The authors have no conflicts of interest to declare.

Prevalence of asymptomatic *Leishmania* infection in people living with HIV (PLHIV) and progression to symptomatic visceral leishmaniasis in Bihar, India

Raman Mahajan, Sophie Owen, Shiril Kumar, Shahwar Kazmi, Pradeep Das, Krishna Pandey, Neena Verma, Vikash Kumar, Amit Harshana, Estrella Lasry, Laura Moretó Planas, Emily Adams, **Sakib Burza**

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Visceral leishmaniasis (VL) in India - background

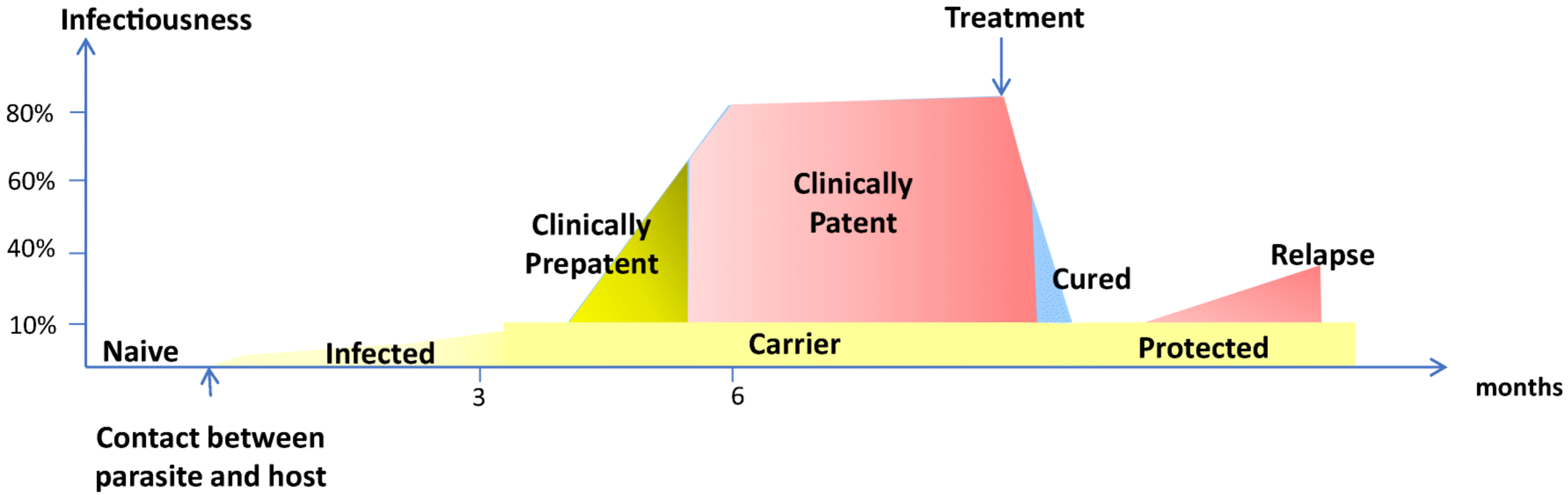
Image credit: Maps of India

- Parasitic disease endemic in northern India, ultimately fatal
- 80-90% of Indian VL cases occur in Bihar
- VL target of an elimination campaign on the Indian Subcontinent (ISC)
- Anthroponotic transmission on the ISC
- Typical NTD, with limited treatments, diagnostics and interest

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Typical evolution of the infection/disease process resulting in an asymptomatic condition (yellow) or clinically diagnosable disease (red)



- Asymptomatic *Leishmania* infection (ALI) 4-17 times more prevalent than symptomatic VL
- Risk of progression to symptomatic VL between 1.5 to 25%

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

Alvar, J., Alves, F., Bucheton, B. et al. Implications of asymptomatic infection for the natural history of selected parasitic tropical diseases. Semin Immunopathol 42, 231–246 (2020). https://doi.org/10.1007/s00281-020-00796-y

SCIENTIFIC DAYS

VL-HIV coinfection in India

- Evolving issue in the Indian setting: up to 20% of newly reported adult VL cases in highly endemic districts
- Most VL-HIV cases present in late stages of illness, all with advanced HIV: mutually reinforcing diseases
  - Challenges in diagnosis, double stigma
  - Higher rates of treatment failure, relapse & mortality
  - VL-HIV co-infection shown to be strongly associated with transmission at the village level
- **No evidence exists in the ISC on asymptomatic leishmania infection (ALI) in PLHIV**

Study aims

To determine the prevalence of asymptomatic *Leishmania* infection in HIV cases registered at ART centres from highly VL endemic areas of Bihar and to determine the rate of progression of asymptomatic *Leishmania* cases into symptomatic VL over a period of 18 months.

This study was approved by the ethics boards of the Rajendra Memorial Research Institute of Medical Sciences, Patna, India, and Liverpool School of Tropical Medicine, UK, and the MSF Ethics Review Board. Clinical Trial Registry-India number, CTRI/2017/03/008120

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Study design

Cross sectional cohort: what is the prevalence of ALI in PLHIV?

- 1,296 consecutive consenting adult PLHIV from 4 highly endemic VL district ART centres in Bihar
- Living in a village that had reported a case of VL in the previous 24 months
- No current or previous diagnosis of VL
- Screened for ALI by serological (rK39 ELISA & rK39 RDT), and/or molecular methods (qPCR)

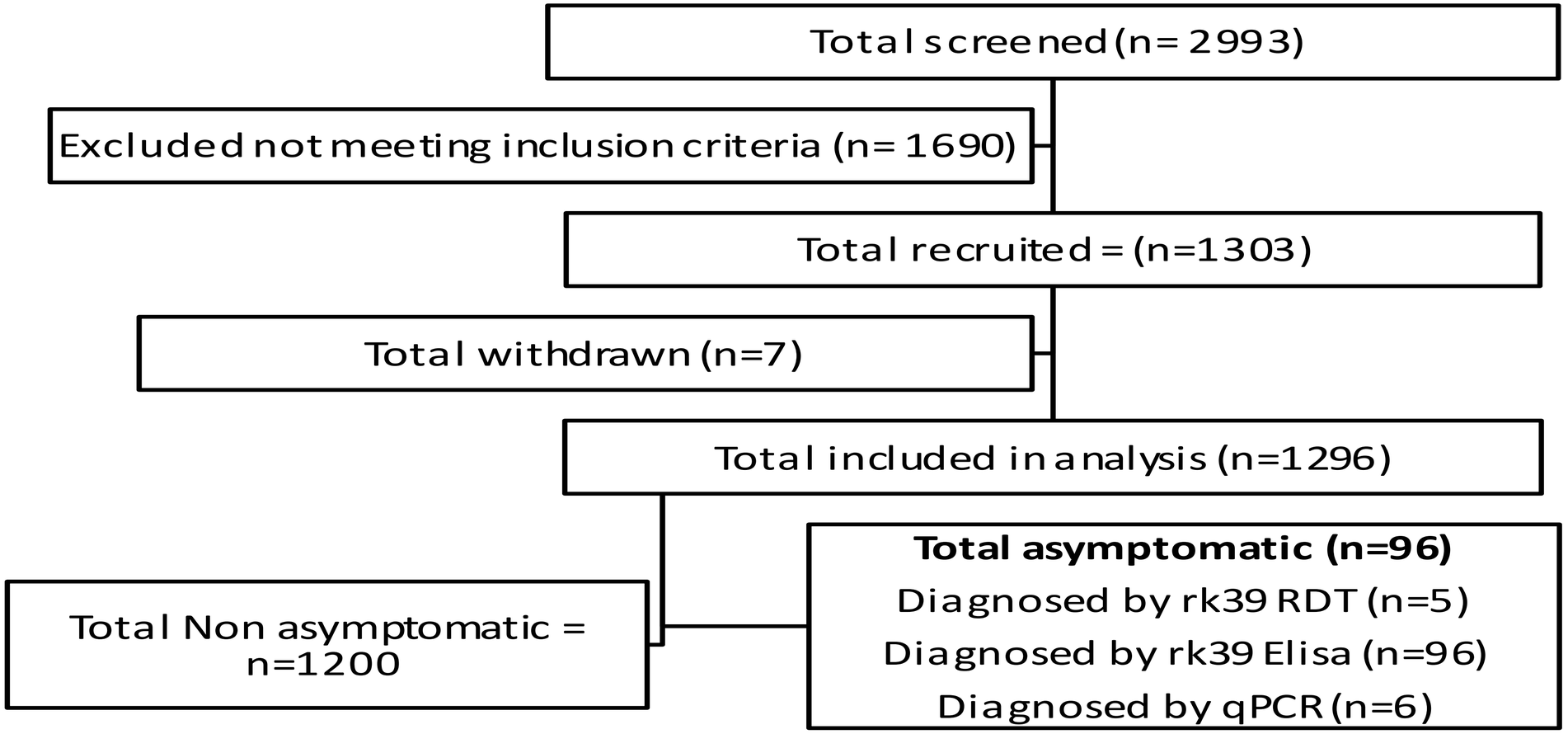
Prospective cohort: What happens to ALI in PLHIV over 18 months?

- Those with ALI to be followed up every 3 months for 18 months in person
- Primary endpoint: Diagnosis as symptomatic VL upto 18 months
- Those without ALI followed up by telephone every 3 months for 18 months to check health status

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Flow Diagram – Cross sectional survey



MEDECINS DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

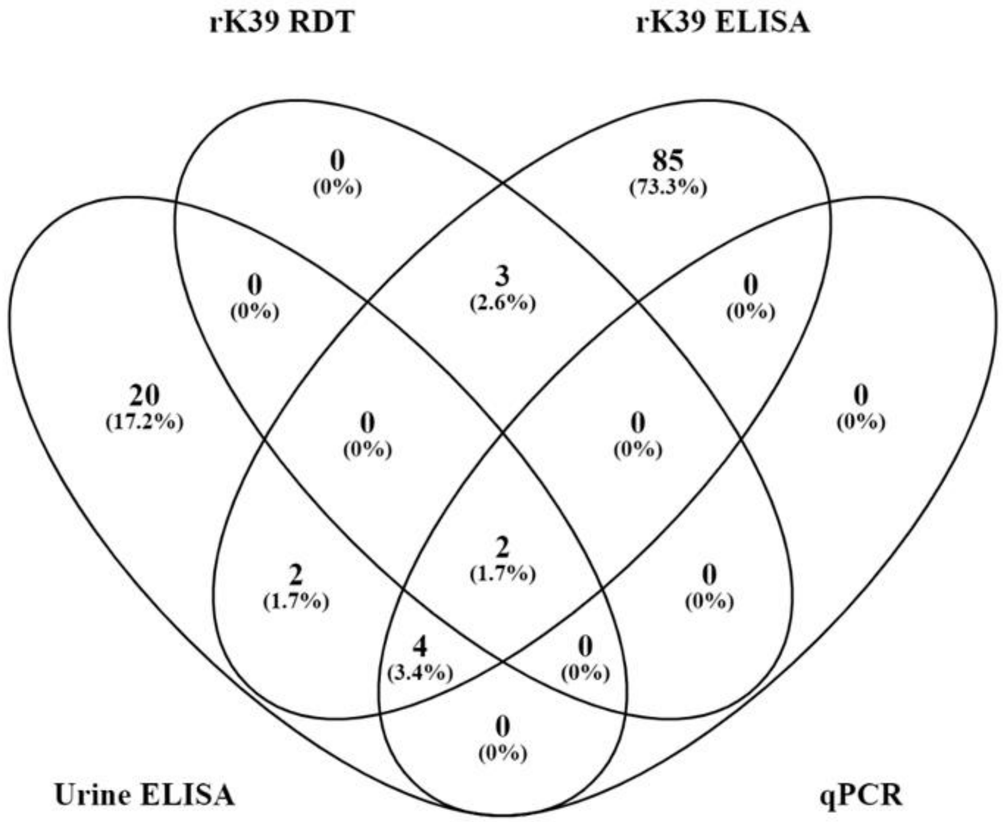
Diagnostics basket of choice

- rK39 RDT and ELISA detect anti-*Leishmania* antibodies present in the blood of patients with *Leishmania* infection. RDT may fail to detect low antibody titres and cannot be used in relapses
- Quantitative polymerase chain reaction (qPCR) considered a proxy for parasite load. Highly sensitive, but currently used only in research as no standardization in VL
- Leishmania antigen ELISA – Urine – novel non-invasive test to detect *leishmania* antigens in the urine

Prevalence of asymptomatic *Leishmania* infection in PLHIV living in highly endemic VL areas

Number of HIV patients testing positive for asymptomatic *Leishmania* infection

	N	%
Total recruited	1296	
Total **asymptomatic *Leishmania* infection**	96	7.4
Total positive with rK39 RDT	5	0.4
Total positive by rk39 ELISA	96	7.4
Total positive by qPCR	6	0.5
(Total positive by *Leishmania* antigen ELISA in urine	28	2.2)



MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Predictors of asymptomatic *Leishmania* infection

	All participants N (%)	Non-*Leishmania* infected N (%)	Asymptomatic infection (ALI) N (%)	Odds Ratio (95%CI)	P value
CD4 (cells / µL)					
< 100	35 (12.7)	29 (2.4)	6 (6.3)	**3.1 (1.2, 7.6)**	**0.012**
100 - 199	104 (8.0)	91 (7.6)	13 (13.5)	**2.1 (1.1, 4.0)**	**0.019**
200 - 299	179 (13.8)	164 (13.7)	15 (15.6)	1.4 (0.8, 2.4)	0.316
≥ 300	978 (75.5)	916 (76.3)	62 (64.6)		
Household size					
< 5	395 (30.5)	376 (31.3)	19 (19.8)		
≥ 5	901 (69.5)	824 (68.7)	77 (80.2)	**1.8 (1.1, 3.2)**	**0.016**
Number of IRS in last 18 months					
0	140 (10.8)	133 (11.1)	7 (7.3)	0.5 (0.2, 1.2)	0.103
1	132 (10.2)	128 (10.7)	4 (4.2)	**0.3 (0.1, 0.9)**	**0.020**
2	642 (49.5)	593 (49.4)	49 (51.0)	0.8 (0.5, 1.3)	0.317
> 2	382 (29.5)	346 (28.8)	36 (37.5)		

Factors <u>not</u> associated with ALI: Age, sex, Socioeconomic status, type of house, proximity to pond/livestock, month of IRS spray, use of bed nets, ART usage status, TB infection, BMI

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Prospective cohort: Progression to symptomatic infection

- 3.7% (4/109) participants progressed from asymptomatic to symptomatic infection over 18 months
- 3 out of 4 progressed within 3 months; 1 at month 12
- Conversion rates of participants identified as positive:
  - rK39 ELISA - 3.7% (4/109)
  - rK39 RDT - 40% (2/5)
  - qPCR - 57% (4/7)
  - *Leishmania* antigen ELISA - 14% (4/29)
- Risk of all-cause mortality in ALI 6.4% (n=7) compared with 2.5% (n=30) in those without (risk ratio, RR, 2.6, 95% CI 1.2-5.7, p=0.018)

Conclusions and recommendations

- PLHIV living in highly VL-endemic areas have a relatively high prevalence of asymptomatic *leishmania* infection in India
- Progression rates to symptomatic infection appear relatively low
- None of currently available diagnostic tools useful in predicting progression
- However, all cause mortality rates higher compared to non-*Leishmania* infected
- More studies needed to investigate impact early ‘prophylactic’ treatment of ALI in PLHIV considering poor outcomes in VL-HIV patients
- Role of screening within advanced HIV diagnostic packages in endemic areas?