Defining endpoints of transmission through measuring *Leishmania donovani* infections in *Phlebotomus argentipes* sandflies

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

Xenomonitoring

- Xenomonitoring = assessing parasite DNA or RNA levels through PCR in the vector population, rather than the host 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
- Shed light on the role of asymptomatics and post-kala azar dermal leishmaniasis (PKDL) patients in maintaining transmission

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

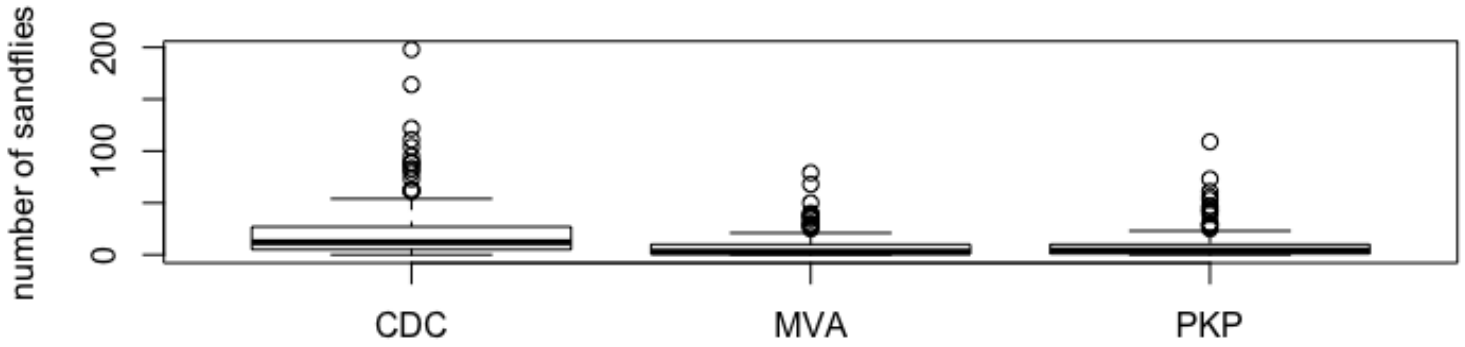
Objective 1

- Pilot study conducted carried out from June – September 2018 to determine the optimal sampling practice for *P. argentipes*:
  - CDC light traps
  - Improved Prokopack aspirators
  - Mechanical vacuum aspirators
- 7156 samples collected over 576 attempted trap nights (563 successful trap nights)
  - 3686 specimens (51.5%) identified as female
  - 3470 specimens (48.5%) identified as male

Objective 1: Results – global data

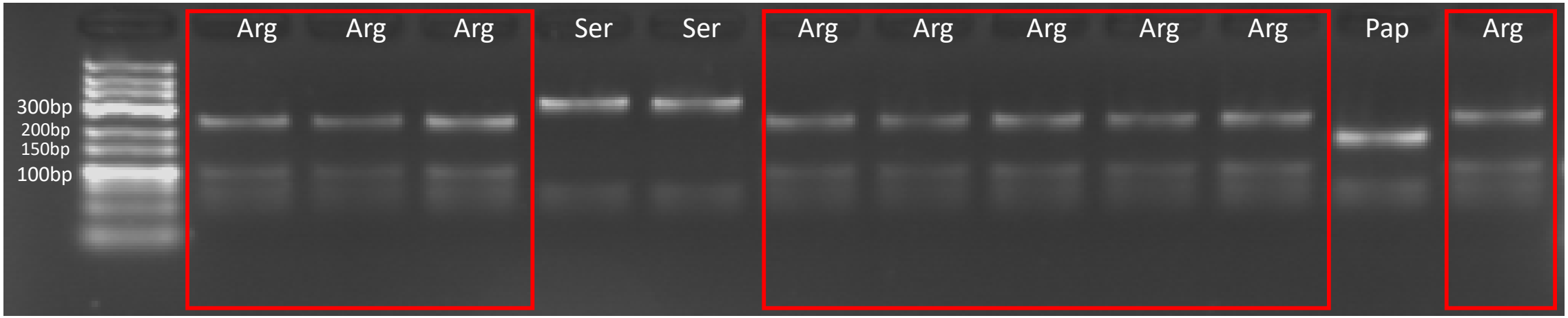
- 7156 sandflies caught in total: 3686 specimens (51.5%) female, 3470 specimens (48.5%) male

Sandfly type	CDC Light Trap	Prokopack	Mechanical Aspirator
Female	1788**	1021	877
Unfed	1452***	455	400
Bloodfed	336	566**	477
Male	2293	619	558
TOTAL	4081**	1640	1435



Molecular analysis methods

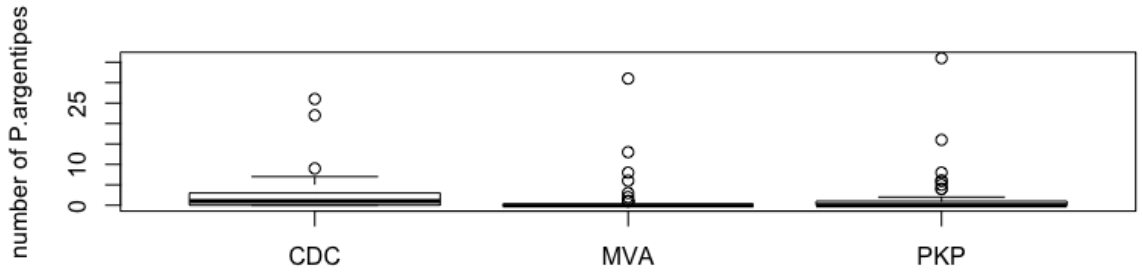
- DNA extraction from **single female SF**
- **PCR-RFLP** to identify SF species (*P. Tiwary et al., J Med Entomol. 2012*):
  - Primers amplify part of 18S rRNA gene
  - Digestion performed at 37 ºC for 10min with enzyme HpaII
    → **discriminate between *P. argentipes*, *P. papatasi* and *S. babu***
  - Digested products were run on 3% agarose gel for 2h



Objective 1: Results –speciated subsample

- Subsample of 1207 sandflies (all sexes) selected to coincide with the first rotation of the randomised Latin Square; each household received treatment type once; 641 specimens (53%) identified as female and speciated

Species	CDC Light Trap	Prokopack	Mechanical Aspirator
P. argentipes	121 (16.8%)[a]	97 (33.1%)[a]	68 (35.4%)[b]
P. papatasi	2	10	1
Sergentomyia	171	58	25
Other species	9	18	20
Unidentifiable	10	16	15
Male	409	94	63
TOTAL	722	293	192



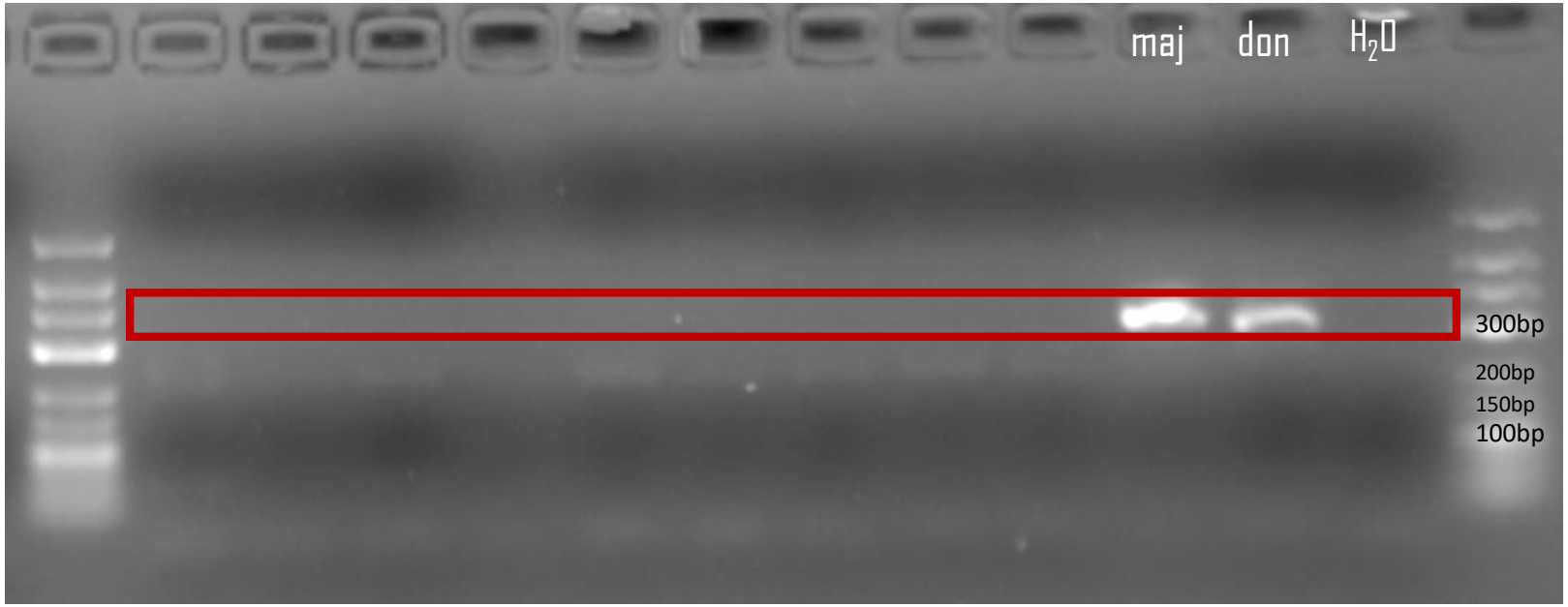
Objective 1: Results –speciated subsample

	Previous samples	New samples
P. argentipes	286	289
P. papatasi	13	20
Sergentomyia	254	462
Total	553	771

1. Pilot study

b. Presence of *L. donovani* infection → PCR-RFLP *(N. Hijjawi et al., 2016)*

- **No infected SF** so far → next: confirmation through **sequencing**



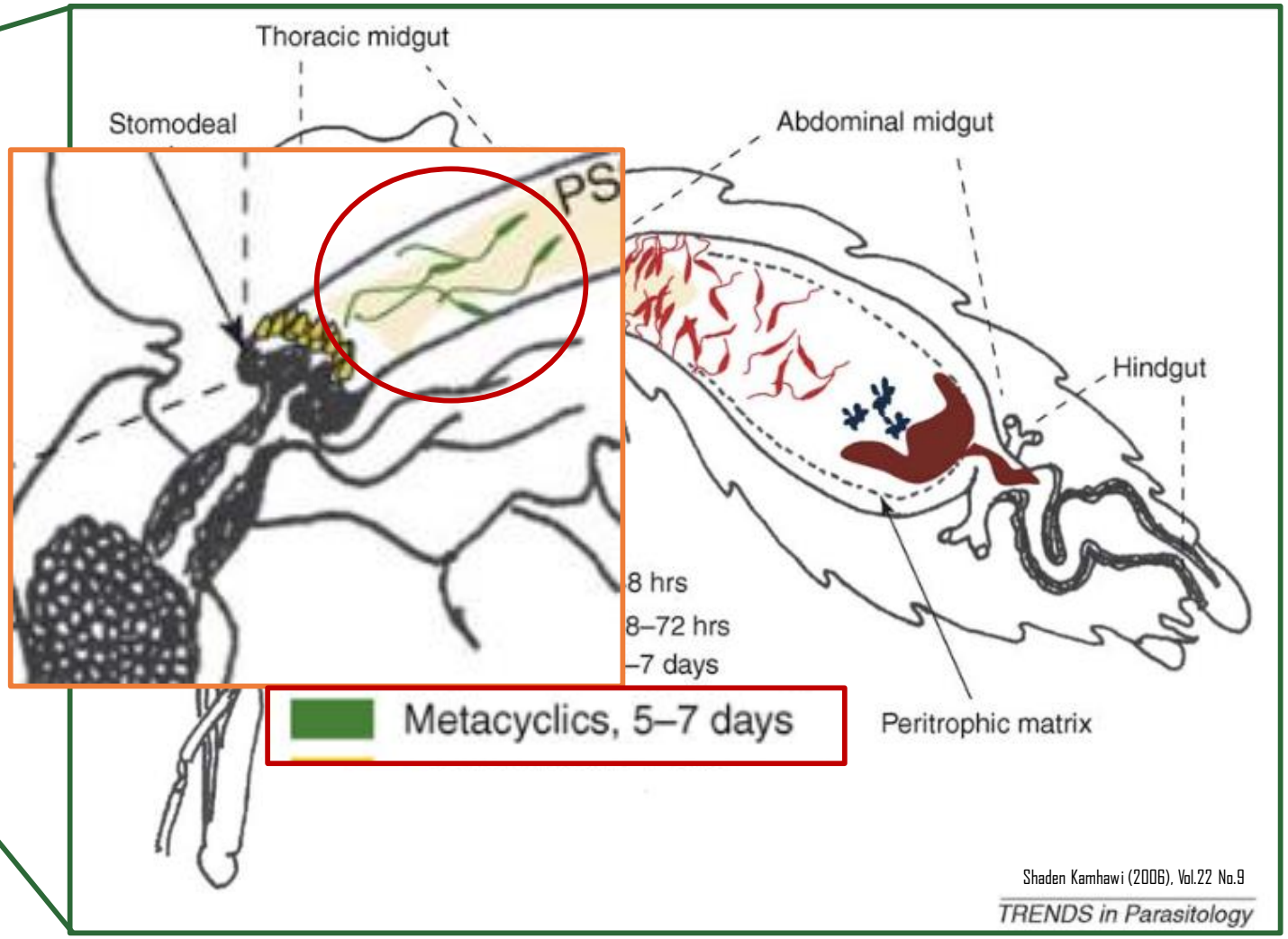
Objective 2

- 120 households recruited in 12 villages, spanning three transmission strata: endemic, meso-endemic, and non-endemic
  - 50 households 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
- Miniature CDC light traps placed indoors overnight to capture *P. argentipes* bimonthly in each household

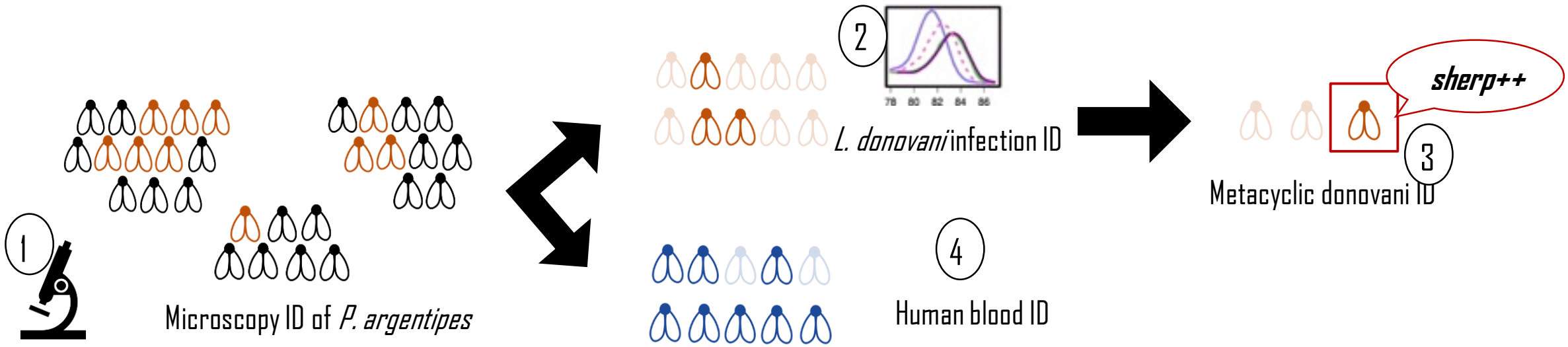
Objective 2 cont.

- 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)

2. Infection vs Infectious



2. Xenomonitoring



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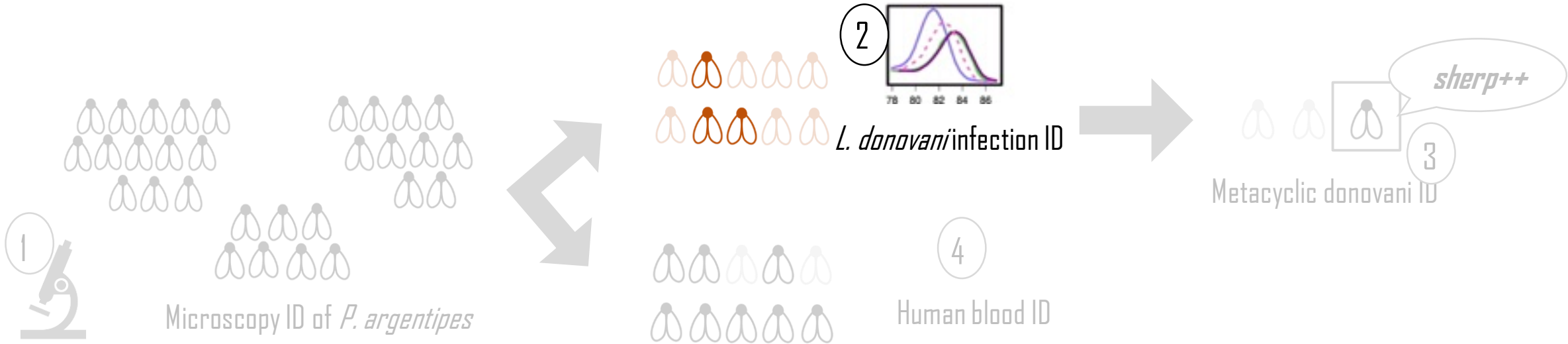
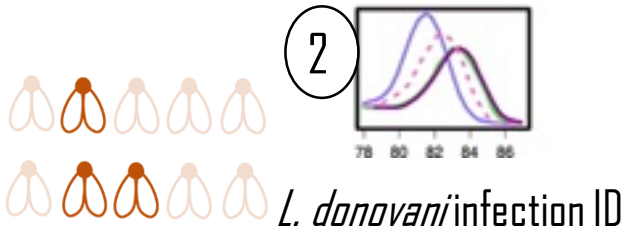


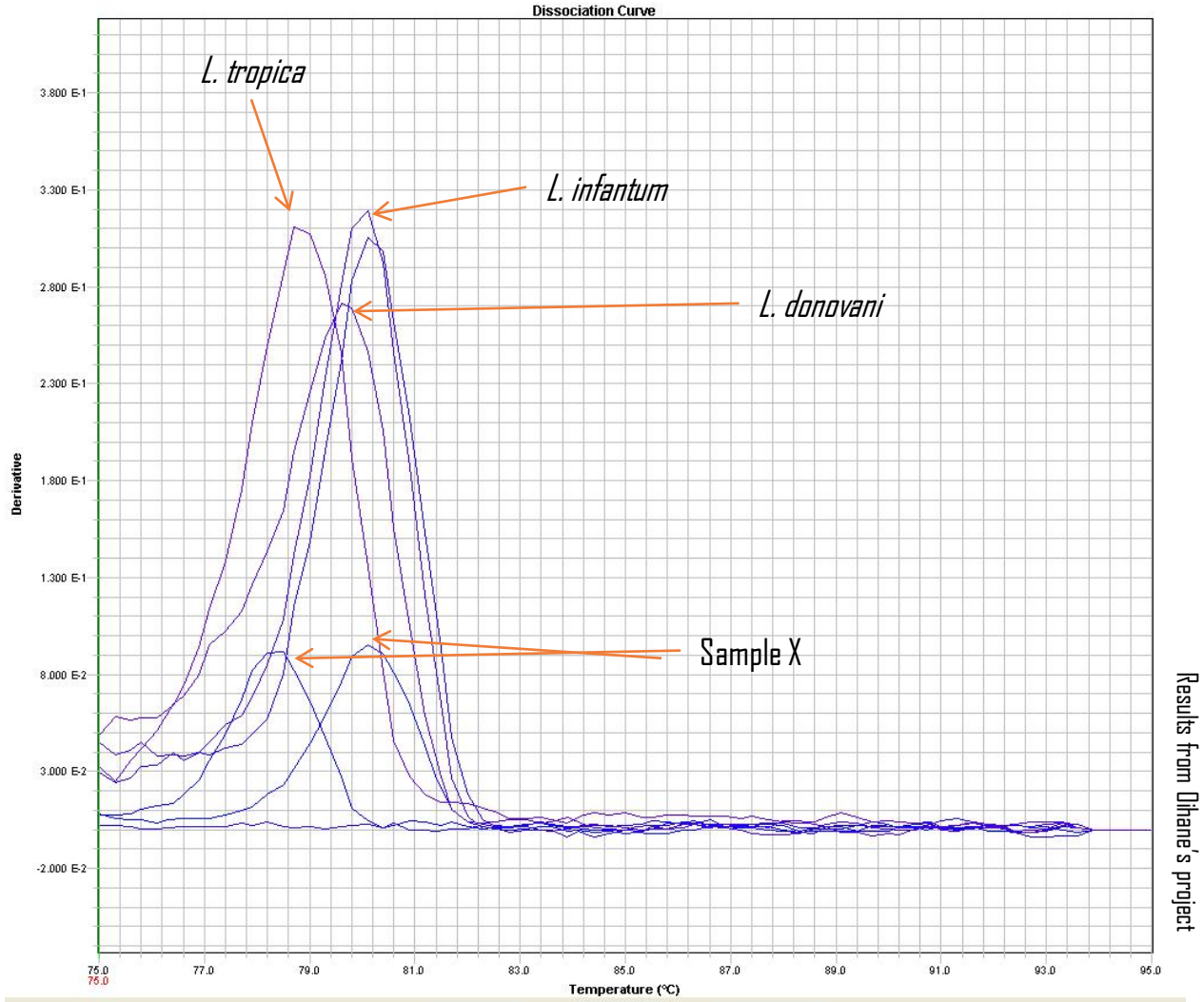
a. Identification *P. argentipes* infected with *L. donovani:*

- Pooling strategy → SFs from same household
- Method: qPCR-HRM (*Jason L. Weirather et al., 2011; Marcos E. de Almeida et al., 2016*) → positive pool for *L. donovani* → single SF analysis

2. Xenomonitoring



L. donovani infection ID



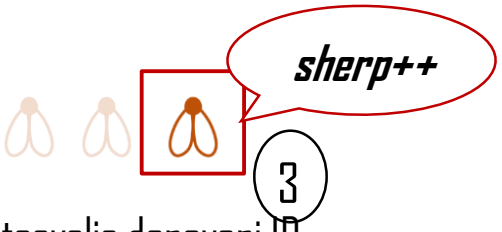
Results from Dihane's project

2. Xenomonitoring

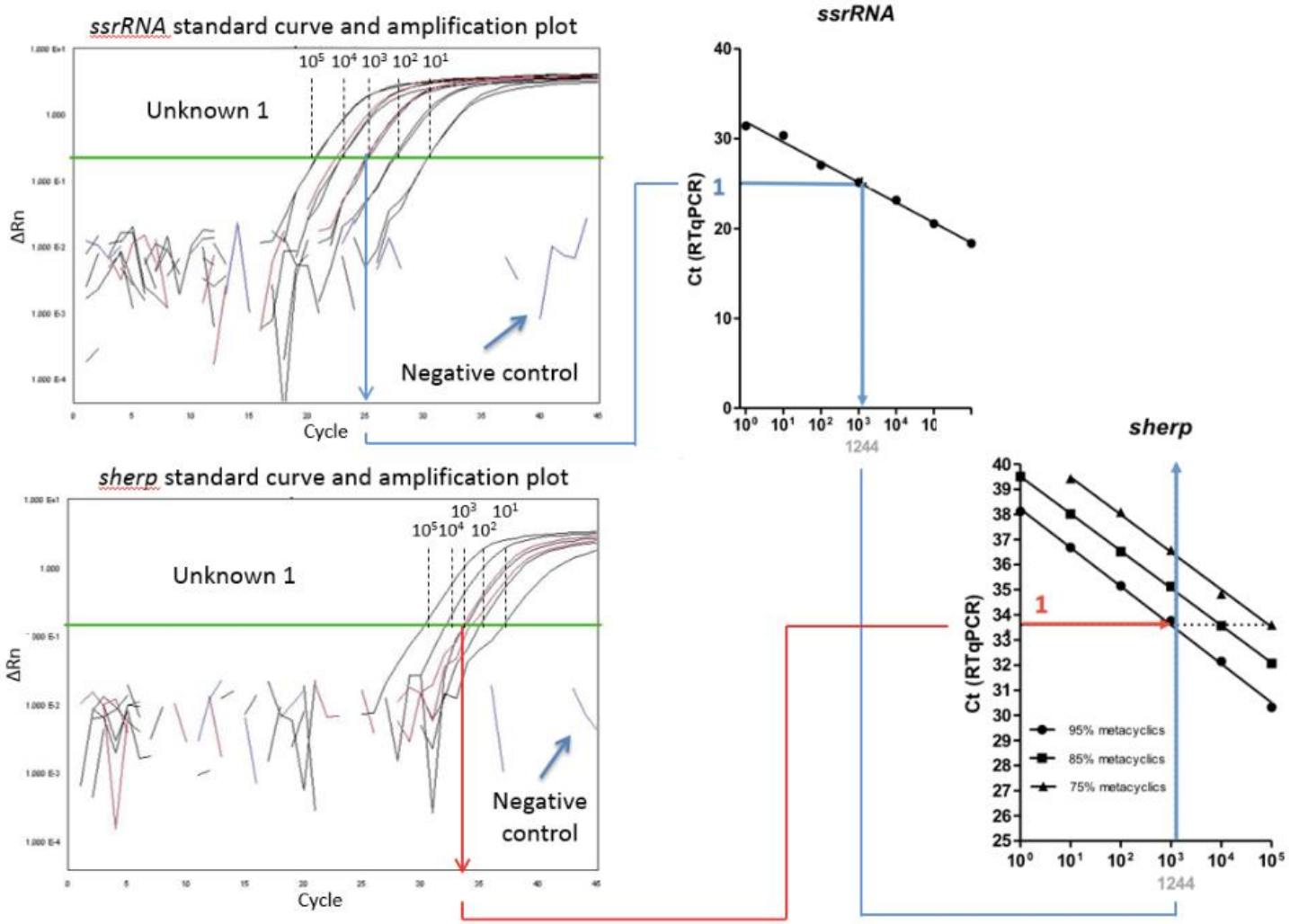
b. Identification of infectious *P. argentipes* - *L. donovani* metacyclic stage:

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

2. Xenomonitoring



Metacyclic donovani ID



Supplementary figure 5: E. Giraud et al., 2018

Objectives 3 & 4

- Collected data used to calculate:
  - Entomological inoculation rate
  - Critical infection thresholds
  - Natural rates of infection and infectiousness
- These measures will be used to inform the development of transmission endpoint assessment guidelines and transmission models

Objectives 3 & 4 cont.

- 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

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