resistance monitoring. DNA samples from *P. argentipes* sand flies were used for detection of *kdr* mutations using PCR and Sanger sequencing. Overall, a high frequency of *kdr* mutations was detected, similar to previously reported data from India. Significant inter-village variation was observed: whereas 56% of samples from the unsprayed village of Dharampur were wild type (L1014=Leucine amino-acid), a high proportion of sand flies with mutations leading to the serine amino acid (L1014S) were detected in the other three villages and only 12% and 8% of samples were wild type in the IRS-sprayed villages.

MAIN XENOMONITORING STUDY TO DETERMINE THE COST EFFECTIVENESS, FEASIBILITY AND SCALABILITY OF XENOMONITORING AS A SURVEILLANCE MECHANISM FOR VL

The study was conducted in 12 villages (endemic, previously endemic and non endemic) located in 5 districts in Bihar (Figure 3). Using previous infection data, a sample size calculation was performed and a target

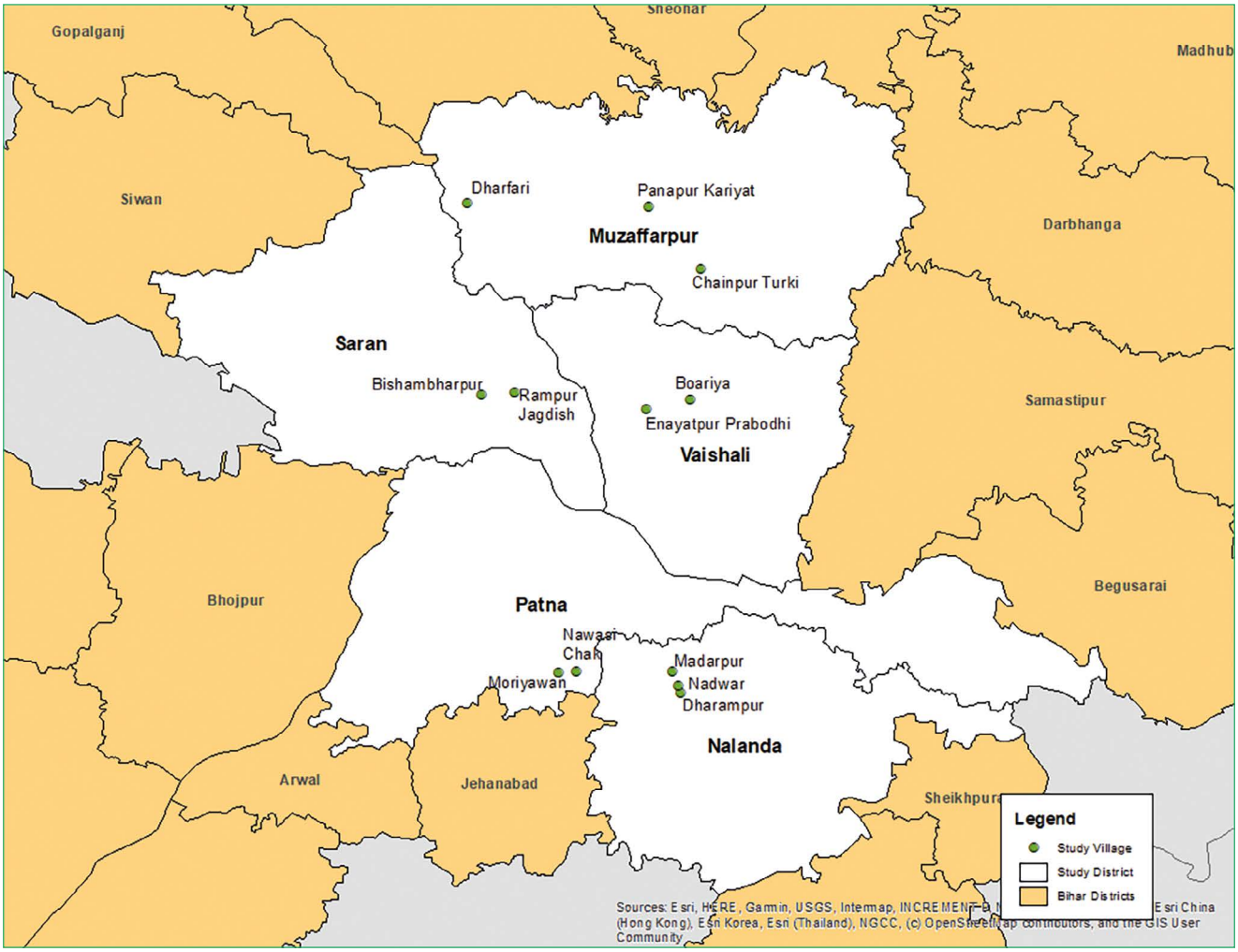


Figure 3. Location of study villages and districts in Bihar.

of 3,750 *P. argentipes* females was required to detect differences in *L. donovani* DNA prevalence rates between treatment groups (levels of endemicity).lance by the National Kala-Azar Elimination programme.

Following a significant sampling effort undertaken between 2018-2021, we collected over 3,900 *P. argentipes* females for *L. donovani* screening. We optimized two protocols that were specific for *L. donovani* DNA detection in sandflies. DNA extractions were performed on 172 pools of lower thorax-abdomen sections of between 4-14 sandflies/pool (pooled by collection date and village). Pools were analysed for the presence of *L. donovani* DNA using a qPCR protocol with Taqman primers and probes. Samples were considered positive if Ct values were lower than the limit of detection by the assay (Ct<31). All samples and controls were run in duplicate. Sequencing was performed on any pools that were preliminary considered to be positive for *L. donovani*.

Following molecular analyses, none of the pools were confirmed as positive for *L. donovani* DNA. The effort required to collect large numbers of female *P. argentipes* for *L. donovani* detection, during this period of low endemicity, was challenging and resource demanding as estimated using a costing tool that itemized human, transportation, equipment, and supply resources (including time).

We conclude that establishing a relationship between *Leishmania* infection rates in human and sandfly populations may not be feasible in elimination scenarios. We recommend that to optimise the use of limited programmatic resources, new field-friendly tools are required with protocols for simultaneous detection of *Leishmania* and other pathogens present in sandfly and mosquito populations. To address this need, we have developed a point-of-need multiplex tool which will be pilot tested in Bangladesh.

* The project was approved by the GOI Health Ministry's Screening Committee (Ref: 2017-4126) on 8th September 2017, and the Ethics committee of RMRIMS (Ref: 39/RMRI/EC/2017) on 11th October 2017. Ethical clearance was provided by the London School of Hygiene & Tropical Medicine (Ref: 1463) on 11th May 2018.

Oral Presentations and Posters

Wednesday 3rd August 2022

Poster Presentation

ID P2-104-422

Estimating resource needs and costs of molecular xenomonitoring of *Phlebotomus argentipes* for detecting *Leishmania donovani* in Bihar, India

Poster Presentation: Miguella Mark-Carew

Authors: Miguella Mark-Carew, Mary Cameron, Rian Snijders, Kundan Kumar, Ashish Kumar, Vijay Kumar

Friday 5th August 2022

Oral Presentation (Session 39) 18:00 – 19:00

ID O39-04-455

Combining human serosurveillance with molecular xenomonitoring in two endemic villages for visceral leishmaniasis surveillance in Saran, Bihar, India

Oral Presentation: Miguella Mark-Carew

Authors: Miguella Mark-Carew, Kristien Cloots, Singh OP, Singh AK, Malaviya P, Epco Hasker, Susana Campino, Mojca Kristan, Kundan Kumar, Ashish Kumar, Vijay Kumar, Shyam Sundar and Mary Cameron

Friday 5th August 2022

Oral Presentation (session 30) 15:30 – 16:30

ID 030-05-522

Knockdown resistance mutations in *Phlebotomus argentipes* from villages with and without indoor residual spraying in Bihar, India (ID 100522)

Oral Presentation: Mojca Kristan

Authors: Mojca Kristan, Mary Cameron, Carlamarita Hazelgrove, Kundan Kumar, Ashish Kumar, Vijay Kumar and Susana Campino

Symposia

Wednesday 3rd August 2022
Satellites symposium (session 17) 11:30 – 13:00

Is molecular xenomonitoring a useful tool for monitoring visceral leishmaniasis (VL) transmission in the peri-elimination phase?

Presenting: Prof Mary Cameron, London School of Hygiene and Tropical Medicine

Authors: Mary Cameron, Susana Campino, Miguella Mark-Carew, Krishna Pandey, Kundan Kumar, Ashish Kumar, Mojca Kristan, Pradeep Das, Vijay Kumar

Wednesday 3rd August 2022
Satellites symposium (session 17) 11:30 – 13:00

Wednesday 3rd August 2022
Oral Presentation (session 24) 18:00 – 19:00
ID 024-02-408

Monitoring of VL transmission in the peri-elimination phase:
the potential of serological surveys

Presenting: Dr Kristien Cloots, Institute of Tropical Medicine Antwerp, Belgium

Authors: Kristien Cloots, Om Prakash Singh, Abhishek Kumar Singh, Paritosh Malaviya, Epco Hasker, Shyam Sundar

Thursday 4th August 2022
Satellites symposium (session 25) 10:00 – 11:30

Friday 5th August 2022
Oral Presentation (session 34) 15:30 – 16:30
ID O34-01-084

Diagnosis of visceral leishmaniasis in an elimination setting: a validation study of the diagnostic algorithm in India

Presenting: Dr Shyam Sundar, Institute of Medical Sciences, Banaras Hindu University, India

Authors: Kristien Cloots, Om Prakash Singh, Abhishek Kumar Singh, Anurag Kumar Kushwaha, Paritosh Malaviya, Sangeeta Kansal, Epco Hasker, and Shyam Sundar

Friday 5th August 2022

Satellites symposium (session 35) 10:00 – 11:30

Modelling spatiotemporal patterns of visceral leishmaniasis incidence in India.

Presenting: Dr. Subramanian Swaminathan, ICMR – Vector Control Research Centre, India

Authors: Swaminathan Subramanian, Rajendran Uma Maheswari, Gopalakrishnan Prabavathi, Adinarayanan Srividya, Ashwani Kumar, Manju Rahi, Emily S. Nightingale, Graham F. Medley, Mary M. Cameron, Nupur Roy, Purushothaman Jambulingam

Friday 5th August 2022

Oral Presentation (Session 39) 18:00 – 19:00

ID O39-01-255

Modelling spatiotemporal patterns of visceral leishmaniasis incidence in India using environment, bioclimatic and demographic data, 2013-2021

Presenting: Dr. Subramanian Swaminathan, ICMR – Vector Control Research Centre, India

Members of the molecular xenomonitoring team.

We are very grateful to the Bill and Melinda Gates Foundation for sponsoring the operational research projects conducted by the SPEAK India consortium. The work would not have been possible without the support of the Indian Council of Medical Research and the National Center for Vector Borne Disease Control. We thank our partners for their commitment and are most grateful to all of the volunteers who participated in the projects.

To find out more, please see the SPEAK India website
https://speakindia.org.in

Preparing a pool of sand flies prior to molecular analyses.

Field team isolating sand flies from a CDC light trap collection.