

Epidemiological and Serological Study of Leishmaniasis in Najran Region, Saudi Arabia

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Abstract

Leishmaniasis is a public health and veterinary hazard. Screening of serum samples of 384 human and 387 domestic animals were carried out by indirect hemagglutination assay (IHAT) to detect antibodies against *L. donovani* and to see the involvement of animal reservoirs in Najran region, Saudi Arabia. The overall prevalence of human Leishmaniasis infection in Najran area, Saudi Arabia was 8.3%. The prevalence rate of human Leishmaniasis was

significantly higher in summer (21.9 %) and spring (8.3%) than in winter and autumn. In addition, the prevalence rate of human Leishmaniasis was significantly higher in old age (17.6%) and young period (7.3%). Out of 53 females and 331 males, 3 (5.7%) females and 29 (8.8%) males were found to be infected with human Leishmaniasis, respectively. The highest titer in human was 1/256 with percentage of 43.8%. Antibody against *L. donovani* in domestic animals was detected and the overall proportion of occurrence was 1.6%. The prevalence rate of Leishmaniasis infection was significantly higher in goats than in sheep, camel and horses. The highest titer (1/512) was in goats. The study was thrown light on infected African and Asian workers as source of Leishmaniasis infection. Also, this study suggests the possibility of varied species of domestic animals to harbor the parasite and hence play a central role in the transmission. Consequently, this may hurdle our clarification of disease epidemiology.

Keywords: Leishmaniasis, Leishmania, Sandfly, Epidemiology, Human, Animals, Serology, Najran, Saudi Arabia

1. Introduction

Leishmaniasis are zoonotic worldwide spectrum, vector-borne communicable parasitic diseases and of public health concern for many countries around the world (Dantas-Torres, 2006). Natural reservoir hosts include man (anthroponotic cycle) (human–sandfly–human) and dogs or wild animals (zoonotic cycle). *L. donovani* is anthroponotic; it is mainly transmitted between people, who act as the reservoir hosts (Roberts et al., 2000). Anthroponotic visceral leishmaniasis, caused by *L. donovani*, occurs mainly in Sudan and Somalia, however, zoonotic visceral leishmaniasis, caused by *L. infantum*, occurs in most countries of the region (Postigo, 2010). Climate and other environmental changes have the potential to expand the geographic range of the sand fly vectors and the areas in the world where leishmaniasis is found (El-naiem et al., 2003). Very little is known about identification of visceral Leishmaniasis in Saudi Arabia but there was many studies in cutaneous Leishmaniasis (Bienzle et al., 1978; Al-Zahrani et al., 1979; Buttiker et al., 1982; Peters et al., 1985; Peters and Al-Zahrani, 1987; Dye et al., 1989; Al-Zahrani et al., 1989, Morsy et al., 1991; Al-Shammari et al., 1992; Al-Tawfiq and Abukhamsin, 2004). In Makkah, Saudi Arabia, Leishmania isolates were characterized from Makkah Al-mukarramah specialized hospital (Morsy et al., 1993). The studied isolates were two *L. donovani zymodeme* LON-41 (VL Indian patient) and LON-46 (VL Sudanese patient), three *L. tropica zymodeme* LON-71 (2 CL Yemenis patients) and LON-22 (CL Egyptian patient) and two *L. major zymodeme* LON-4 (2 CL Saudi patients). Several reports concerning with the isolation of *L. tropica* from *P. sergenti* sandfly in Middle-Eastern countries (Al-Zahrani et al., 1988 and Pazarbasi et al., 2006). Also, isolation of *L. major* from *P. papatasi* sandfly was reported (Abou El-Ela et al., 1995 and Oshaghi et al., 2010). Serologically, high antibody levels are observed prior to detection of parasite specific T cell responses (Ghose et al., 1980). Most widely used serological techniques of Leishmaniasis are indirect immunofluorescent antibody test (IFAT) (Mancianti & Meciani, 1988), direct agglutination test (DAT) (Harith et al., 1988), enzyme-linked immunosorbent assay (ELISA) (Amin et al., 1985 and Zijlstra et al., 1998), dot-ELISA (Fisa et al., 1997), latex agglutination test (Lockwood & Sundar, 2006). The

purpose of the present study was to detect the prevalence of Leishmaniasis in Najran region, Saudi Arabia and control of it by knowing the reservoir of the disease.

2. Methods

2.1 Geographic Area of Research Study

Najran is a city in southwestern Saudi Arabia near the border with Yemen. It is the capital of Najran Province. Najran enjoys three different geographic landscapes, and they are the oases, mountains, and desert at its eastside. The average temperature in Najran ranges from 14.6 to 30.9. The average annual rainfall is 83-mm. (figure 1).



Figure 1. Map of Saudi Arabia showing the geographic location of Najran (arrow), region of Saudi Arabia, located in the south of the country along the border with Yemen.

2.2 Ethical Consideration

The University Ethical Board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board.

2.3 Collection of Human Serum Samples

384 human serum samples were obtained from the different nationality people of both genders and different ages, and from different parts and environmental seasons in Najran. The samples will be obtained from apparently healthy human to make serological survey (Table 1).

Table 1. Different nationality people in Najran region, Saudi Arabia.

Nationality	Season*				Age**			Sex		total
	1	2	3	4	A	B	C	♀	♂	
Indian	38	18	31	34	0	97	24	8	113	121
Saudi Arabian	4	25	9	11	14	33	2	5	44	49
Kenyan	5	0	0	0	0	5	0	0	5	5
Filipino	7	0	9	7	0	22	1	2	21	23
Egyptian	11	19	9	16	2	47	6	11	44	55
Yemeni	8	10	14	3	0	30	5	10	25	35
Sudanese	12	13	10	12	0	41	6	13	34	47
Ethiopian	8	3	4	5	0	17	3	3	17	20
Sri Lankan	2	0	0	3	0	5	0	0	5	5
Jordanian	1	0	0	0	0	1	0	0	1	1
Moroccan	0	0	1	1	0	2	0	0	2	2
Neapolitan	0	0	5	0	0	3	2	1	4	5
Pakistani	0	0	0	4	0	4	0	0	4	4
Bangladeshi	0	8	4	0	0	10	2	0	12	12
Total	96	96	96	96	16	317	51	53	331	384

* Season: (1) summer, (2) autumn, (3) winter and (4) spring.** age: (A) between 1 year and 19 year, (B) between 20 year and 39 year and (C) between 40 year and 59 year.

2.4 Collection of Animal Serum Samples

387 animal serum samples (116 goat, 104 sheep, 140 camel and 27 horse) were collected from different farm localities in Najran to detecting the reservoir and the site of high infection. Human and animal blood samples were collected and transported to the Department of Applied Medical Sciences Lab, Community College, Najran University. All sera were kept in deep freeze at -20°C until used for serological diagnosis.

2.5 Serological Test

The collected sera were examined for detection of specific antibodies to *Leishmania donovani* using the indirect hemagglutination assay (IHAT) with a commercially available kit (Cellognost Leishman, Siemens Healthcare Diagnostic Products GmbH, 35041 Marburg/Germany) according to the manufacturer's instructions. Serum dilution of 1:32 and higher provide diagnostically useful titers. Mid-range serum titers lie between 1:256 and 1:2048. Positive and negative controls were included in each test (Sundar and Rai, 2002).

2.6 Statistical Analysis

The data were analyzed According to The t-test and one-way Analysis of Variance (ANOVA) analysis. Differences were considered significant when $P \leq 0.05$.

3. Results

The overall prevalence of human Leishmaniasis infection in Najran area, Saudi Arabia was

8.3% (Table 2). The prevalence rate of human Leishmaniasis was significantly higher ($P < 0.05$) in summer (21.9 %) (21/96) and spring (8.3%) (8/96) than in winter and autumn (Table 3). In addition, the prevalence rate of human Leishmaniasis was significantly higher in old age (17.6%) and young period (7.3%) (Table 3). Out of 53 females and 331 males, 3 (5.7%) females and 29 (8.8%) males were found to be infected with human Leishmaniasis, respectively. Human Leishmaniasis infection titers were 1/32, 1/64, 1/256 and 1/512 with percentage of 12.5%, 25%, 43.8% and 18.8%, respectively, (Table 3). The prevalence rate of human Leishmaniasis was significantly higher ($P < 0.05$) in Kenyan (40%), Sri Lankan (40%), Ethiopian (25%), Bangladeshi (16.7%) and Sudanese (10.6 %). The infection in Indian, Saudi Arabian, Filipino, Egyptian and Yemeni was 4.9%, 6.1%, 4.3%, 7.3% and 5.7%, respectively (Table 3 and figure 2,3,4,5).

There was no infection in Jordanian, Moroccan, Neapolitan and Pakistani (Table 3). The highest titer in human was 1/256 with percentage of 43.8%. According to The t-test and one-way Analysis of Variance (ANOVA) analysis, there was no statistically significant differences in Leishmaniasis seroprevalence in relation to human nationality and the sex (p -value >0.05). There was statistically significant differences in Leishmaniasis seroprevalence in relation to season variation and the age (p -value <0.05), but confidence intervals are usually expressed with 95% confidence. Prevalence of animals Leishmaniasis infection in Najran area, Saudi Arabia in sheep, goats, camels and horses was 0.96%, 4.3%, 0% and 0%, respectively (Table 4 and Figure 6). The prevalence rate of Leishmaniasis infection was significantly higher ($P < 0.05$) in goats than in sheep, camel and horses. The overall prevalence in animals Leishmaniasis infection in Najran area, Saudi Arabia was 1.6% (6 out of 387). The titer 1/512 was significantly highest ($P < 0.05$) in goats.

Table 2. Seroprevalence of *Leishmaniasis* infection in human in relation to Nationality

Human Nationality	Examined Numbers	Number of positive	Percentage of positive (%)
Indian	121	6	4.9
Saudi Arabian	49	3	6.1
Kenyan	5	2	40
Filipino	23	1	4.3
Egyptian	55	4	7.3
Yemeni	35	2	5.7
Sudanese	47	5	10.6
Ethiopian	20	5	25
Sri Lankan	5	2	40
Jordanian	1	0	0
Moroccan	2	0	0
Neapolitan	5	0	0
Pakistani	4	0	0
Bangladeshi	12	2	16.7
Total	384	32	8.3

Table 3. Seroprevalence of Leishmaniasis infection in human in relation to season, age and sex.

Nationality	Season*				Age**			sex		Titer***			
	1	2	3	4	A	B	C	♀	♂	I	II	III	IV
Examined Numbers	96	96	96	96	16	317	51	53	331				
Number of positive	21	1	2	8	0	23	9	3	29	4	8	14	6
Positive (%)	21.9	1.04	2.08	8.3	0	7.3	17.6	5.7	8.8	12.5	25	43.8	18.8

* Season: (1) summer, (2) autumn, (3) winter and (4) spring.

** Age: (A) childhood, (B) young period and (C) old age.

***Titer: (I) 1/32, (II) 1/64, (III) 1/256 and (IV) 1/512.

Table 4. Seroprevalence of Leishmaniasis infection in farm animals.

Species	Examined	Positive	Positive (%)	Titer									
				1/32		1/64		1/128		1/256		1/512	
				N	%	N	%	N	%	N	%	N	%
Sheep	104	1	0.96	1	100	0	0	0	0	0	0	0	0
Goats	116	5	4.3	1	20	1	20	2	40	0	0	1	20
Camels	140	0	0	0	0	0	0	0	0	0	0	0	0
Horse	27	0	0	0	0	0	0	0	0	0	0	0	0
Total	387	6	1.6	2	33.3	1	16.7	2	33.3	0	0	1	16.7

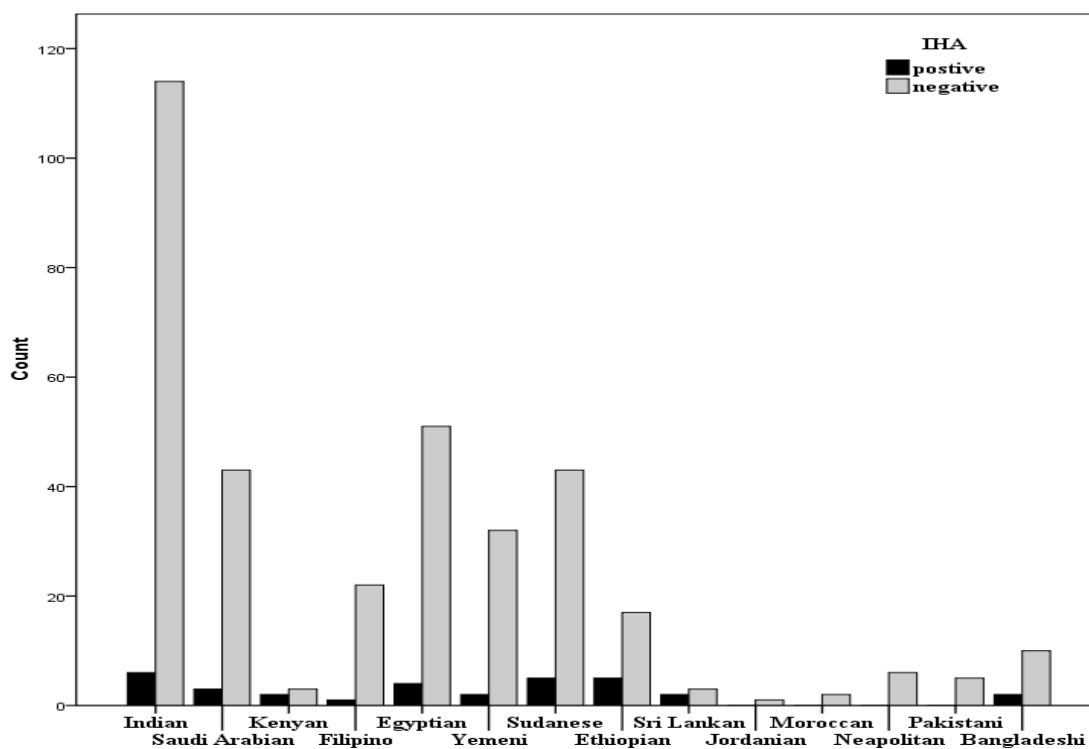


Figure 2. Seroprevalence of Leishmaniasis infection in human in relation to Nationality.

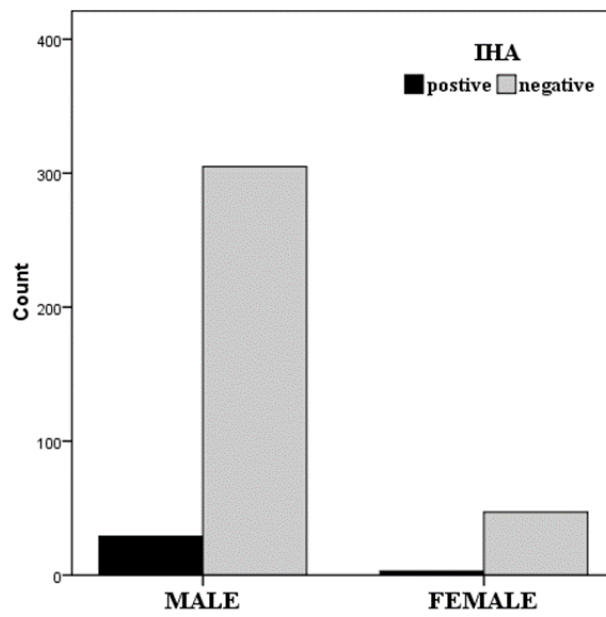


Figure 3. Seroprevalence of Leishmaniasis infection in human in relation to sex.

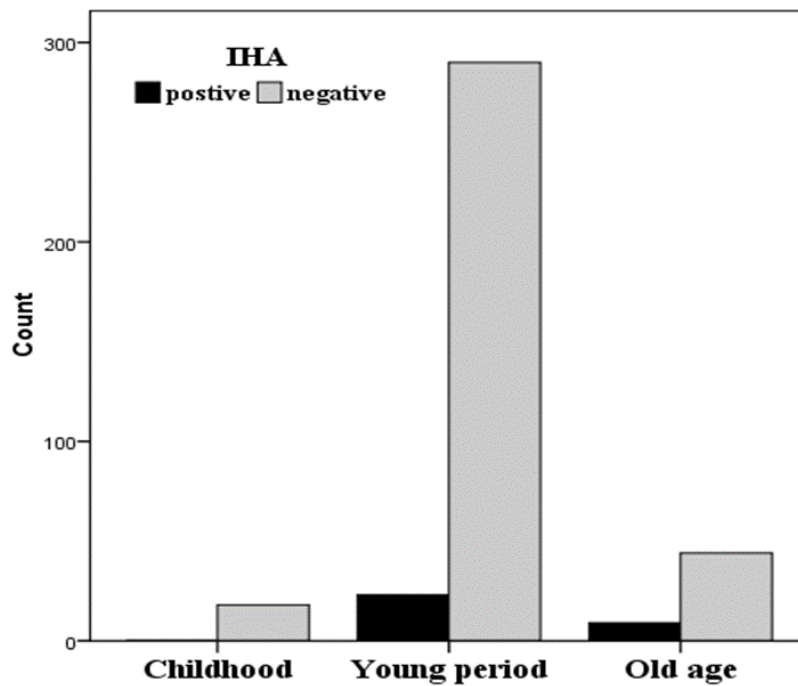


Figure 4. Seroprevalence of Leishmaniasis infection in human in relation to age.

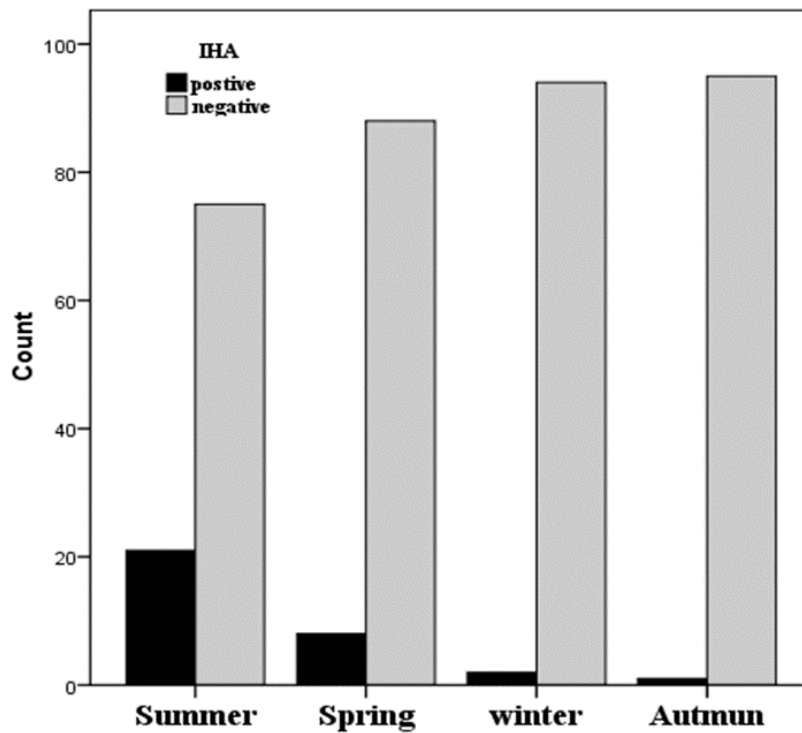


Figure 5. Seroprevalence of Leishmaniasis infection in human in relation to season.

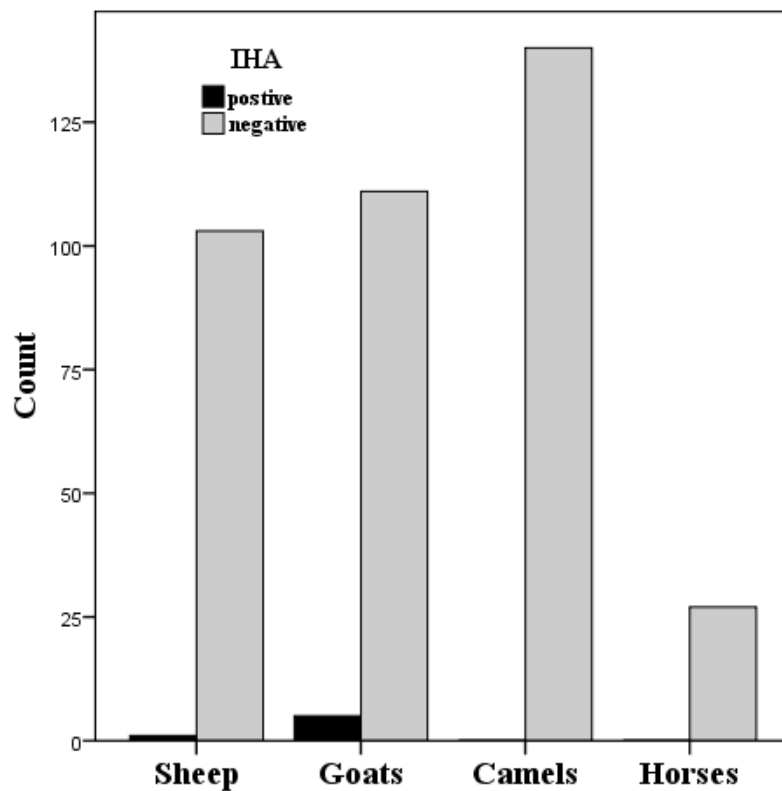


Figure 6. Seroprevalence of Leishmaniasis infection in farm animals.

4. Discussion

From the epidemic point of view, Leishmaniasis is the third-major vector-borne disease killer in the world after malaria and sleeping sickness (Bern et al., 2008). It is estimated about 500,000 infections each year worldwide (Desjeux, 2001).

The data in corresponding to our study show that all age groups are susceptible to infection with *Leishmania* (Kubba, 1987). Seasonal pattern of the disease correlates with the known activity of the vector (Buttiker & Lewis, 1979 and Al-Cindan et al., 1984). In our study in Najran, southern region in Saudi Arabia, *L. donovani* Sero-positive persons (8.3%) was recorded, Similar study in Gizan province, south-west Saudi Arabia by Ibrahim et al. (1992) in which *L. donovani* s.l. LON42 was isolated from human visceral Leishmaniasis patients living in this area. The disease peaks in late spring and summer, then declines sharply in winter. This is similar to the variation seen in the Mediterranean variety (Al-Orainey et al., 1994). We found *Leishmania* serologically positive in domestic animals (sheep and goats) from Najran area, Saudi Arabia, mostly in goats (4.3%). Similar conclusion was detected by Bhattarai et al. (2010) from Dharan-17, where infection was mostly in goats (16%). Even if our results indicate that goats might be involved in the epidemiology of Visceral Leishmaniasis, they do not necessarily mean that these animals constitute a reservoir host for *L. donovani*. Criteria for the definition of *Leishmania* reservoir hosts were recently reviewed by Chaves et al. (2007) and include sand fly foraging behaviour and feeding preferences and the dynamics of infections in assumed reservoir hosts; a key question is the clearance times (chronicity) of infections. Whether the phenomenon observed here can be extrapolated to other VL-endemic foci should also be explored.

Najran area, Saudi Arabia is a new emerging focus, and in the absence of immunity, human and animal populations could be more sensitive to *Leishmania* infections. Our observations warrant further investigation and a close monitoring of goats and other peridomestic animals. If the role of these animals in the transmission cycle is confirmed, the potential implications could affect Visceral Leishmaniasis control programs in the region. Comparison of the results from animals with those from human samples from Najran area, Saudi Arabia provided additional interpretation of the animal results. *Leishmania* spp. positivity was found to be $\approx 4\times$ lower among animals than among persons (1.6% vs. 8.3%, respectively). Although these data weren't consistent with data on the feeding behavior of *Phlebotomus argentipes* blood-sucking flies, reported previously by Palit et al. (2005) those attributed to the most of seropositive persons was in old age. *Phlebotomus argentipes* blood-sucking flies seems to breed essentially in cattle sheds (Singh et al., 2008) and are $5\times$ more attracted to cattle than to persons (Dinesh et al., 2001) and feed more on animals (62.80%) than on persons (24.92%), according to a study in India. The occurrences of antibody for *L. donovani* differ among different animal species in the present and previous studies (Mukhtar et al., 2000). In our study the highest seropositivity of 4.3% was found in goat while other similar study (Kenubih et al., 2014) in north West Ethiopia found cattle have high seropositivity 41.9%.

5. Conclusion

The study was thrown light on infected African and Asian workers as source of Leishmaniasis

infection in south area of Najran, Saudi Arabia. Also, this study suggests the possibility of varied species of domestic animals to harbor the parasite and hence play a central role in the transmission. Consequently, this may hurdle our clarification of disease epidemiology. Health education activities are still required to sensitize people living in endemic areas, medical and public health managers, and individuals at risk on leishmaniasis. Also, control measures against sand flies and reservoir hosts need to be further evaluated in terms of their effect on disease incidence.

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