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The epidemiology of *Leishmania donovani* infection in high transmission foci in India

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Summary

OBJECTIVE Visceral Leishmaniasis (VL) is highly prevalent in Bihar, India. India and its neighbours aim at eliminating VL, but several knowledge gaps in the epidemiology of VL may hamper that effort. The prevalence of asymptomatic infections with *Leishmania donovani* and their role in transmission dynamics are not well understood. We report data from a sero-survey in Bihar.

METHODS Demographic and immunological surveys were carried out in July and November 2006, respectively in 16 highly VL endemic foci in Muzaffarpur district in Bihar. Household and individual information was gathered and capillary blood samples were collected on filter papers. Direct agglutination test (DAT) was used to determine infected individuals (cut-off titre 1:1600). DAT results were tabulated against individual and household variables. A multivariate generalized estimating equation (GEE) model was used to study the prevalence of serologically positive individuals taking into account the clustering at household and cluster levels.

RESULTS Of study subjects 18% were DAT positive, and this proportion increased with age. Women had a significantly lower prevalence than men >14 years old. Owning domestic animals (cows, buffaloes or goats) was associated with a higher risk of being DAT positive [OR 1.16 (95% CI 1.01–1.32)], but socio-economic status was not.

CONCLUSIONS Prevalence of leishmanial antibodies was high in these communities, but variable. Demographic factors (i.e. marriage) may explain the lower DAT positivity in women >14 years of age. Within these homogeneously poor communities, socio-economic status was not linked to *L. donovani* infection risk at the individual level, but ownership of domestic animals was.

keywords epidemiology, kala-azar, India, *Leishmania donovani*, infection, direct agglutination test (DAT)

Introduction

Visceral Leismaniasis (VL), known as kala-azar (KA) in the Indian subcontinent, is a neglected vector-borne parasitic disease caused by *Leishmania donovani* and affecting an estimated 500 000 new cases with 59 000 deaths per year worldwide (Davies *et al.* 2003). More than 90% of the VL cases are reported from the Indian subcontinent and Sudan. Within India, the State of Bihar accounts for nearly 90% of the cases (Desjeux 1996). For more than three decades, there has been a continuous transmission of *L. donovani* in Bihar, and more than 200 000 cases have been reported in this state since 1977 (Ostyn *et al.* 2008). However, these officially notified cases are a serious underestimate of the real incident cases as shown by a study conducted in 2003 in Muzaffarpur district in Bihar, which documented a VL incidence rate of 2.5/1000, 8 times the official figure (Singh *et al.* 2006). Various authors have pointed to the gaps in the understanding of the VL epidemiology in the Indian subcontinent where risk factors for VL are not fully understood (Bern *et al.* 2000, 2005; Schenkel *et al.* 2006). VL in Bihar seems to have a focal distribution in place and time and occurs in small clusters with poor socio-environmental conditions and poor access to the health system

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(Boelaert *et al.* 2009). Post-Kala azar Dermal Leismaniasis (PKDL) has been incriminated as a factor contributing to the persistence of *L. donovani* transmission in India (Addy & Nandy 1992); however, there is little data on asymptomatically *L. donovani* infected individuals in India and their potential role in the VL cycle. There are no studies in India documenting the ratio between asymptomatic infections to clinical cases but the ratio in other regions ranges from 1:2.4 to 18:1 (Ali & Ashford 1994; Badaro *et al.* 1986; Evans *et al.* 1992; Zijlstra *et al.* 1994). All these factors are crucial to understanding the transmission dynamics of *L. donovani* and to design more effective control programs which are currently based on passive case detection and treatment of VL cases and vector control using insecticide residual spraying (IRS). In this study, we used demographic and serological data collected in a cross-sectional survey in 16 high endemic VL villages in Bihar to describe the *L. donovani* infection patterns and to understand the individual and household risk factors associated with *L. donovani* infection.

Materials and methods

Selection of clusters

Sixteen high VL endemic clusters (corresponding to a hamlet or a complete village) in Muzaffarpur district (Bihar state) were selected in a two-step approach. The first step in the selection of clusters was based on records of VL cases kept by public and private health facilities (Primary Health Centres, District hospitals, private and charitable hospitals) in Muzaffarpur district. In this first step, 35 clusters were selected, all with an estimated approximate annual incidence rate of 2 or more per thousand population during the previous 12 months. The final selection of clusters was carried out using the data collected in a house to house survey conducted in May 2006 to record the number of VL cases in the previous 3 years in those villages. Sixteen clusters with the highest VL incidence were selected out of 37 when the following conditions were met: (i) at least one case per year was reported since 2003, (ii) a minimum annual average VL incidence of 0.8% in the past 3 years, (iii) a population ranging from 350 to 1500 habitants and (iv) a minimum distance of 1 km between clusters. These 16 clusters were included in the KALANET community trial (Clinicaltrials.gov CT-2005-015374) aiming to assess the effectiveness of long lasting insecticidal nets (LN) to prevent clinical and subclinical VL infection in the Indian subcontinent. Figure 1 shows the location of the 16 selected clusters in Muzaffarpur district, Bihar. Data reported in this article correspond to the baseline data, collected prior to the intervention. All the activities were organized from the Kala-azar Medical Research Centre (KAMRC), a charitable hospital exclusively dealing with VL cases.

Demographic survey

In July 2006, a demographic survey was carried out in the 16 clusters to collect household and individual information. A structured questionnaire was used to gather socio-economic data in each of the households (i.e. household characteristics, head of the household information, household assets including domestic animals owned). Individual information (i.e. age, gender, anthropometric measures) was also collected. The data were collected by trained interviewers. The household data were used to build a composite index describing the socio-economic status of the households in the clusters, based on principal component analysis (PCA). The index was constructed using variables describing ownership of consumer durables (i.e. ownership of bicycle, radio and television), housing characteristics (i.e. type of house, availability of electricity and number of rooms in the house) and demographic variables (i.e. age head of household, number of household members). Households were categorized into five evenly distributed quintiles on the basis of the scores obtained from the PCA to visualize and analyse the distribution of wealth in the 16 communities. A detailed description of the method used to define household wealth has been described previously elsewhere (Boelaert *et al.* 2009).

Age, gender and anthropometric measures; height in centimetres and weight in kilograms recorded using an electronic scale (SECA 872, Hamburg, Germany) and measuring board (Promes-MSF-Holland, the Netherlands), respectively, were used to calculate individual nutritional indices. Adults (above 19 years old) were considered moderately or severely malnourished when their body mass index (BMI) was between 16 and 17 or below 16, respectively, as defined by WHO (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html) (WHO 2006). Similarly, children and teenagers were classified using z-scores: weight-for-height z-scores for children 0–5 years old and BMI-for-age z-scores for individuals from 6 to 19 years old based on WHO references (WHO Multicentre Growth Reference Study Group 2006; De Onis *et al.* 2007). Individuals with z-scores between −3 and −2 and below −3 were considered moderately and severely malnourished, respectively. Z-scores beyond ±5 were considered outliers and were not included in the analyses. No outliers were defined for BMI.

Immunological survey

Only individuals >2 years old who had lived in the cluster at least 6 months during the past one year prior to the

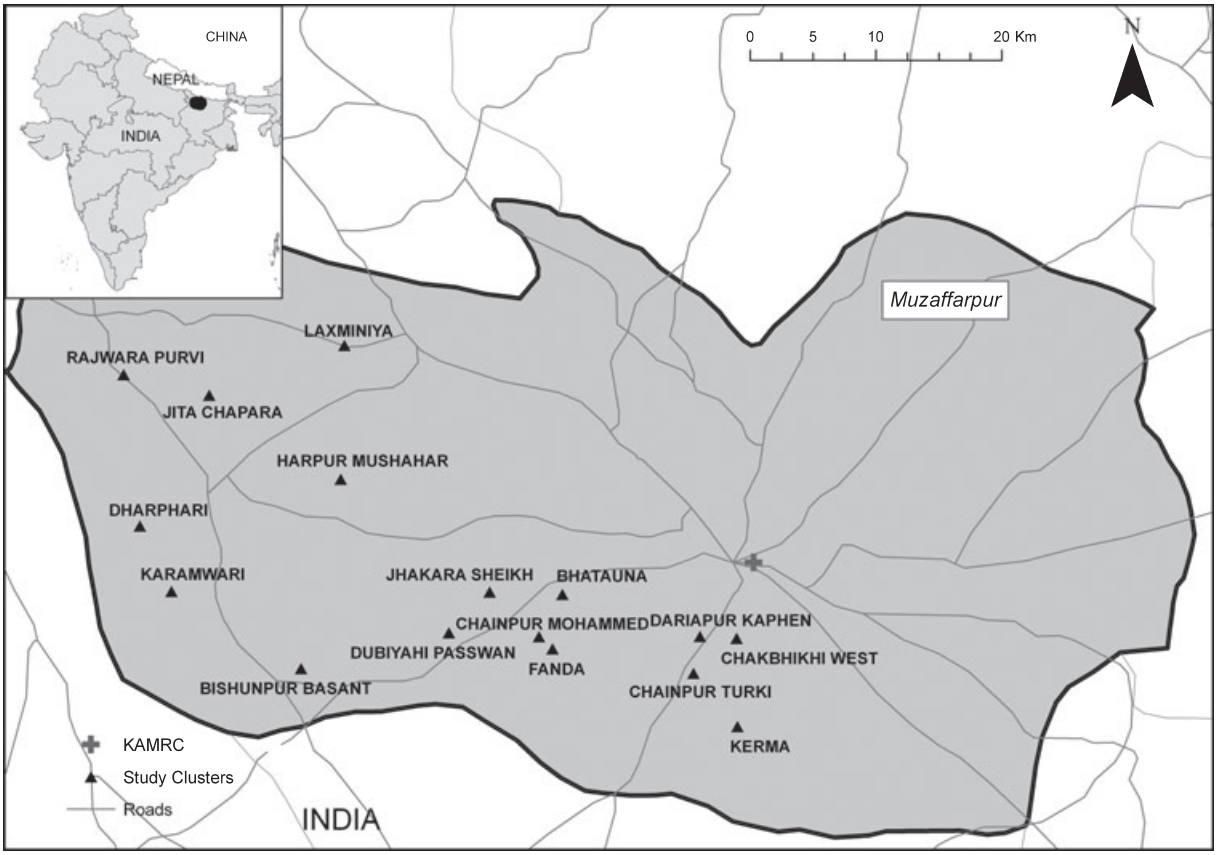

Figure 1 Map of selected clusters in Muzaffarpur district, India.

survey were eligible to participate in the immunological study. In November–December 2006, consenting individuals >2 years old were asked to provide a capillary blood sample collected on a filter paper (Whatman #3) by finger prick. Individual history on past VL was gathered; a questionnaire was completed for each one of the suspected cases to collect information on history of symptoms, diagnostic procedures, duration, mode of administration and type of drug use for treatment. All this information was verified using clinical records (i.e. discharge documents, prescriptions, hospital/health post records) when possible. All suspected cases were ascertained by a trained physician. Filter papers labelled with unique identifiers were transferred to the Banaras Hindu University (BHU), Varanasi for laboratory examinations. Blood samples were kept at −20 °C until the direct agglutination test (DAT) to detect anti-*L. donovani* antibodies was conducted. Filter papers were eluted, and the DAT was performed as described elsewhere (Jacquet *et al.* 2006; Bhattarai *et al.* 2009) using freeze-dried antigen suspension of trypsin-treated, fixed and stained promastigotes of *L. donovani* (Institute of Tropical Medicine – Antwerp). The cut-off for *L. donovani* infection was set at a titre of 1:1600 as used in previous epidemiological studies (Davies & Mazloumi Gavgani 1999, Saha *et al.* 2009). All data were double entered, checked and corrected using Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Statistical analyses

We described the serological status of the study population by cluster and other demographic factors (e.g. age group and gender). The chi square (χ^2) test was used to compare groups. A generalized estimating equation (GEE) model was used to study the prevalence of serologically positive individuals taking into account the clustering at household and cluster levels. The GEE model included DAT results (response variable) and age group, gender, composite index quintile, nutrition category and whether the household

owned cows, buffaloes or goats as explanatory variables, taking into account that the data were correlated within household and cluster. Past kala-azar was purposely not included in the model because of its strong collinearity with DAT positivity (Gidwani *et al.* 2009). The GEE used was a population-average model; comparing two randomly selected persons from the study population estimating the odds for the person with the exposure of interest compared to the person without the exposure but with the same values of the adjusting variables, taking into account that observations from the same cluster are likely to be correlated. A stepwise regression approach was used to get the final model by using a Wald type test and removing not significant (P-value<0.05) variables. All statistical analyses were carried out using Stata 10 (StataCorp LP, College Station, TX, USA) or SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Table 1 Characteristics of households in 16 study clusters in Bihar, India, July 2006 (n = 2047)

Household Characteristics	Value	%*
Gender of head of household		
Men	1903	93.0
Women	144	7.0
Age of head of household	45.3†	13.7‡
Religion of head of household		
Hindu	1823	89.1
Muslim	224	10.9
Education of head of household		
Illiterate	1374	67.1
Primary school	350	17.1
Beyond primary	322	15.7
Missing	1	0.1
Occupation of head of household		
Farmer	580	28.3
Business/service/skilled worker	383	18.7
Unskilled worker	977	47.7
Other	107	5.2
Type of house		
Thatch	997	48.7
Mud	425	20.8
Full or mixed cement	598	29.2
Other and Missing	27	1.3
Number of rooms in house	1.7†	1.1‡
Household with cows, buffaloes or goats		
Yes	1187	58.0
No	860	42.0
Households with Cows	481	23.5
Households with Buffaloes	330	16.1
Households with Goats	839	41.0

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

Ethical approval

Ethical approval was obtained from the Institutional Review Board (IRB) of the Institute of Medical Sciences, Banaras Hindu University and from the IRB of the Institute of Tropical Medicine, Antwerp, Belgium. Informed consent was obtained from the head of households as well as from the concerned individuals after fully explaining the purpose of the study and the extent of their involvement.

Results

A total of 2047 households and 12176 people were registered during the demographic survey in the 16 clusters. The main characteristics of the households in the selected clusters are summarized in Table 1. Nine out of 10 of the heads of household were Hindu and the majority (67%) was illiterate. They were mainly unskilled workers (48%) or farmers (28%) living in thatched (49%) houses with domestic animals, as 58% of them owned at least one cow, buffalo or goat. The average family size was 5.9 individuals per household. Further details on the socio-economic status of the households in those 16 clusters have been described elsewhere (Boelaert *et al.* 2009). Figure 2 presents the flow of participants: children <2 years old (n = 605) and 1248 who had migrated for more than 6 months prior to the survey were non-eligible. Men constituted 87.6% of this migrant population, with a mean age of 26.7 years, whereas in the registered population the proportion of men was 52.6% and mean age was 23.6 years. Of the 10323 eligible individuals, 8051 (78%)

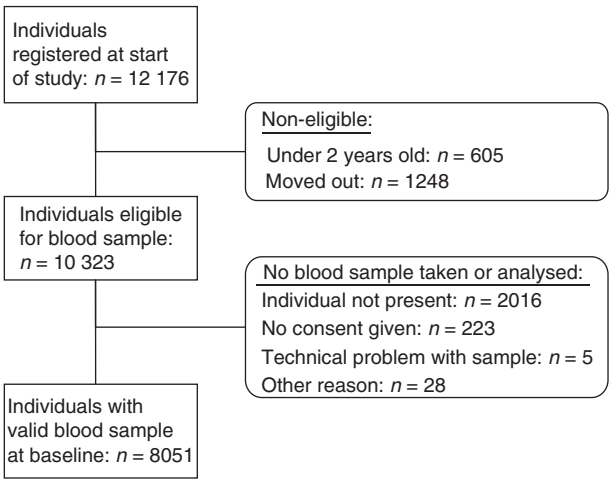

Figure 2 Flow diagram of individuals registered in the KALANET community trial and providing valid blood sample during the initial immunological survey, India, 2006.

gave a valid blood sample. The 2272 non-participating individuals were either not present on the day of survey (n = 2016), did not give consent (n = 223) or had other reasons (n = 33). Among non-participants, 62.5% were men, compared to 44.6% among the participants ($P < 0.001$) (Table 2). The main reason for under-inclusion of men in the study was that young men leave early in the morning for their job assignments and return only by late evening. The mean age of participants and non-participants was 24.6 and 24.3 years, respectively ($P = 0.45$). Logistic (i.e. distance from the nodal centre – KAMRC) and demographic (i.e. employment outside the villages) factors may explain the variation in participation rates (69–89%) observed in the study clusters (Table 3).

Among the population from which a blood sample was obtained, 11% and 6% were moderately and severely malnourished. Past VL was reported by 7.8% of the individuals, and 3.3% had had VL in the past two years.

Overall 18.5% of the blood samples were DAT positive, and the positivity ranged from 11% to 29% in the different clusters as shown in Table 3. The DAT results were undetermined in 6 (0.07%) cases, and these were excluded from further analysis. Men had a higher VL infection prevalence (20.7%) compared to women (16.8%) ($P < 0.001$). The prevalence of infection increased with age in both genders ($P < 0.001$); however, the prevalence was lower in women between 14 and 24 years of age compared to the previous age group as shown in Table 4. There were slightly more cases of severe malnutrition in the DAT-positive individuals (21%) compared to normal and moderately malnourished people (18% in both groups) ($P = 0.283$).

The DAT results of individuals with past VL are presented in Table 5; 93% of the persons with VL since

Table 2 Characteristics of individuals included in the sero-survey in Bihar, India, November–December 2006 (n = 8051)

Subject Characteristics	Value	%*
Gender		
Men	3589	44.6
Women	4462	55.4
Age	24.6†	19.9‡
Age groups (years)		
2–6	1567	19.5
7–13	1930	24.0
14–24	1096	13.6
25–39	1484	18.4
40+	1974	24.5
Nutritional status		
Normal	6650	82.6
Moderate malnutrition	876	10.9
Severe malnutrition	477	5.9
Missing	48	0.6
Moderate and severe malnutrition by age group		
2–6	153	9.8
7–13	357	18.5
14–24	140	12.8
25–39	182	12.3
40+	521	26.4
Kala-azar ever		
Yes	627	7.8
No	7424	92.2
Kala-azar since November 1, 2004		
Yes	269	3.3
No	7782	96.7

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

Table 3 Proportion of individuals with a positive DAT in Bihar, India, November–December 2006, by study cluster

	Individuals with valid blood sample, as % of eligible population		Positive DAT	
Cluster	N valid	% of eligible	N	%
C01	520	77.15	68	13.08
C02	642	84.14	122	19.00
C03	698	79.23	144	20.63
C04	810	78.03	92	11.36
C05	423	69.00	58	13.71
C06	325	80.65	51	15.69
C07	435	80.71	126	28.97
C08	352	69.29	76	21.59
C09	542	76.12	87	16.05
C10	309	69.59	74	23.95
C11	391	79.63	53	13.55
C12	794	85.38	171	21.54
C13	303	73.19	63	20.79
C14	437	69.26	96	21.97
C15	324	72.65	50	15.43
C16	746	89.23	159	21.31
Total	8051	77.99	1490	18.51

Table 4 DAT results by age group and gender, India

	Positive DAT					
	Men		Women		Missing	
Age Group	N	%	N	%	N	%
2–6	83	9.96	56	7.64	1	0.06
7–13	168	17.00	174	18.51	2	0.10
14–24	102	22.67	96	14.91	2	0.18
25–39	121	25.74	179	17.65	0	0.00
40+	268	31.68	243	21.56	1	0.05
Total	742	20.67	748	16.76	6	0.07

November 1, 2004 had a DAT-positive result compared to 85% for older cases and 13% for people with no past history of VL. Having suffered from kala-azar is strongly associated to a DAT-positive result. However, the socio-economic status was not associated with the serological status; the prevalence of DAT positives varied from 18, 19, 20, 19 and 17 per cent in the five quintiles of the wealth index in increasing order, from poorest to less poor.

Table 6 shows the distribution of DAT positivity by ownership of animals. Ownership of goats and buffaloes was significantly associated with DAT positivity, with *P*-value of 0.003 and 0.002, respectively, but ownership of cows was not ($P = 0.096$). When cows, buffaloes and goats are combined, there is still a slightly higher prevalence of DAT positives in the group with animals when compared to those without animals ($P < 0.001$).

The GEE approach modelled the probability of being DAT positive taking confounders and the data structure into account. The initial model included the main effects of cluster, age group, gender, cow-buffalo-goat, composite index score and nutrition. The latter two factors were removed from the model because they were not significant. The effect of cows, buffaloes or goats was borderline significant so it was retained. The three interactions between age group, gender and domestic animals were then added one at a time to the model with the main effects, and the only interaction found to be significant was the interaction between age group and gender. The final model for the probability of positive DAT included main effects of cluster and domestic animals (cow, buffalo or goat) and the interaction between age group and gender while taking the clustering in households and community into account. The estimated odds ratio for positive DAT was 1.16 (95% CI: 1.01, 1.32) for somebody in a household with cows, buffaloes or goats compared to a person of the same gender, age group and cluster but without domestic animals. Figure 3 represents the estimated probability of positive DAT (with 95% confidence intervals) plotted for gender and age group adjusted for cluster and cows, buffaloes or goats.

Table 5 DAT results by previous Kala-azar status, pre and since November 1, 2004

	Positive DAT		Negative DAT		Undetermined DAT	
Kala-azar	*N*	%	*N*	%	*N*	%
KA since November 1, 2004	250	92.94	19	7.06	0	0.00
KA before November 1, 2004	304	84.92	54	15.08	0	0.00
No KA	936	12.61	6482	87.31	6	0.08

Discussion

This is one of the first large cross sectional studies on *L. donovani* infection in India (Bihar) which may contribute to understanding the epidemiology of VL in high endemic foci. With the limited resources available, those are the villages that need to be targeted to control VL in the region. Eighteen per cent of the study population, resident in 16 VL foci, was DAT positive, pointing to infection at some point in the past by *L. donovani*. This figure is twice as high as in Nepal (Rijal *et al.* 2010) which may reflect different levels of VL endemicity, disease control or infection dynamics in high endemic foci between the two countries. In India, the present VL epidemic has been ongoing since the last 4-5 decades whereas in Nepal, the disease was first reported in 1980 (Chelala 2004). The strong variation in the prevalence of DAT positivity among clusters highly endemic for VL is congruent with the spatial clustering also observed in clinical KA cases (Bern *et al.* 2005).

Table 6 DAT results by ownership of animals in India

	Positive DAT		Negative DAT		Undetermined DAT		
Ownership of animals	*N*	%	*N*	%	*N*	%	*P* (χ^2 test)
No cows	1019	18.04	4626	81.89	4	0.07	0.096
Cows	471	19.61	1929	80.31	2	0.08	
No goats	746	17.29	3564	82.60	5	0.12	0.003
Goats	744	19.91	2991	80.06	1	0.03	
No buffaloes	1137	17.83	5236	82.11	4	0.06	0.002
Buffaloes	353	21.09	1319	78.79	2	0.12	
No cows/buff/goats	444	16.49	2247	83.44	2	0.07	0.001
Cows/buff/goats	1046	19.52	4308	80.40	4	0.07	

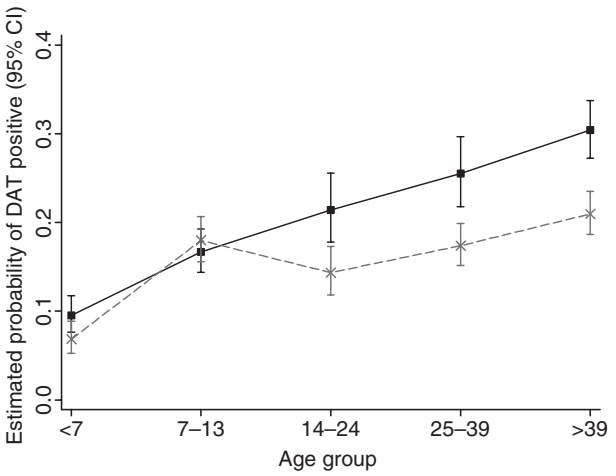

Figure 3 Estimated Prevalence of positive DAT by gender and age group adjusted for cluster and cows, buffalos or goats (Black squares = men, red crosses = women).

The marker of infection used in this study, the DAT, was extensively validated in the diagnosis of disease (Chappuis *et al.* 2006) and has been used in population surveys (Zijlstra *et al.* 1991; Chowdhury *et al.* 1993). We observed a strong association between DAT-positive results and past kala-azar history, as 93% of those who reported VL in the previous two years were still DAT positive on November 2006, a phenomenon described by Zijlstra *et al.* (1991) and others. Many people were DAT positive (12.6%) but did not have a history of past VL and thus, had an asymptomatic infection either present or past.

Only three factors remained significant risk factors in the multivariate model: gender, age and ownership of animals. Men had a higher risk of being DAT positive than women, possibly related to sleeping habits (i.e. use of less clothing during sleep, sleeping outdoors and close to animals), occupation (i.e. farming) or other behavioural factors (i.e. less use of protective measures against sandfly bites). The gender effect showed an interaction with age: there was a steady increase in the prevalence of DAT-positive individuals in both the genders over the age groups but there was a drop in prevalence among women between 14 to 24 years (Figure 3). In Bihar the residence rule for newly married couples is patrilocal, and thus the observed pattern could be linked to emigration of young newly married women to their husband's village, and immigration of fully susceptible women who join their husbands' family living in the high endemic cluster. According to the National Family Health Survey (NFHS-3) in the state of Bihar, 65% of rural women in the age group of 20–24 years were married below the age of 18 years (International Institute for Population Sciences and Macro International 2007). As per National Census 2001, the average age at marriage in the state was 17.2 years, rural and urban areas combined together (Office of the Registrar General & Census Commissioner, India, 2001). The epidemiological implication of this demographic dynamics could result into geographical expansion of the disease to areas where it is not endemic or less endemic, as the sandfly vector is quite ubiquitous in Bihar as suggested for other vector-borne diseases (Stoddard *et al.* 2009).

Owning domestic animals (which can be related to economic wealth) was related to an increased risk of DAT positivity in this survey, somewhat contrasting to other studies from the region. In Nepal, Bern *et al.* (2000) identified cows and buffaloes as protective factors, and in a subsequent study, in Bangladesh, the protective effect of cattle ownership did not reach statistical significance, but cattle density around the house was strongly protective (Bern *et al.* 2005). In a recent age- and neighbourhood-matched case–control study in India, specially designed to examine the role of keeping animals inside compounds, no association was found either to ownership or keeping animals inside the sleeping room (Singh *et al.* 2010). However, these studies cannot be compared to this study where we have estimated the association of animals with *L. donovani* infection, and not with clinical cases alone.

As already reported by Boelaert *et al.* (2009), VL-affected villages are the poorest of the poor in Bihar, but within these poor communities, socio-economic status does not seem to be related to prevalence of infection at the individual level.

Our study found high prevalence rates of infection in VL endemic foci in Bihar. The relationship between infection and clinical cases needs further study. Age, gender and ownership of animals were found as risk factor for leishmanial infection, but before the latter can lead to any intervention, the exact role of domestic animals in transmission needs further study, given the contrasting results found in the literature.

Acknowledgements

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Conflicts of interest

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

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