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RISK FACTORS FOR VISCERAL LEISHMANIASIS IN INDIA; FURTHER EVIDENCE ON THE ROLE OF DOMESTIC ANIMALS

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Summary

INTRODUCTION—Studies investigating risk factors for Visceral Leishmaniasis (VL) on the Indian Subcontinent have shown contradictory results related to the role of domestic animals. In some studies having animals in or around the house was a risk factor, in others it was protective. We investigated the specific hypothesis that keeping domestic animals inside the house at night is a risk factor for VL.

METHODS—The study was designed as an individually matched case control study. All VL cases diagnosed in the study area in Bihar, India between March 1st, 2007 and December 1st, 2008 were eligible. For each case we selected 2 random controls, with no history of previous VL; matched on sex, age group and neighborhood. Cases and controls were subjected to a structured interview on the main exposure of interest and potential confounders; a conditional logistic regression model was used to analyze the data.

RESULTS—We enrolled 141 cases and 282 controls. We found no significant associations between VL and keeping domestic animals inside the house (OR of 0.88 for bovines and 1.00 for 'any animal') or ownership of domestic animals (OR of 0.97 for bovines and 1.02 for 'any animal'). VL was associated with housing conditions. Living in a thatched house (OR 2.60, 95% CI 1.50–4.48) or in a house with damp floors (OR 2.60, 95% CI 1.25–5.41) were risk factors, independently from socio economic status.

CONCLUSION—Keeping animals inside the house is not a risk factor for VL in Bihar, India. Improving housing conditions for the poor has the potential to reduce VL incidence.

Introduction

Several studies have been conducted on the Indian subcontinent (Bangladesh, India, Nepal) trying to identify factors associated with Visceral Leishmaniasis (VL); some using *Leishmania donovani* infection as end point, others VL disease. Damp floors or 'dampness in the home' were risk factors in a study by Bern *et al.* (2000) looking at clinical disease and in a study by Saha *et al.* (2008) looking at infection documented by a positive direct agglutination test (DAT). Sleeping under a bednet was protective in the above mentioned study by Saha e*t al.* (2008) and in 2 studies by Bern *et al.* (2000, 2005); both studies by Bern *et al.* used clinical disease as end point. Having a previous case of VL in the household was a risk factor for clinical

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VL disease in studies by Bern *et al.* (2005) and Ranjan *et al*. (2005). Living in a mud house was a risk factor for VL infection in a study by Schenkel *et al*. (2006), documented by a positive DAT; Ranjan *et al*. (2005) identified mud-plastered walls as risk factor for VL disease. Other risk factors identified for VL infection are large family size (≥ 6 members) (Schenkel *et al*. 2006) and proximity to bodies of water and Muslim religion (Saha *et al.* 2008). 'Granary inside the house', 'presence of bamboo trees around the house' and 'house not sprayed with DDT in past 6 months' were found to be risk factors for clinical disease by Ranjan *et al.* (2005). Boelaert *et al*. (2009) found a strong association between VL disease and poverty.

The role of domestic animals as a risk factor for VL is still controversial. In contrast to Latin America and Europe where the host reservoir of VL is the domestic dog, humans are assumed to be the only reservoir on the Indian subcontinent (WHO 1990). Yet domestic animals can play a role in the transmission of VL on the Indian subcontinent because of their association with the sandfly vector. Animals may either attract sandflies, thereby increasing vector density and transmission to humans; or they may serve as an alternative bloodmeal source, thereby decreasing transmission.

Livestock ownership was protective against VL in Nepal (OR 0.34, p=0.001, Bern *et al*. 2000) but was a risk factor for VL (OR 2.0, p=0.089, Barnett *et al.* 2005) and *L. donovani* infection (RR 2.1, 95% CI 1.5–3.8, Saha et al. 2008) in India. A second study by Bern *et al.* (2005) did not confirm the protective effect of owning livestock on VL in Bangladesh (OR 0.89, p=0.180). Having small animals around the house was protective against *L. donovan*i infection in the study of Schenkel *et al.* (2006) (OR 0.4, 95% CI 0.2–1.1); the presence of cows around the house was protective against VL in Bangladesh (Bern *et al* 2005). The latter study even showed a "dose response" effect in the association between VL and cows with an odds ratio 0.81 (p=0.005) for every additional cow per 1000m^2.

Proportions of households owning livestock were high but variable in all these studies. Cow ownership varied from 32% (Barnett et al, 2005) to 70% (Bern et al. 2000); livestock ownership varied from 54% (Bern et al. 2005) to 78% (Saha et al. 2008). VL incidence expressed per 100,000 person years at risk varied from 888 in the Bangladeshi villages studied by Bern et al. (2005) to 600 in Uttar Pradesh (Barnett *et al.* 2005) and an estimated 20–80 in Nepal (Bern *et al*, 2000).

In the impoverished rural communities of Bihar (India), domestic animals are highly valued assets kept in close proximity to the houses. There is a commonly held believe that sleeping in the same room as domestic animals increases the risk for VL as it attracts more sandflies into the house (Bern *et al.* 2000). This led us to the research hypothesis that having animals around the house may be protective because of animals acting as preferred bloodmeal source for sandflies (Mukhopadhyay *et al.* 1987, Palit *et al.* 2005), while keeping animals inside the house might be a risk factor because of animals attracting more sandflies indoor. In case the protective effect of domestic animals prevails, they could be used as 'zooprophylaxis'. Zooprophylaxis is defined as the use of animals to deviate vectors from humans (Chelbi *et al.* 2008), a concept already known in malaria control (WHO 1982).

The primary objective of this study was to investigate the association between VL and keeping domestic inside the house at night. Other known risk factors for VL were included in our study primarily because of their potential as confounders, with special attention for the role of socio-economic status.

Materials and methods

Study area and population

The study forms part of a larger ongoing community-based study, funded by the National Institutes of Health 'Tropical Medicine Research Centre' (NIH/TMRC) grant program. For this study a VL endemic area has been selected in Muzaffarpur district of Bihar State, India. The study area is an impoverished rural area comprising of 50 villages with a total population of 73,024. Most inhabitants are subsistence farmers or daily wages earners. VL incidence during 2008 was 78 new cases, giving an estimated annual incidence of 107/100,000.

Study design and case definitions

The study was designed as an individually matched case-control study; we selected 2 controls for each case. For ethical reasons we included only persons aged 2 years and above. Cases were identified during an initial household survey covering the entire study area as well as from records of government PHC and private medical facilities. Cases identified were ascertained by examining medical case records. The case definition includes all parasitologically confirmed VL cases as well as all probable VL cases residing in the study area; all diagnosed between March 1st, 2007 and December 1st, 2008. A probable case of VL was defined as a person with the combination of a clinical history typical for VL (fever of more than 2 weeks' duration, not responding to anti malaria treatment), a positive result of the rK39 test (Inbios International, Seattle, WA, USA) and a good response to specific VL treatment.

Controls were individually matched on neighborhood, age group and sex. Only persons who had never suffered from or been treated for VL, and were not living in the same household as study cases, were eligible. Though there had been no VL cases in control households since the start of the larger study in March 2007, controls were asked whether there had ever been VL cases in their households previously. Each control was subjected to an rK39 dipstick test (Inbios International, Seattle, WA, USA). Since there is no clear relation between asymptomatic rK39 positivity when documented in a cross-sectional way and subsequent clinical VL disease (Gidwani 2009), we did not reject rK39 positive individuals as controls provided they had no history of prior VL or no other current signs of VL. We used 5 age groups: 0–4 years, 5–14 years, 16–29 years, 30–44 years, and 45 years and above.

Sample size

The sample size was calculated based on animal ownership data from another ongoing trial in Bihar in which 26% of households owned animals. Assuming that 10% of controls keep their animals inside the house at night and assuming a correlation r of 0.2, with a 95% confidence level and a power of 0.8, a sample of 139 cases and 278 controls would be enough to detect an OR of 2.5.

Study procedures

Controls were selected and recruited in the villages from the study area by the field trial coordinator. Per household a maximum of 1 control was selected. Control households were selected starting from the 2 houses nearest to the house of the case. If no suitable control was available in the house selected, the next house was chosen. For some cases the house of the immediate neighbor was an attached thatched house with only a single thatched wall separating the living quarters of case and control. As sandflies can easily penetrate a thatched wall, such households were excluded. Instead a control was selected from the next house.

The field study coordinator prepared a list of all eligible controls, which was provided to the field teams. After obtaining individual written informed consent, the field teams conducted structured interviews and performed rK39 tests. The questionnaire contains detailed questions

on ownership of animals, keeping animals inside the house and sleeping outside in the vicinity of animals. It also contains questions on potential confounders such as socio-economic status, housing conditions, bednet use and presence of (other) cases of VL in the household.

Dampness of floors was assessed by the field investigator who touched the floor with the back of the hand. Socio economic status was assessed for the household, based on a previously validated asset index (Boelaert *et al.* 2009). Included in the asset index are ownership of: land, motorcycle(s), bicycle(s), television set(s), radio(s), mobile phone(s), watch(es), fan(s), mattress(es) and bed(s). The assets index was converted into an assets score using principal component analysis. Based on the assets scores, households were divided into 4 socio economic layers.

Data was double entered in a Microsoft Access database independently by 2 data entry clerks. Upon completion of the 2nd entry, the 2 files were compared. In case of discrepancies corrections were made after reviewing the original questionnaire forms.

Ethical approval for the study was obtained from the ethics committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Statistical analysis

For data analysis we used Stata/ IC V10.1 (Stata Corp., College Station Tx, USA). Observed associations were assessed through conditional logistic regression. All variables with a p-value <0.10 in uni-variate analysis were included in the multivariate logistic regression model. Variables for the final model were selected using the hierarchical backward elimination strategy. The probability of removal was set at p = 0.10.

A sensitivity analysis was performed to assess the effect of (potentially) including rK39 positive controls, of including controls with past history of VL in the household, and of excluding as controls persons living in attached thatched houses.

Results

One hundred and forty one cases and 282 controls were enrolled in the study, 219 males (52%) and 204 females (48%). Ages ranged from 2–75 years, median age was 15 years, interquartile range (IQR) 10 - 31 years.

Of 141 VL cases enrolled, 93 (66%) were parasitologically confirmed; the remaining 48 cases all had a positive rK39 result and had been successfully treated. Of 282 controls enrolled, 33 did not agree to give blood for rK39 testing. Of the remaining 249, only 1 had a positive rK39 dipstick result without showing any signs of disease. Eighty four out of 282 controls (30%) reported ever having had a VL case in the household, prior to March 2007.

Eighty eight out of 141 case households (62.4%) and 176 out of 282 (62.4%) control households owned domestic animals. Bovines (cows and buffaloes) were owned by 61 out of 141 case households (43.3%) and 124 out of 282 control households (44.0%). With the exception of poultry, the majority of animal owners owned only 1 animal; the maximum number of animals owned was 5 for bovines, 6 for goats and 10 for poultry. Animals were sometimes kept inside the house, especially at night, by 29 out of 142 cases (20.6%) and 58 out of 282 controls (20.6%); for bovines the figures are 9 out of 142 for cases (6.4%) and 20 out of 282 (7.1%) for controls. Those who keep animals inside the house, do so for a minimum of 45 days and at maximum throughout the year. Details on animal ownership and keeping animals inside the house are provided in table 1.

We calculated odds ratios for 'ownership of animals', 'keeping animals inside the house' and 'sleeping in the same room as animals' between cases and controls. All odds ratios observed were close to 1; 1.02 (95% CI 0.65–1.60) for ownership of 'any animal', 0.97 (95% CI 0.62–1.51) for ownership of bovines and 1.00 (95% CI 0.59–1.70) for keeping 'any animal' inside the house. For sleeping in the same room as animals the odds ratios were 1.33 (95% CI 0.75–2.33) for 'any animal' and 1.13 (95%CI 0.49–2.61) for bovines (table 2).

We checked whether the number of animals owned or the time an animal is kept inside the house modify the risk. For this purpose we split the data according number of animals owned (0/1 or more than 1) and according to the median duration an animal is kept inside among controls (except for poultry because the vast majority of those keeping poultry inside do so throughout the year). There were no substantial changes in odds ratios (table 3).

Two hundred forty six out of 282 controls (87%) and 128 out of 142 cases (91%) slept outside the house in summer. Of those sleeping outside, 36 cases (28%) and 68 controls (28%) slept within 5 meters of domestic animals. The odds ratio for VL when sleeping within a 5 meters distance of domestic animals was 0.96 (95%CI 0.57–1.63).

Among the other factors we examined, type of housing, damp floors and socioeconomic status were associated with VL (table 4). Type of housing was divided into 3 categories, brick houses with windows, brick houses without windows and thatched houses. With brick houses with windows as referent, we found odds ratios of 2.44 and 2.92 for brick houses without windows and thatched houses respectively in uni-variate analysis. For 'damp floors' we found an odds ratio of 3.10. In all there were 405 non-cemented and 18 cemented floors. Of the 405 non-cemented floors, 350 (86%) were damp; of 18 cemented floors only 1(5.6%) was damp.

In uni-variate analysis socio economic status was associated with VL, the odds increasing by a factor of 1.26 (95% CI 1.02–1.54) per level down the scale. Sleeping on a bed rather than on the floor and using a bednet were protective but not statistically significant at the 5% level.

In the final model we tested all variables found to have a p-value of 0.10 or less in uni-variate analysis. The effect of assets decreased after controlling for housing conditions (OR 1.15, 95% CI 0.93–1.42). The only variables retained were 'type of housing' and 'damp floor'. Both variables remained statistically significant at the 5% level, there were no major changes in odds ratios (table 5). Including socio economic status in the model changed the odds ratios associated with housing conditions by less than 5% and did not significantly increase precision of the model as a whole.

Excluding from the analysis the 34 controls that had no rK39 result or were rK39-positive did not significantly change any of the odds ratio's observed, neither did excluding the 84 controls from households that reported VL cases prior to March, 2007.

On 6 occasions, a control in a brick house was chosen instead of a control in a thatched house because the intended control household was separated from the case household only by a thatched wall. Excluding these controls from the analysis did not substantially change any of the housing type related results (OR 2.35, 95% CI 1.18–4.68 for brick walls, no windows; OR 2.41, 95% CI 1.38–4.18 for thatched walls).

Discussion

This study was designed in the first place to investigate the association between domestic animals and VL at individual and household level. By individually matching on age group, sex and neighborhood, we eliminated much of the variance related to other factors, notably back ground level of transmission intensity of VL in the village. VL incidence was high but well

within the range observed in the other studies; the same applies to the observed proportion of households owning animals. Whereas most of the earlier studies investigated only one aspect of the role of animals, along with a wide array of other risk factors; in this study we considered animals in much more detail. We differentiated between the different kinds of domestic animals; we considered ownership; we documented the numbers of animals owned, whether or not animals were kept inside the house and for how long; we also examined 'sleeping outdoors in the vicinity of cattle' as a risk factor. The number of cases enrolled in our study is comparable to the number enrolled by Bern (2005) in Bangladesh (155) and Saha (2008) in India (150 infected persons) but higher than the number enrolled by Bern (2000) in Nepal(84), Barnett (2005) (49) or Schenkel (2006) (28 infected persons). We used a rigorous case definition for VL, accepting only rK39 positive and/or parasitologically confirmed cases. We used VL disease rather than infection as our endpoint because disease is the main outcome of interest; moreover a recent study by Gidwani *et al.* (2009) showed that cross-sectionally measured seropositivity among asymptomatic persons is not a predictor for development of clinical VL.

Yet our study failed to show any relation at individual level between the presence of domestic animals in compounds and occurrence of VL in humans. Odds ratios for 'ownership', 'keeping animals inside the house' and 'sleeping in the same room as animals' were at times unstable for individual animal species because of low numbers but were stable when considering bovines or 'any animal'; none showed any significant risk or protection effect. The 95% confidence interval of the Odds ratio for ownership of 'any animal' was between 0.65 and 1.60, making it highly unlikely that keeping animals inside the house makes a difference for VL risk at the individual level.

Our study was not designed to measure the protective effect of the animal density around houses as described by Bern (2005); since our controls were neighborhood matched there was no difference in exposure between cases and controls to cows in the neighborhood. We can therefore not rule out that some association exists at a higher level, that of the community or neighborhood.

In contrast, housing conditions clearly emerged as risk factors for VL at the individual level. We divided brick houses into 2 categories, 'with windows' and 'without windows', to distinguish those built properly from those built on a low budget. Typically, the latter used mud as mortar instead of cement and had no windows. Living in a properly constructed brick house reduces the risk of VL, irrespective of other socio economic factors. This finding is not surprising, as breeding conditions of sand flies are optimal in humid environments and moist soils (Singh *et al*, 2008; Sivagnaname and Amalraj, 1997). Our findings suggest that housing schemes which target those living below the poverty line (Govt. of Bihar, 2009) could help in reducing incidence of vector borne diseases.

Dampness of floors, assessed by palpation, was a strong risk factor in the study of Bern et al in Nepal(2000) but not confirmed in another study by Bern et al in Bangladesh (2005). In our study we equally found a strong association between palpably damp floors and VL (OR 2.60, 95% CI 1.25–5.41); moreover we also found a strong association between dampness and type of floor. These findings should be confirmed by a more objective measurement of dampness. If confirmed it would be recommended for subsidized housing schemes to ensure that houses do not only have brick walls but cemented floors as well.

Since we were primarily interested in the association between domestic animals and VL, we did not accept as a control a person living in a house separated from the house of the case by only a thatched wall. If animals affect sandfly density, this might very well be the case on either side of a thatched wall. Accepting controls from such households would have caused us to

underestimate the association between keeping animals inside the house and VL; not accepting controls from these households might have caused us to overestimate the effect of housing conditions because the next control household may have been a brick house. We tested this hypothesis by identifying all instances in which a thatched house had been skipped and a control had instead been selected from a brick house. There were only 6 such cases; excluding them from our sample did not significantly change any of the associations related to housing conditions.

Conclusion

At individual and household level, we did not find any association between domestic animals and risk for VL. Based on the results of this study and considering the findings from previous studies, there is no rationale for any recommendations of changing animal husbandry practices at the household level to reduce VL risk. In contrast, housing conditions are important factors related to the risk of VL, independently from poverty. Living in a proper brick house and having dry floors reduces the risk of VL though the importance of the latter factor needs to be confirmed using objective measurements.

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Table 1

Animal ownership and keeping animals inside the house

Animal	No. of owners		No. owning more than 1 animal		No. keeping inside house		Median days/nights inside	
	Case (%)	Control (%)	Case (%)	Control (%)	Case (%)	Control (%)	Case	Control
Any animal	88(62.4)	176(62.4)	41(29.1)	80(28.4)	29(20.6)	58(20.6)	175	173
- Bovines	61(43.3)	124(44.0)	16(11.4)	35(12.4)	9(6.4)	20(7.1)	160	150
Cow/Oxen	*35(24.8)*	*84(29.8)*	*13(9.2)*	*28(9.9)*	*5(3.6)*	*16(5.7)*	*160*	*150*
Buffaloes	*27(19.1)*	*48(17.0)*	*3(2.1)*	*6(2.1)*	*4(2.8)*	*4(1.4)*	*170*	*98*
- Goats	38(27.0)	66(23.4)	11(7.8)	22(7.8)	19(13.5)	28(9.9)	160	185
- Pigs	0	1(0.4)	0	1(0.4)	0	1(0.4)	0	85
- Dogs	1(0.7)	4(1.4)	1 (0.7)	2(0.7)	0	1(0.4)	0	145
- Poultry	12(8.5)	21(7.5)	10(7.1)	13(4.6)	5(3.6)	13(4.6)	240	360

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Table 2

Unadjusted odds ratios for factors related to animals (part 1)

Animal	Ownership		Keeping inside house		Sleeping in the same room	
	OR	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
Any animal	1.02	0.65–1.60	1.00	0.59–1.70	1.33	0.75–2.33
- Bovines	0.97	0.62–1.51	0.88	0.37–2.08	1.13	0.49–2.61
Cow/Oxen	*0.75*	*0.45–1.23*	*0.55*	*0.18–1.68*	*0.71*	*0.22–2.31*
Buffaloes	*1.17*	*0.68– 2.01*	*2.00*	*0.50–8.00*	*1.80*	*0.57–5.69*
- Goats	1.28	0.75– 2.17	1.44	0.76–2.73	1.46	0.70–3.02
- Poultry	1.20	0.52–2.76	0.70	0.21–2.35	1.20	0.29–5.02

Table 3

Unadjusted odds ratios for factors related to animals (part 2)

Animal	Ownership of more than 1 animal		Keeping inside house longer than median among controls		Sleeping in the same room more often than median among controls	
	OR	95%CI	OR	95%CI	OR	95%CI
Any animal	1.04	0.65–1.66	1.05	0.51–2.15	1.22	0.58–2.55
- Bovines	*0.90*	*0.48–1.70*	*1.27*	*0.40– 4.10*	*1.12*	*0.36–3.49*
- Goats	*1.00*	*0.47–2.14*	*0.91*	*0.33–2.54*	*1.31*	*0.48–3.62*
- Poultry	*1.65*	*0.67–4.07*	*NA*	*NA*	*NA*	*NA*

Table 4

Unadjusted Odds Ratios for other factors associated with VL

Risk factor	No. exposed		OR	95%CI
	Case (%)	Control (%)		
Type of housing				
- *Thatched walls*	82 (58)	120(43)	2.92	1.71–4.97
- *Brick walls, no windows*	22(16)	34(12)	2.44	1.24–4.79
- *Brick walls with windows*	37(26)	128(45)	referent	
Damp floor	128(91)	223(79)	3.10	1.51–6.34
Socio economic status			1.26	1.02–1.54
- *Level 1(highest)*	26(18)	77(27)	referent	
- *Level 2*	27(19)	53(19)	1.48	0.80–2.77
- *Level 3*	42(30)	75(27)	1.75	0.95–3.19
- *Level 4*	46(33)	77(27)	2.00	1.05–3.83
Ownership of bednet	49(35)	120(43)	0.62	0.37–1.03
Use of bed net	41(29)	101 36)	0.79	0.60–1.02
Sleeping on a bed (vs. on the floor)	102(72)	214(76)	0.72	0.39–1.33
History of VL case in household	50(35)	84(30)	1.32	0.85–2.07
Insecticide spraying in 06/07	33(23)	52(18)	1.70	0.88–3.30

Table 5

Adjusted Odds Ratios for other factors associated with VL

Risk factor	OR	95%CI
Type of housing		
- *Thatched walls*	2.60	1.50–4.48
- *Brick walls, no windows*	2.38	1.20–4.72
- *Brick walls with windows*	referent	
Damp floor	2.60	1.25–5.41