The need for reassessing dogmas: exploration of the role of animals in the transmission cycle of *Leishmania donovani* in Nepal

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Visceral leishmaniasis (VL) also known as kala-azar, is a vector-borne parasitic disease caused by two species of the *Leishmania donovani* complex: *L. donovani* in the Indian subcontinent and East Africa, and *L. infantum* around the Mediterranean basin and in Latin America (1). The parasites are transmitted by the bite of infected female sandflies and the disease is fatal if left untreated.

Two different eco-epidemiological patterns have been documented in VL across the world: (i) zoonotic visceral leishmaniasis (ZVL) in the Euro-Mediterranean region and in Latin America and (ii) anthroponotic visceral leishmaniasis (AVL) in East Africa and the Indian sub-continent

The possible role of domestic animals in AVL has been studied in the past but there are no clear conclusions on their involvement as risk factor or reservoir. Correct identification of the Leishmania reservoir is crucial for the design of VL control programmes.

Molecular and GIS tools now offer new opportunities to better document and re-assess transmission patterns in the region. We performed an extensive study in an active transmission focus of VL in Nepal, mapping Leishmania infections among healthy human individuals and domestic animals in order to explore the potential role of the latter in transmission.

METHODOLOGY

Study Area:

The study was conducted in Dharan-17; an active VL transmission community in the Terai region, Eastern Nepal. Dharan-17 is a peri-urban ward of Dharan municipality, located in the foothill of Mahabharata hill. It has 515 inhabitants living in 105 households. Dharan-17 has a VL incidence rate of 1.61% (average calculated on the period of 2004 to 2006). Control samples were collected in Dhankura-3, a ward is situated in a hilly area where no VL cases were reported so far.

Sample collection:

Human subjects

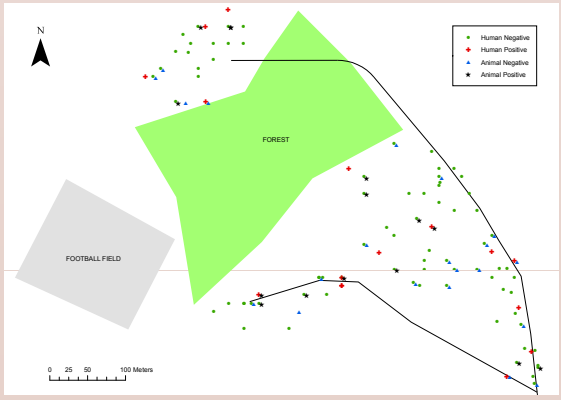
278 blood samples from individuals above 5 years of age who agreed to join the study were collected in October 2007.

Domestic animals

144 goats, 24 buffaloes and 20 cows were sampled from the 37 households in Dharan-17 that had at least one bovine or goat in October 2007. Samples from 25 goats, 17 buffaloes and 21 cows were collected in the control ward.

DNA extraction and PCR amplification:

Blood samples were stored in AS1 buffer. The QIAamp DNA mini kit was used to extract DNA. The samples were analysed by a PCR assay targeting the small ribosomal genes of *Leishmania* able to detect 1 parasite in 180µl of blood (1). PCR reaction was carried out as described by Bhattarai *et al* (2).



Map 1: Location of human and animal PCR+ households in Dharan-17.

Spatial clustering of PCR-positive households

Each household (unit of analysis) was geo-referenced by GPS and mapped using ArcGIS 9.2 (ESRI, USA) and was identified as positive or negative for animal and human samples (Map 1).

The space K-function (3) was used to study if PCR-positive households for (i) human, (ii) animals, (iii) goats and (iv) both human and animals, were clustered.

$$K(d) = \frac{\text{average number events within dist } d}{\text{mean number events per unit area}}$$

1. The spatial clustering of PCR-positive households was assessed by calculating the difference of K-functions between PCR-positive households (for each one of the four groups) and the total population (all sampled households in the cluster) (3)
2. The bivariate K-function was used to examine the spatial relationship between human and animal (and goat) positive households (3).

- 10000 permutation simulations were used to assess the statistically significance of the results.
- K-functions calculated using the package Splancs in R .

	Dharan-17		Control ward	
	total	% PCR+ (n)	total	% PCR+ (n)
Animals	188	13% (25)	63	0% (0)
Goats	144	16% (23)	25	0% (0)
Cows	20	5% (1)	21	0% (0)
Buffaloes	24	4% (1)	17	0% (0)
Humans	278	6% (17)	xx	xx

Table 1: PCR results from human and animal samples

RESULTS

- **Goats** (and other domestic animals) can be infected with *Leishmania donovani* (Table 1)
- There is no clustering of PCR+ households (humans or animals) as shown by K-function difference (Figure 1)
- There is no clustering of human PCR+ around animal PCR+ households as shown by the bivariate K-function (Figure 2)

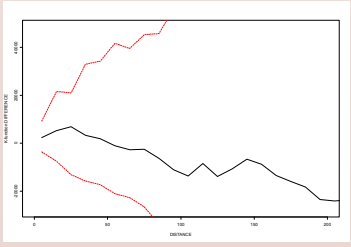


Figure 1: K-function difference

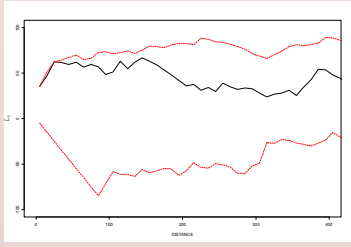


Figure 2: Bivariate K-function

DISCUSSION

- *Leishmania* DNA found in domestic animals in Indian subcontinent for the first time
- What is the role of domestic animals in the *L. donovani* cycle.
  - Part of the transmission cycle? Cannot be ruled out but no spatial clustering was detected
  - Risk factor as already described (4)? → multivariate analyses are ongoing using the PCR results from Dharan-17

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