Visceral leishmaniasis transmission in India: the role of xenomonitoring in a post-elimination setting.

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SPEAK India

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

INDIAN COUNCIL OF MEDICAL RESEARCH NEW DELHI

Molecular Xenomonitoring (MX)

- MX = assessing parasite DNA or RNA levels

through PCR in the vector population

- Potential for use as a passive surveillance system for VL
  - Non-invasive = fewer ethical implications than screening human populations
  - Potentially more cost effective than active case detection and more sensitive than traditional passive case detection systems
  - Indicates circulating parasite levels

Objectives

1. Generate *Phlebotomus argentipes* sampling framework and standard operating procedures

2. Collect and analyse *P. argentipes* from sites across blocks with no, low, and high VL transmission in Bihar
3. Use data to develop transmission endpoint assessment guidelines for India's VL control program
4. Utilise xenomonitoring and sero-surveillance data collected in the same place, at the same time, to improve transmission models

Objective 1: Protocol

- Three sampling methods:
  - CDC light trap
  - Improved Prokopack Aspirator
  - Mechanical Vacuum Aspirator

Aspirations standardised at a rate of 30s/m^2

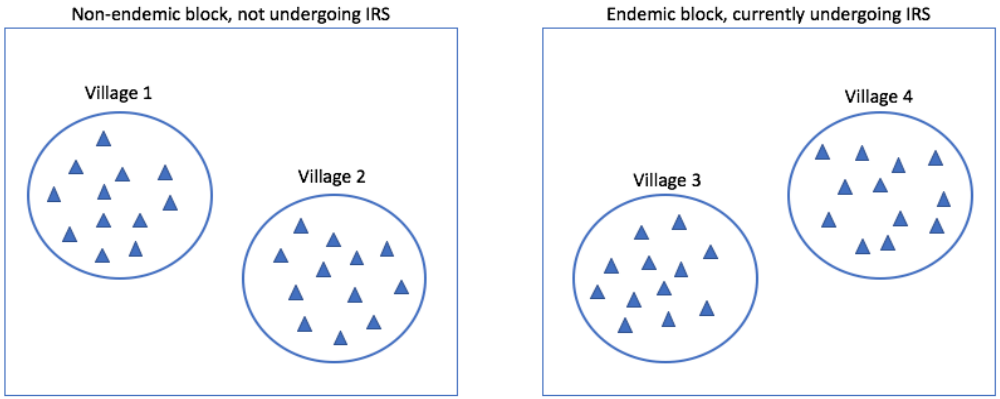
$$n = \frac{2(Z_a + Z_{1-\beta})^{2\delta 2}}{\Delta^2}$$

α = 0.05
ß = 0.20
Δ^2 (estimated effect size) = 1.0, 1.25, 1.5
(standard deviation) = 3.2512

Effect size	Trap nights required
1.0	166
1.25	106
1.5	74

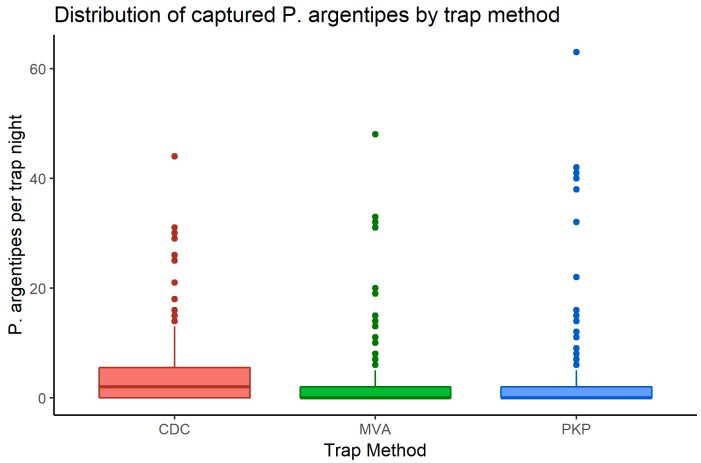
Objective 1: Protocol

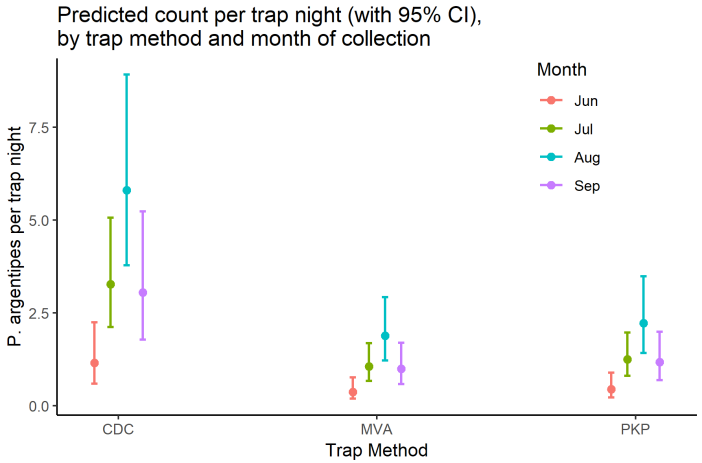
- 48 households across four villages (Dhampura, Ruchanpura, Rampur Jagdish & Bishambhapur) split between two districts (Nalanda & Saran) recruited in April 2018
- Household surveys examining known risk factors for VL completed in June 2018
- Sampling of households commenced in June 2018
  - Follows a randomised Latin Square design balanced for carry-over effects (4 x 12 x 12 = 576 'trap nights' - much greater than 498 required).



Objective 1: Results

	CDC LT	Mechanical aspirator	Prokopak
Total Sandflies	4076	1433	1645
Females	1781	876	1025
P. argentipes females	866****	501	568





Objective 2: Study Design

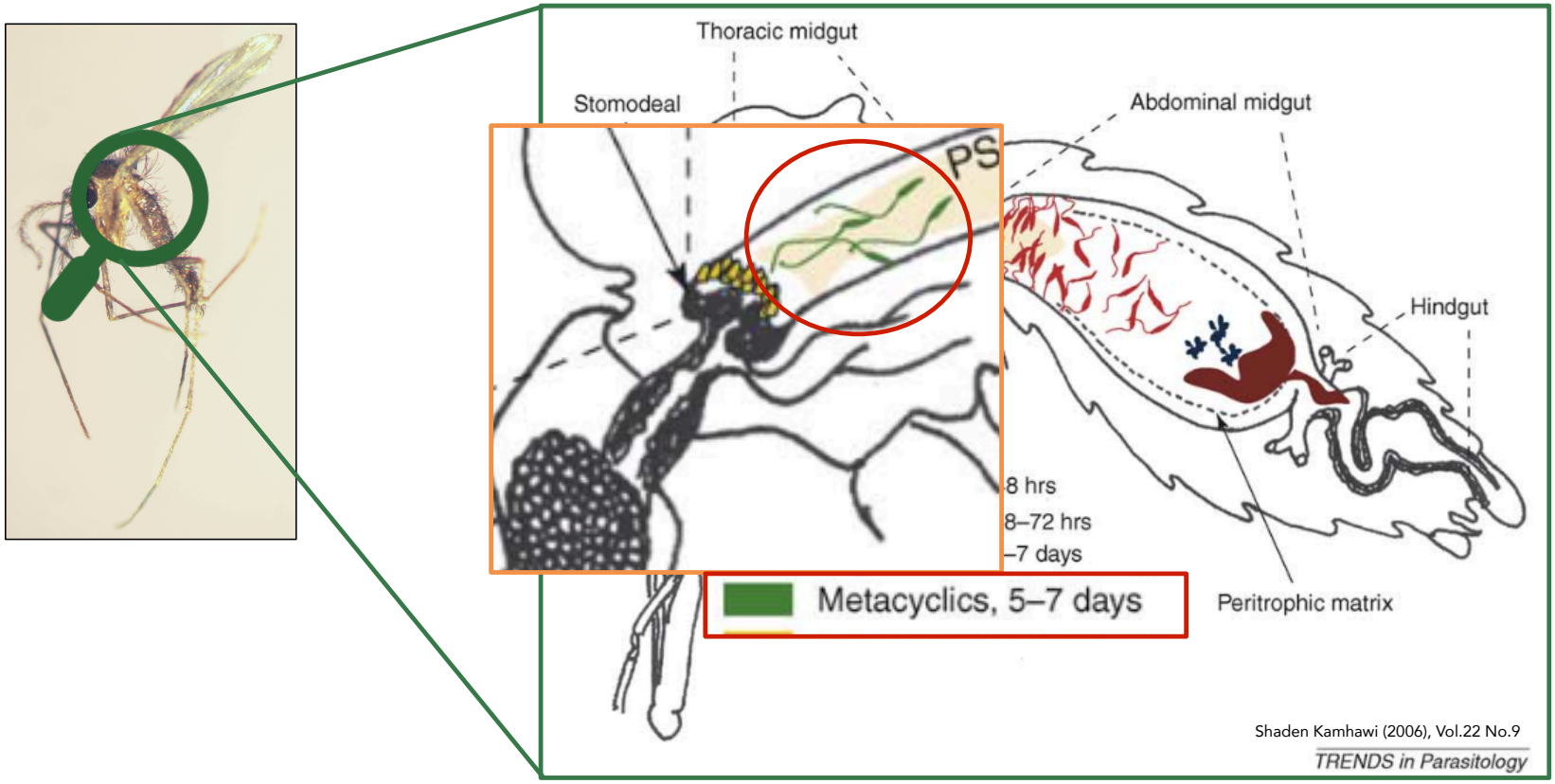
- 120 HH recruited in 12 villages, 6 endemic and 6 non-endemic
  - 50 HH to overlap with the collection of human serum samples by the SPEAK India Surveillance project
- Surveys assessing household composition, building materials, and known risk factors for VL carried out in advance of entomological sampling commencement
- CDC light traps placed indoors overnight to capture *P. argentipes* bimonthly in each HH

District	Village
1=Vaishali	1=Enayatpur Prabodhi
1=Vaishali	2=Boariya
2=Patna	3=Nawasichak
2=Patna	4=Moriyawan
3=Nalanda	5=Dharampur
3=Nalanda	6=Nadwar
3=Nalanda	7=Madarpur
4=Saran	8=Rampur Jagdish
4=Saran	9=Bishambarpur
5=Muzzafarpur	10=Panapur Kariyata
5=Muzzafarpur	11=Dharfari
5=Muzzafarpur	12=Chainpur Turki

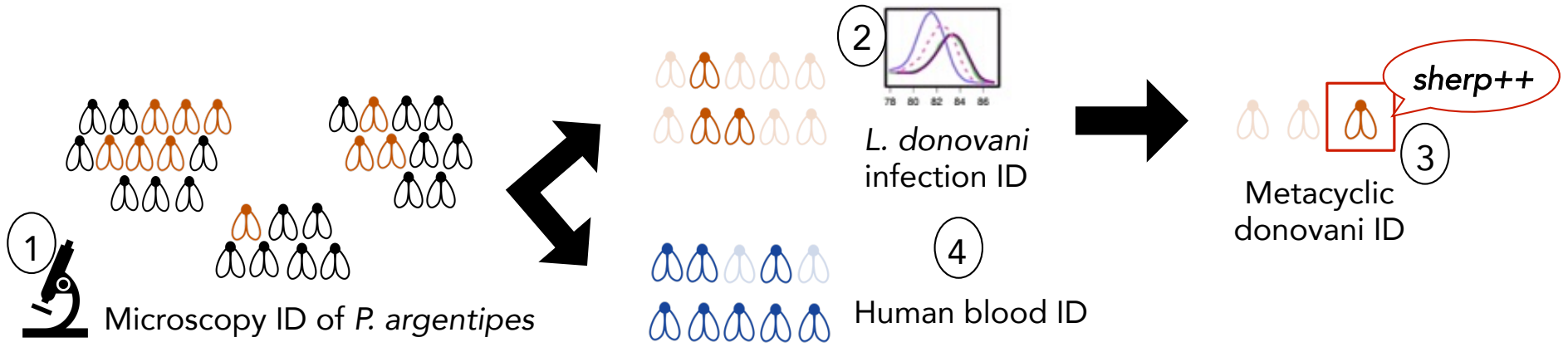
Study Design

- Female sandflies, male sandflies, female mosquitoes, and male mosquitoes separated from CDC light trap captures
  - Female sandflies: speciated, counted, stored in ethanol at -80^{o}C
  - Male sandflies: counted, stored in ethanol at -80^{o}C
  - Female mosquitoes: counted, stored in ethanol at -80^{o}C
  - Male mosquitoes: counted
- All capture data recorded against date and household identification number
- PCR to detect presence of *L. donovani* (infection) and *L. donovani* metacylics (infectiousness)

Infection vs Infectious



Study design

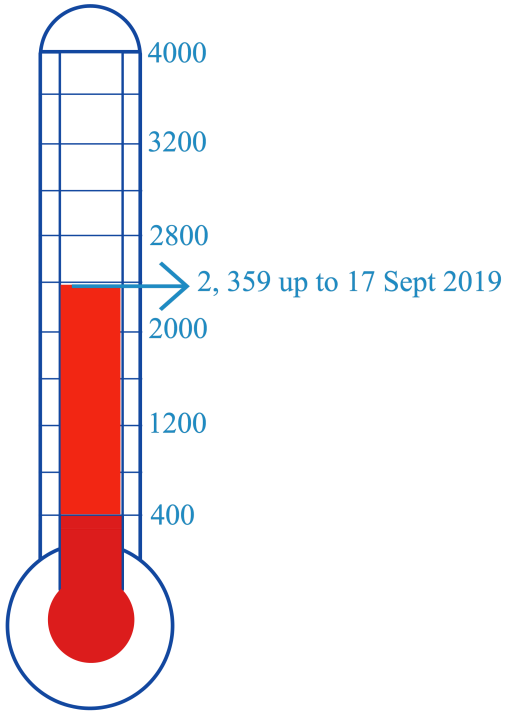


2. Xenomonitoring

b. Identification of infectious *P. argentipes* - *L. donovani* metacyclic stage:

- Single SF infected with *L. donovani*
- Method: specific qPCR for *sherp* expression (*E. Giraud et al., 2018*)

Problem – Flooding – DNA (infection) - ~~RNA~~



Objective 2: Revised Study Design

- 144 HH recruited in 12 villages, 6 endemic and 6 non-endemic
  - 60 HH to overlap with the collection of human serum samples by the SPEAK India Surveillance project
- Surveys assessing household composition, building materials, and known risk factors for VL carried out in advance of entomological sampling commencement
- CDC light traps placed indoors overnight to capture *P. argentipes* bimonthly in each HH
- +CDC light traps placed outdoors (varanda) overnight to capture *P. argentipes* bimonthly in 3 HH/village
- Samples stored in RNA*later*-ICE at -20°C.

District	Village
1=Vaishali	1=Enayatpur Prabodhi
1=Vaishali	2=Boariya
2=Patna	3=Nawasichak
2=Patna	4=Moriyawan
3=Nalanda	5=Dharampur
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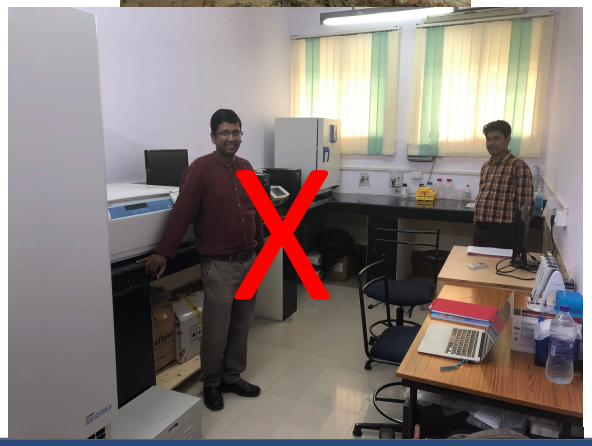
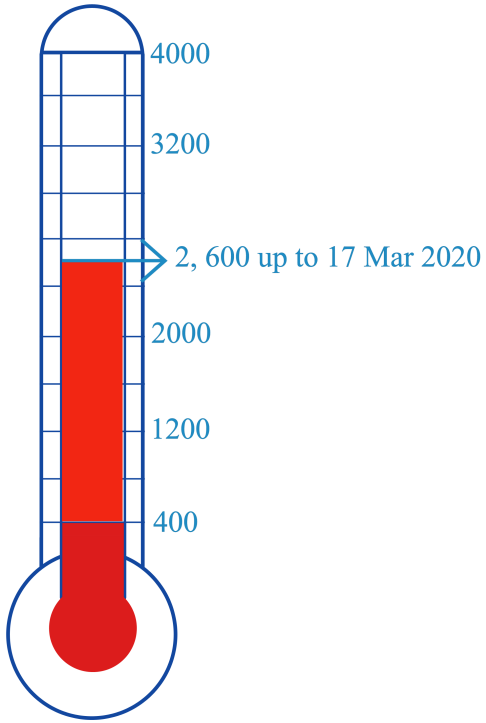
Problem – COVID-19 Pandemic - lockdown





Female *P. argentipes* Collected	**Total**
Flood-Affected Specimens (Replicates 1-6)	2,359
18 June – 18 September, 2019	
Post-flood, frozen at -20 C and stored in ethanol (Replicate 7)	200
5 November – 18 November, 2019	
Post-flood, frozen at -20 C and stored in RNALater Ice (Replicate 8)	41
3 March – 17 March, 2020	
Includes both indoor (n=31) and veranda (n=10) totals	
Total	2,600

Mitigation

 DNAExtraction_Video1.mp4

 DNAExtraction_Video2.mp4

 LeishIDqPCR_Video.mp4

Objectives 3 & 4

- Human sero-surveillance data to be collected at the same time in five of the 12 study villages
  - Asymptomatic VL
  - Clinical VL
  - Post-Kala azar dermal leishmaniasis (PKDL)
- Data on humans, parasites & vectors at the household level used to improve transmission models

Conclusions

- Integrating MX into India's existing passive surveillance system could help to overcome sensitivity constraints and indicate whether transmission within a given region is ongoing to assist in the deployment of VL control methods.
- MX could shed light on unknown aspects of VL transmission, including the entomological inoculation rate, natural rates of *P. argentipes* infection and infectiousness.
- This information could be used to formulate transmission assessment surveys similar to those in use for lymphatic filariasis, to determine when transmission has been interrupted.

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