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Epidemiology of *Leishmania donovani* infection in high-transmission foci in Nepal

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Summary

OBJECTIVE Nepal reports a visceral leishmaniasis (VL) incidence of 5 per 10 000 per year on the basis of notification by health facilities, but little community-based epidemiological information exists. We report data on prevalence rates of *Leishmania donovani* infection in ten communities in East Nepal.
METHODS Ten clusters with highest VL incidence rates were purposefully selected in Nepal. All households were mapped and socio-demographic data and data on past VL incidence were collected. An exhaustive serological survey was performed of individuals aged >2 years, by collecting finger prick blood on filter paper in November–December 2006. The samples were tested by direct agglutination, and a titre ≥1:1600 was taken as marker of infection. A generalized estimating equation (GEE) model was used to assess risk factors for Direct Agglutination Test (DAT) positivity taking into account the clustering at household and village level.
RESULTS The sero-survey (n = 5397) showed an infection prevalence rate of 9%, (range 5–15% per cluster) with higher prevalence in men (9.9%) than in women (8.3%) (P = 0.049). Male gender, increasing age and poverty were significant risk factors in the final GEE model.
CONCLUSION *Leishmania* infection rate in high-transmission areas in Nepal is associated with gender, age and socio-economic status.

keywords *Leishmania donovani,* infection, epidemiology, Nepal, kala-azar, DAT

Introduction

Visceral leishmaniasis (VL) or kala-azar, first officially recorded in Nepal in 1980 from one district (Bista 1998), is now endemic in 12 districts in the central and eastern Terai lowlands bordering North Bihar with an estimated eight million population at risk. In Nepal, VL is caused by *Leishmania donovani* and is transmitted by *Phlebotomus argentipes*. Its transmission cycle is considered to be anthroponotic with humans being the only known reservoir (Lawyer 1992). A national kala-azar control programme has been operational in Nepal since 1993 with as main strategies early diagnosis and treatment along with vector control using indoor residual spraying (IRS). However, control efforts conducted between 1993 and 2005 have not had any detectable impact on the incidence of the disease (Figure 1). Since 2005, the country participates in a regional kala-azar elimination initiative that is jointly implemented with Bangladesh and India (TDR 2005). The target of this campaign is to reduce the incidence rate of VL below 1:10 000 per year by 2015 in endemic districts.

Asymptomatic *Leishmania* infections are described in various foci over the world, and the ratio of asymptomatic to clinical cases has varied from 1:2.4 to 18:1 (Badaro *et al.* 1986; Zijlstra *et al.* 1994). The role of these asymptomatic infections in the transmission of VL is not well understood. They have been associated with high PCR positivity (Fakhar *et al.* 2008; Bhattarai *et al.* 2009), and transmission to hamsters by inoculation has been demonstrated in experimental animal studies (Moshfe *et al.* 2009). A proportion of the asymptomatic infections may subsequently progress to clinical disease (Badaro *et al.* 1986). So far, no specific control strategy is targeting asymptomatic infected persons.

There is sparse information available on the epidemiology of VL or *Leishmania* infection in Nepal, except for two small studies from the eastern region. In a sero-survey conducted in 1996–1997 in two villages, a *Leishmania*

[†]Deceased.

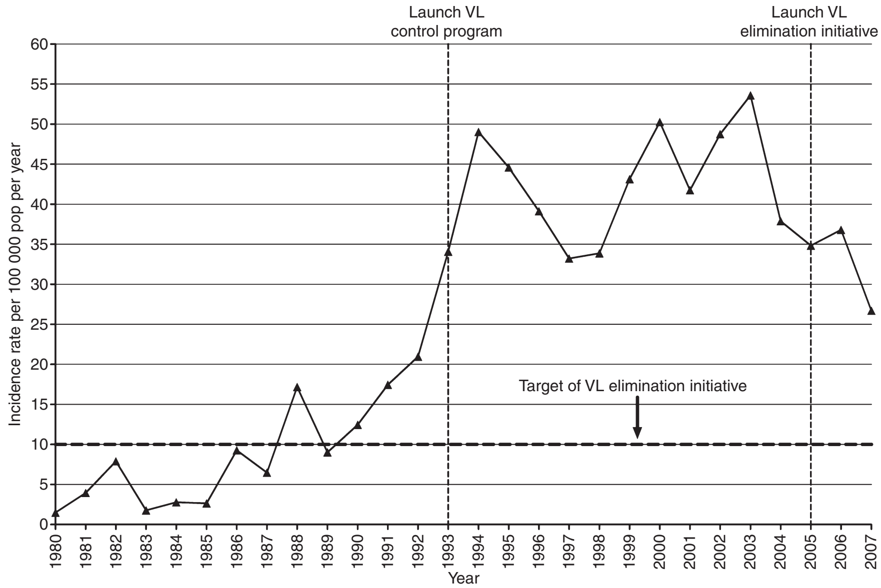

Figure 1 Evolution of visceral leishmaniasis (VL) reported yearly incidence in Nepal from 1980 to 2007. Source: Annual Reports, Ministry of Health and Population, Nepal.

infection rate of 6.1% (n = 1083) was found with a male to female ratio of 1.44:1 (Koirala *et al.* 2004). Another survey on a random sample of 373 persons from one village in the same region found an infection rate of 7.5% (Schenkel *et al.* 2006). Lack of adequate epidemiological data is considered as a major handicap in the proper implementation of the control programme in Nepal (Bista 1998). We describe here the epidemiology of *Leishmania* infection and disease in high-transmission areas in eastern Nepal, on data obtained within the framework of a community intervention trial.

Materials and methods

Background

House spraying with residual insecticide is recommended by the WHO (WHO 1990) against endophilic sand fly species. However, the effectiveness and sustainability of this intervention are of concern. Alternatively, insecticide-treated nets (ITNs) are one of the most effective methods of reducing peridomiciliary transmission of vector borne diseases. A community intervention trial of ITNs, named KALANET, was launched in Nepal and India in 2005 (http://www.ClinicalTrials.gov CT-2005-015374). Prior to the intervention, we assessed the epidemiology of *L. donovani* infection by cross-sectional survey in the study clusters, as reported below.

Selection of clusters

In Nepal, the study was conducted in the Terai (lowlands) region of three eastern districts (Morang, Saptari and Sunsari). Based on records of VL notifications from the previous years kept at the District Public Health Offices, the twenty most endemic wards (corresponding to a subunit of a village development committee, the smallest administrative unit in Nepal) were identified in September 2005. From February to April 2006, trained field workers undertook a house-to-house survey in these 20 wards to record the number of VL cases over the previous 3 years. Within those wards, a study cluster of around 500 persons–usually corresponding to a hamlet or an entire village–was defined on the basis of existing boundaries e.g. road, stream, etc. The VL incidence in each cluster was recorded. In May 2006, the ten clusters with the highest VL incidence rates were selected from the list of 20, on the basis of the following criteria: (i) population between 300 and 1500 (ii) a minimum distance of ≥1 km between clusters, (iii) VL cases being reported during each of the previous years, (iv) a minimum average 0.8% VL incidence rate over the past 3 years, and (v) being accessible by road.

Data collection

House-to-house demographic and immunological surveys were conducted in the 10 selected clusters in July–October and November–December 2006, respectively. The immunological survey was timed to take place at the end of the second annual *P. argentipes* density peak (i.e. October) (Picado *et al.* 2010). Informed written consents to participate in the KALANET study were obtained from the heads of households and from each of the individuals over 2 years of age in the demographic and immunological surveys, respectively. For children under 16, the consent of the parents or guardians was obtained. Data were collected by trained field workers and nurses using a semi-structured questionnaire. The study population included inhabitants of all the households above 2 years of age. During the survey, a unique household identification number was given to each household. Data on age, gender, family size, occupation, household characteristics, livestock and past history of VL were recorded. All participants were identified with a unique code.

Socio-economic index

A composite index describing the socio-economic status of households in VL endemic foci in Nepal was constructed through the use of principal components analysis (PCA). Households were then divided into quintiles of equal size based on the values of the index. The index was calculated on the basis of variables describing ownership of consumer durables, dwelling characteristics and demographic variables. Variables on consumer durables included ownership of a bed, a bicycle, a car, a scooter, a radio and a television. Dwelling characteristics included the total number of living rooms in the house, whether the house had electricity and the type of house (*kacha*, semi-*pakka* or *pakka*). Demographic variables included the total number of household members and the age of the household head. We also included a variable describing the type of cooking fuel used in the household. A complete description of the methods is given by Boelaert *et al.* (2009).

Nutritional status

Age, gender and anthropometric measures (height and weight) of consenting individuals were noted. Weight was measured in kilograms using an electronic scale (SECA 872, Hamburg, Germany) and height was recorded in centimetres using a measuring board for infants, children and adults (Promes-MSF-Holland, the Netherlands).

Individuals were classified as moderately or severely malnourished using 3 indices according to their age. Body Mass Index (BMI) was calculated for adults (>19 years). For 0- to 5- year-old children, weight-for-height *z*-scores were computed and for individuals aged 6 to 19 , BMI-for-age *z*-scores were calculated using WHO growth references (WHO 2006). Zero to 19- year-old individuals were categorized as severely or moderately malnourished when *z*-scores were less than -3 and between -3 and -2, respectively (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html). Similarly, adults were classified using BMI below 16 and between 16 and 17 as severely and moderately malnourished, respectively. *Z*-scores above 5 or below -5 were considered outliers and were not included in the analyses.

Ascertainment of past or present kala-azar

Subjects with fever lasting for 2 weeks or more were examined by a physician and tested with a rapid diagnostic test for VL (Kala-azar Detect™ Rapid Test; InBios International, Seattle, WA). Past history of VL treatment was recorded after verification of patient's prescriptions and case records.

Laboratory analysis

Only individuals older than 2 years who lived permanently in the cluster (defined as more than 6 months a year) were included in the serological study. A trained nurse obtained capillary blood by fingerprick from each eligible person and collected the sample onto pre-printed Whatman # 3 filter paper. The dried filter papers were placed in plastic bags containing silica gel and transported on into storage at −20 °C at the B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan. In the BPKIHS laboratory, the DAT was performed by using a freeze-dried antigen of fixed, trypsin-treated and stained promastigotes of *Leishmania donovani* obtained from ITM-Antwerp as described by Jacquet *et al.* (2006). A DAT titre ≥1:1600 was taken as marker of infection.

Statistical analyses

The characteristics of the study population are described, and DAT results were tabulated by cluster, VL history and demographic variables. Groups were compared by chi-square (χ^2) test. The prevalence of DAT positive was modelled by logistic regression using a generalized estimating equation (GEE) approach to take the clustering in households and villages into account. The prevalence of positive DAT was modelled by including an effect of village (cluster) and explanatory variables (age group, gender, asset score group, nutrition and whether the household had cows, buffaloes or goats) while taking into account that the data are not independent but correlated within the household and, to a lesser extent, within the

Cluster name	No. of households	Total population	VL cases (year wise) 2003	2004	2005	Total VL cases	VL incidence rate
Amahibelha	230	1153	18	7	3	28	0.81
Aurabani	125	528	12	19	6	37	2.34
Bhokraha	118	612	17	8	4	29	1.58
Dharan -14	172	804	1	40	19	60	2.49
Dharan -17	92	455	1	13	8	22	1.61
Duhabi	94	464	19	8	2	29	2.08
Joganiya	170	870	15	9	2	26	1.00
Pathari	138	712	1	7	21	29	1.36
Sanischare	102	578	1	5	10	16	0.92
Tanmuna	90	489	6	2	4	12	0.82
Total	1331	6665	91	118	79	288	1.50

Table 1 Characteristics of ten study clusters included in the KALANET community trial, East Nepal, 2006

cluster. The initial model was simplified by stepwise testing (using a Wald type test) and removing variables that were not statistically significant (P-value < 0.05). All statistical analyses were performed with STATA 10 (StataCorp LP, College Station, TX, USA)

Ethical approval

The study was conducted as part of the KALANET community intervention trial (Clinicaltrials.gov CT-2005-015374). Ethical approval was obtained from the Institutional Review Board (IRB) of the B.P. Koirala Institute of Health Sciences and from the IRB of the Institute of Tropical Medicine, Antwerp, Belgium.

Results

The characteristics of the 10 study clusters are shown in Table 1. There were two clusters from Morang, one from Saptari and seven from Sunsari district (Figure 2). The mean annual incidence rate of VL in the clusters over the 3 years prior to the study varied from 0.81% to 2.49%. The initial census registered 1331 households and 6665 people in total, ranging from 96 to 241 households per

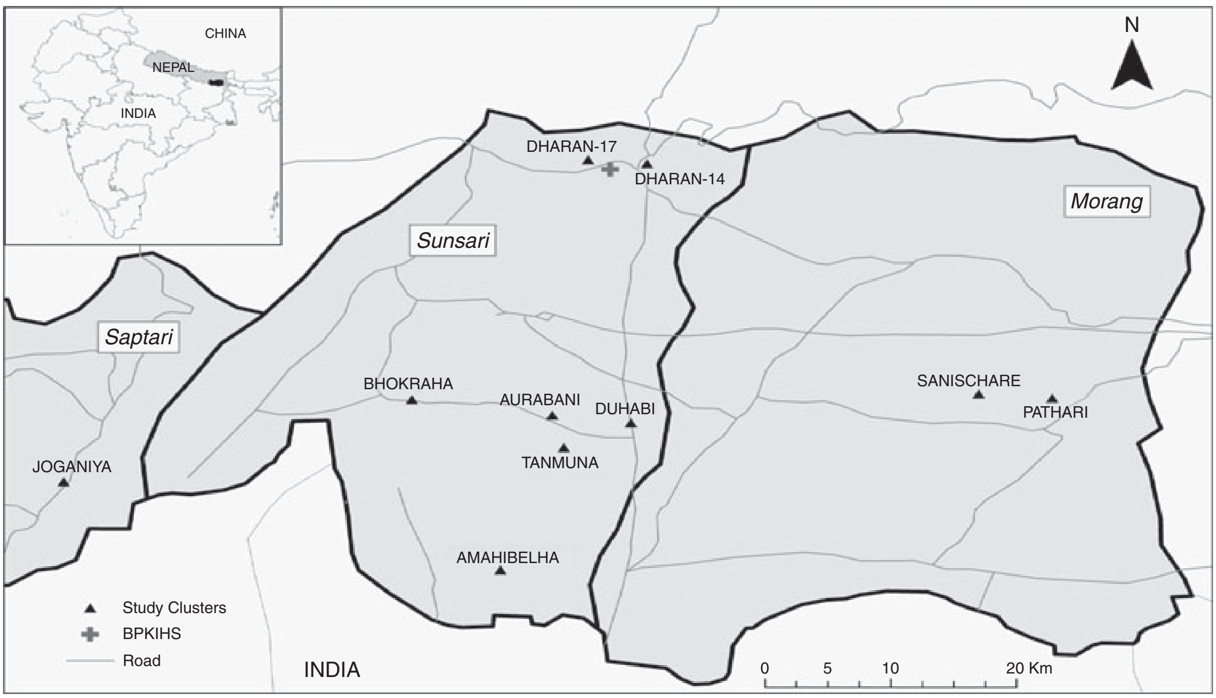

Figure 2 Geographical location of the 10 study clusters selected in the KALANET community trial, East Nepal, 2006.

cluster and a mean of 4.9 individuals per household finally included in study. The characteristics of the households included in the study are summarized in Table 2. During the subsequent demographic survey, some extra households were registered, bringing the total study population to 6985.

A DAT result was obtained for 5397 of 6985 (77.2%) registered persons (Figure 3). The individuals who refused to participate were evenly distributed by gender but were mainly children (36% were aged <6) and over 40 years old (27%). In the population finally studied, there were more women than men, the median age was 22 , 5.1% reported a past history of VL and 13.8% of the study population were malnourished (4.9% severely) (Table 3).

Overall, 9% of the study persons (n = 5397) were DAT positive, varying between 4.7% and 15.2% in clusters (Table 4). Men had a higher percentage of DAT positivity (9.9%) than women (8.3%) (P = 0.049). The prevalence of infection increased with age group from 2.7% in 2 to 6 years, 7.5% in 7 to 13 years, 7.8% in 14 to 24 years, 10.8% in 25 to 39 years to 13.3% in the over 40 years age group when men and women were combined (P < 0.001). No clear association was found between nutritional status and DAT results with 8.9% DAT positivity in normal (n = 4570), 10.2% in moderately (n = 479) and 8.0% in severely malnourished individuals (n = 263)) (P = 0.547).

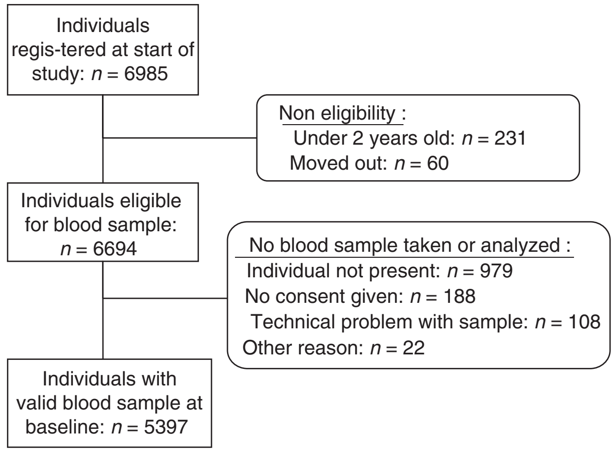

Figure 3 Flow diagram of individuals registered in the KALANET community trial and providing valid blood sample during the initial immunological survey, East Nepal, 2006.

Table 2 Household characteristics in the study clusters included in the KALANET community trial, East Nepal, 2006

Household characteristics	N	%*
Education of head of household		
Illiterate	686	49.00
Primary School	462	33.00
More than primary	251	17.93
Missing	1	0.07
Occupation of head of household		
Farmer	428	30.57
Business/service/skilled worker	154	11.00
Unskilled worker	707	50.50
Other	111	7.93
Type of house		
Thatch	190	13.57
Mud	903	64.50
Mixed or full cement	120	8.57
Wooden and others	185	13.21
Missing	2	0.14
Number of rooms in house	2.2†	1.4 (1–12)‡
Cows		
No	857	61.21
Yes	543	38.79
Buffaloes		
No	1292	92.29
Yes	108	7.71
Goats		
No	759	54.25
Yes	641	45.79

*Proportion (%) unless specified otherwise.
†Mean; ‡Standard deviation (range).

Table 3 Characteristics of individuals who provided a blood sample (n = 5397) during the KALANET initial immunological survey, East Nepal, 2006

Subject characteristics	N	%*
Gender		
Male	2499	46.30
Female	2898	53.70
Age	25.7†	18.30‡
Age Groups (years)		
2–6	677	12.54
7–13	1157	21.44
14–24	1109	20.55
25–39	1166	21.60
40+	1288	23.87
Nutrition		
Normal	4570	84.68
Moderate malnutrition	479	8.88
Severe malnutrition	263	4.87
Missing	85	1.57
Kala-azar ever		
Yes	277	5.13
No	5120	94.87
Kala-azar since 1 November 2004		
Yes	93	1.72
No	5304	98.28

*Proportion (%) unless specified otherwise.
†Mean; ‡Standard deviation.

Table 4 Prevalence of positive DAT by cluster during the KALANET initial immunological survey, East Nepal, 2006

	Positive DAT	
Cluster	*N*	%
C51	83	8.74
C52	58	13.88
C53	67	11.63
C54	62	9.13
C55	16	5.26
C56	60	15.19
C57	73	11.13
C58	28	4.76
C59	18	4.69
C60	24	5.37
Total	489	9.06

Of 5397 individuals with a valid DAT result, 317 were past VL cases and of them, 277 (88%) had a DAT result (Table 5). No active VL cases were detected during the survey.

Having a VL case in the household in the previous 24 months was a strong predictor for a positive DAT result in the bivariate analysis. Thirty-nine per cent of individuals (out of 439 people) living in houses with at least one kala-azar case from November 2004 to November 2006 were DAT positive. In contrast, only 7.1% of individuals (of 5058 people) in households without VL cases since November 2004 were serologically positive ($P < 0.001$). Ownership of domestic animals (i.e. cows, buffaloes and goats) was associated with a reduced risk of DAT positivity ($P < 0.001$), with fewer positive DAT in households owning animals (7.6%) than in households not owning them (12.0%), but this effect could be confounded by socio-economic status.

To model the prevalence of positive DAT while adjusting for potential confounders, a logistic regression model was fitted using GEE methods to take clustering within households and villages into account. The initial model included an effect of village (cluster) and the explanatory variables: age group, gender, asset score group, nutrition and whether the household owned cows, buffaloes or goats. This initial model was simplified by removing nutrition and cows, buffaloes or goats from the model as their effects were not statistically significant when adjusting for cluster, age group, gender and asset score group. Gender was borderline significant and was retained in the model. The remaining factors cluster, age group and asset score group all had a highly significant effect. The resulting population odds ratios are seen in Table 6. Men had 21% higher odds of a positive DAT than women in the same cluster, age and asset score groups, but the effect is only borderline statistically significant (OR 1.21; 95%CI 1.00, 1.47). The odds of being positive increases with age and decreases with asset score group (Table 6).

Table 5 Prevalence of positive DAT results in individuals with or without past history of visceral leishmaniasis (VL), eastern Nepal, 2006

	Positive DAT		Negative DAT		Undetermined DAT	
Past history of VL	*N*	%	*N*	%	*N*	%
Past VL after 01.11.2004	90	96.8	3	3.2	0	0.0
Past VL before 01.11.2004	173	94.0	9	4.9	2	1.1
No past VL	226	4.4	4,894	95.6	0	0.0

Table 6 Adjusted population odds ratio for positive DAT during the KALANET initial immunological survey, East Nepal, 2006

	Adjusted odds ratio	95% CI
Gender		
Male	1.21	1.00–1.47
Female	1	
Age group (years)		
2–6	1	
7–13	3.40	2.04–6.00
14–24	4.42	2.65–7.81
25–39	5.06	3.08–8.82
40+	7.29	4.49–12.64
Asset score group		
1 (poorest)	3.86	2.78–5.39
2	2.91	2.12–4.03
3	2.12	1.53–2.94
4	1.28	0.90–1.81
5 (least poor)	1	

Discussion

For the first time in Nepal the epidemiology of *Leishmania donovani* infection has been described in an extensive community-based study. The overall *Leishmania* infection rate was 9% but there was wide variation among endemic clusters. This seroprevalence may be an underestimate of the true number of individuals who were exposed to *L. donovani*, as it is based on a single serological marker (i.e. DAT) and many exposed individuals may be only positive to Leishmanin Skin Test (LST) or may lose their seropositivity (Gidwani *et al.* 2009). In a previous survey in the same region, Schenkel *et al.* (2006) found a DAT seroprevalence of 7.5% and LST positivity of 13.2%. The rate of infection observed in Nepal is much lower than that in the neighbouring districts in Bihar State, India (Sundar *et al.* 2009). In India, kala-azar has been endemic for much longer with outbreaks noted since the late 19th century (Peters 1981). In contrast, the earliest report of the disease being endemic in Nepal was in the mid-20th century (Shrestha & Pant 1994). Similarly, VL is considered to be less endemic in the eastern region of Nepal, where the study clusters were located, than in the districts bordering the Indian state of Bihar in the central region. Although the control programme implemented since 1993 has failed to lower the incidence of VL in Nepal, it may have prevented the number of cases to flare-up.

In our study we found a higher infection rate in men, and similar ratio has been observed in relation to VL cases (Bern *et al.* 2005). Women in these communities wear long dresses which could prove to be protective to some degree from sand fly biting.

Kala-azar has been always known to be a disease of the poorest of the poor, as proved in a recent study from Bihar, India (Boelaert *et al.* 2009). In our study too, the odds of *Leishmania* infection rose with decreases in the asset score group. Housing conditions (mud plastered house) and the environment (damp soil and organic debris) in these poor communities provide an excellent breeding site for sandflies. The relation of poverty and leishmaniasis is complex and involves multiple factors (Alvar *et al.* 2006), but lightening the leishmaniasis burden would help to alleviate poverty.

In our study malnutrition had no association with the infection rate, though we cannot yet conclude that it has no association with clinical VL. Malnutrition diminishes cell-mediated immunity and is a risk factor for VL in *L. infantum* endemic areas (Cerf *et al.* 1987). Follow-up of these infected individuals to determine the proportion of those who develop disease may provide a clearer picture in the future.

Kala-azar is a focal disease. In our study 39% of individuals living in a house with at least one recent VL case were DAT positive. The strategy for case finding in the current control programme is passive (i.e. only those who present themselves to the public health facilities). Given the high degree of clustering of infections, an active case finding strategy targeting family members of cases with VL may help in the early diagnosis and treatment. The efficacy and feasibility of this strategy, which would not only be beneficial for the individual case but also for the community by reducing disease transmission, deserve further studies. The role of asymptomatic infections in the transmission of kala-azar in the Indian subcontinent is not clear; thus, future research in this direction is warranted from the elimination initiative.

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Conflicts of interest

The authors have declared that they have no conflicts of interest.

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