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Risk factors for cutaneous leishmaniasis in Cukurova region, Turkey

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ABSTRACT

We conducted a case-control study to evaluate risk factors for cutaneous leishmaniasis caused by *Leishmania infantum* outbreaks in villages in the Cukurova region, South Anatolia, Turkey. 282 respondents from eight villages were interviewed using structured questionnaires. Epidemiological and clinical characteristics, personal protection and knowledge of leishmania were analyzed. Young people, aged from 5–19 years, were found to be the most endangered group of villagers. The concurrent presence of both lesions and scars in nine persons may indicate repeated infections. Sleeping without bed nets, ownership of a dog and cattle ownership (living close to a barn and storage of dried dung according univariate analyses) were associated with a significantly increased risk of leishmania infection. Non-impregnated bed nets provided only partial protection, but their use decreased the risk approximately 1.6 times. Further research on the role of dogs in the transmission cycle and the effect of suitable interventions are needed to design the best strategy for disease control. Results suggest that personal protection should be increased, particularly among outdoor sleepers, with insecticide-treated bed nets suggested as the best choice.

1. Introduction

Leishmaniases are diseases with a wide spectrum of clinical forms, from relatively mild cutaneous lesions to life-threatening visceral diseases. In Turkey, cutaneous leishmaniasis (CL) is endemic in several regions and is attributed mainly to *Leishmania tropica*[1] although the sporadic and exceptional occurrence of *L. major*[1,2] and the focus of *L. infantum*[3] has also been reported. Our group[3] reported a new focus of CL in the Cukurova region (South Anatolia, Turkey), where hundreds of human cases continue to occur every year, most of them suffering from relatively small, non-ulcerating lesions. Surprisingly, no cases caused by *L. tropica*, the typical causative agent of CL in the nearby Sanliurfa region,[4] were found. The causative agent in the Cukurova region was identified as a new *L. donovani/infantum* zymodeme MON-309 using multilocus enzyme electrophoresis analyses (MLEE; Jean-Pierre Dedet, personal communication), which is similar to MON-188 in multilocus sequence typing (MLST) analyses.[3]

According to local health authorities no visceral leishmaniases have been reported from the study area; correspondingly the application of dipstick tests during the previous study did not reveal any visceral leishmaniasis (VL) cases.[3] *Phlebotomus tobbi* was incriminated as being a vector, since late-stage infections were repeatedly found in this sand fly and dozens of isolates (identical with the human one) were successfully isolated from *P. tobbi*.[3] The increasing number of CL cases in South Anatolia may be ascribed to a number of factors such as improvements in the official surveillance system, migratory movements, changes in cultivation with impact on the natural equilibrium of vectors and possible reservoirs.

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Identifying the risk of leishmania transmission is essential to designing an appropriate public health response to epidemics, and prevents future cases. Even though CL cases had been reported in the Cukurova region before, a rapid accumulation of patients in villages there within the last two decades demonstrates that the outbreak likely began in the foothills of the Taurus and Anti-Taurus Mountains. Our previous studies suggest that the transmission cycle could be anthroponotic[3] and led us to hypothesize that villagers might be at higher risk from sleeping outdoors without bed nets. The moderate manifestation of CL has been overlooked in this rural area, and disease control strategies that target possible reservoirs and phlebotomine vectors have never been put into effect. Furthermore, the knowledge of local people about the transmission cycle of leishmaniasis, including the role of sand flies and the correct use of insecticides, is relatively low.

The purpose of the present investigation was to improve the knowledge of villagers about leishmaniasis and to identify the risk factors associated with *L. infantum* infections using a case-control study, in order to understand the determinants of human outbreak-related CL acquisition in this rural area of the Cukurova region.

2. Material and methods

2.1. Study area

This case-control study was performed in the northwest part of the Cukurova region, South Anatolia, in 2006–2007. South Anatolia is bound by the West Taurus Mountains, the mountain range of Taurus and Anti-Taurus to the north, and the Amanos Mountains to the east. The study area, in the northern part of Cukurova region (South Anatolia, Turkey),[3] is in the western part of the focus and comprises eight villages approximately 55 km northeast of Adana City at altitudes from 150–280 m a.s.l.: Tepecikören, Zerdali, Camili, Bagtepe, Kizillar, Damyeri, Aydın and Akarca. Tepecikören (37°21′46″N, 35°37′40″E) is the only village with a part-time health office (service one day per week). The Kozan Health Center is the only health care facility in the area with leishmania diagnostic capability and anti-leishmania drugs. The numbers of patients treated for CL are approximately 250 per year (specifically: 258 patients in 2006, 301 in 2007, 210 in 2008, 322 in 2009, and 177 in 2010), however it is estimated that most patients are untreated (Dr. S. Ozdemir, Kozan Health Center, personal communication).

Most of the study area is fertile ('mollisol' soil) and used for agricultural activities, but pine (*Pinus*) and fir (*Abies*) forests are also cultivated. The mean annual precipitation is 636.8 mm with 66% relative humidity, and the mean annual temperature is 18.7 °C. During the sand fly season, from May to October, the mean relative humidity is 66% and mean temperature is 27.9 °C (min 13.7 °C and max 45.7 °C). Residents live in single-family houses built from concrete, bricks, adobe, stone and cement, surrounded by gardens with henhouses and sheep or cattle sheds.

2.2. Case-control study

Patients were diagnosed by physicians at the Kozan Health Center or by local doctors visiting villages. A case was defined as a person having at least one leishmania lesion and/or a typical scar. Most case patients had been treated before the case-control study began, following standard practice and treatment of patients with CL, i.e., multiple weekly injections of Glucantime (meglumine antimoniate) for at least two months. Case patients were sought in their homes, based on name lists from health centers and the familiarity of local health doctors. The controls were chosen preferentially according to the site where the cases occurred (i.e., from the neighboring house); however the age and gender of cases and controls was also taken into account. No significant difference in mean age between cases and controls was found and the results of statistical analysis of epidemiological data were adjusted for age.

The study group of 282 persons (141 males and 141 females) was composed of 83 cases (38 males and 45 females) and 199 controls (103 males and 96 females). The age structure was as follows: 71 children (up to 13 years; 19 [22.9%] cases, 52 [26.1%] controls), 40 youngsters (14–19 years; 19 [22.9%] cases, 21 [10.6%] controls), 71 adults (20–39 years; 19 [22.9%] cases, 52 [26.1%] controls), and 100 persons older than 40 years (26 [31.3%] cases, 74 [37.2%] controls).

Because replies to questions may sometimes be biased to include what the respondent perceives is a socially desirable response, data were collected using a structured interview with data entered into questionnaires. The interviewer did not know the patient's leishmaniasis status. Data collected by this risk factor questionnaire included the presence of domestic animals and where they were kept at night, storage of dried dung, house construction materials, toilet location, job travel to other areas, and socioeconomic indicators (age, gender, occupation, education, time of living in village and family size). The remaining questions focused on epidemiological and clinical characteristics, such as sleeping outdoors, the number of lesions and/or scars and their bodily locations, and the time that lesions appeared. Participants were asked about methods they used to prevent sand fly bites (use of bed nets and insecticides), and their knowledge of leishmania transmission. For young children, parents provided consent and responded to the questionnaire.

Data collection was conducted during September 2006 and during August-September 2007, by the same field workers using the same questionnaires. Case-control data were analyzed using fourfold tables with Fisher's exact test and stepwise multiple logistic regressions with backward elimination. Results are presented as the proportion of exposure in cases and controls; associations are expressed as odds ratios with 95% confidence limits. Variables with $p < 0.10$ in the univariate analyses were consequently tested in multivariable models. All statistical tests were performed at a significance level of 0.05. The statistical packages SPSS, release 9.0 (SPSS Inc., Chicago, IL, USA), and Stata, release 9.2 (Stata Corp LP, College Station, TX, USA), were used for the analyses.

Table 1
Risk factors for cutaneous leishmaniasis caused by *Leishmania infantum* in the Cukurova region, South Anatolia, Turkey, based on univariate conditional logistic regression models

Factor	Cases n = 83		Controls n = 199		Odds ratio	95% CI	p-value
	n	%	n	%			
Owns dog	24	28.9	34	17.1	1.97	1.03–3.74	0.035
Owns cattle	68	81.9	140	70.4	1.91	0.98–3.89	NS
Owns goat/sheep	7	8.4	17	8.5	0.99	0.33–2.63	NS
Owns poultry	40	48.2	104	52.3	0.85	0.49–1.47	NS
Living near barn	71	85.5	147	73.9	2.09	1.02–4.58	0.042
Storage of dried dung	18	21.7	24	12.1	2.02	1.03–3.96	0.045
House from concrete	51	61.4	197	99.0	1.37	0.79–2.40	NS
Plastered walls	57	68.7	143	71.9	0.86	0.48–1.57	NS
Water inside the house	73	88.0	178	89.4	0.86	0.37–2.15	NS
Toilet outside the house	66	79.5	143	71.9	1.52	0.80–3.01	NS
Sleeping outside	74	89.2	181	91.0	0.82	0.33–2.17	NS
Not using bed net	42	50.6	78	39.2	1.59	0.92–2.75	NS

NS: nonsignificant p-values.

3. Results

Groups of cases consisted of 83 persons with a lesion and/or a scar indicative of CL. In 15 (29.4%) of them, lesions appeared in the current year, while other patients had lesions and/or scars from previous years. Lesions appeared in the period of year from January to September, with peaks in January/February and July/August; no lesions were reported in the last quarter of the year. Lesions and scars were generally localized on faces (55 and 47%, respectively), arms (26 and 31%, respectively), and legs (16 and 22%, respectively). In nine cases, five males (3.5%) and four females (2.8%) aged 10–40 years, both lesions and scars were found. In all analyses, patients with lesions and/or scars are grouped together. All studied demographic characteristics were comparable among cases and controls.

Among cases, children and young people aged 5–19 years predominated. One-third of cases were younger than 16 years and the median age of infected persons was 20 years, not differing whether their diagnosis was based upon finding lesions or scars. The oldest person with a lesion was 90 while that with scars was 85 years old. The age of the youngest infected children was five years for a lesion and ten years for a scar. Gender, occupation, education, and family size had no significant influence on leishmania infection. Due to the low number of newcomers to the villages, 21 (7.4%) arriving within five and 35 (12.4%) within ten years, the influence of the length of time living in the current village was not evaluated. The same is true for people regularly traveling outside the region for their job: only four (1.4%) respondents.

In univariate analyses, owning a dog (OR: 1.97; 95% CI: 1.03–3.74), storage of dried dung for fuel (OR: 2.02; 95% CI: 1.03–3.96), and the existence of barns situated near houses (OR: 2.09; 95% CI: 1.02–4.58) were associated with a significantly higher risk of CL (Table 1). Dog ownership is the most important risk factor, as confirmed by the multiple logistic regression (Table 2); 28.9% of cases had and fed a dog within their garden, while only 17.1% of controls did. On the other hand, the regression model did not retain indicators for storage of dried dung and living near a barn, though both of these factors show strong correlation with the raising of cattle ($p < 0.001$).

Table 2
Results of multivariable logistic regression model

Factor	Odds ratio	95% CI	p-value
Regular contact with dog	2.14	1.15–3.96	0.016
Raising cattle	1.93	1.01–3.69	0.046
Sleeping without bed net	1.32	1.01–1.72	0.040

Among other potential risk factors, cattle ownership and sleeping without bed nets also showed correlation with CL (though only marginally statistically significant). These correlations appeared significant in the regression model (Table 2), but not in the univariate analysis (Table 1). Bed nets are the most frequently used means of protection against insect bites. Using mosquito nets was mentioned by 152 (53.0%) and using of various insecticides (also in combination with bed nets) by 60 (21.0%) respondents, while 115 (40.0%) did not use any protection. We did not find any significant effect of gender, occupation, education, family size, house construction materials, water and electricity in the house, location of the toilet or keeping animals like sheep, goats and poultry.

4. Discussion

Our study revealed that three major risk factors for acquiring CL by the *L. donovani* complex exist in the Cukurova region: dog ownership, raising cattle and sleeping without a bed net. At first glance, some or all of these factors could be a characteristic outline of life in the Anatolian countryside reflecting a real (possibly unknown) source of risk. However, our analysis revealed that they are, to a great extent, independent predictors of the disease.

Dog ownership had the greatest correlation with leishmaniasis. In areas where *L. infantum* has a zoonotic cycle, dogs can serve as a reservoir and source of human leishmaniasis, including the endemic area where most human infections caused by *L. infantum* are subclinical or asymptomatic.[5] However, an association of *L. donovani* with dogs has not yet been demonstrated, and visceral

leishmaniases caused by *L. donovani* on the Indian subcontinent is considered an anthroponosis[6,7] while in East Africa the reservoir is unknown, even though dogs are considered reservoirs in some studies.[8,9]

Keeping cattle typically implies other factors, such as having a barn and storage of dried dung, which was confirmed by statistical analysis. Therefore, it might be assumed that all these factors act together providing optimum conditions for vector propagation in the vicinity of the house. This is indicative of the short flight distance of *P. tobbi* sand flies, a zoo-anthropophilic and endo-exophagic species.[3,10–12] *Phlebotomus tobbi* is known to be associated with cattle[13] and cattle blood was frequently found in *P. tobbi* females during our previous study.[3]

Sleeping outside may place people at risk of sand fly exposure and the use of bed nets is usually very important in protecting against leishmaniasis transmission.[9] Ownership of a mosquito net appeared to lower the risk, but it is known that classical mosquito bed nets are not dense enough to provide sufficient protection against sand flies.[14] However, our results may not simply reflect the effect of sleeping outside with or without bed net protection. Whether bed nets are protective against leishmania infection depends on a number of factors besides use, including whether the nets are impregnated with insecticide, net shape and size, and wear and tear.[14,15]

Outdoor sleeping during summer months is very common in the studied area. From June to September, 80–90% of respondents sleep outside almost every night, depending on the weather. They use wooden beds in the courtyards, sometimes with traditional cotton bed nets. A previous study has demonstrated that insecticide impregnated bed nets are effective in reducing the outdoor human-biting rate of *P. papatasi* and *P. sergenti* in the Sanliurfa province, southeast of Anatolia.[14] Those results indicate that field trials in an epidemiologically similar area of Turkey such as the Çukurova region could be fruitful. In two previous studies[12,16] focused on entomological aspects, the activity of sand flies was analyzed in the study area. Sand fly season started in May and ended in October; the sand fly numbers increased through June and July, peaked in August, and decreased through September. Studies on nocturnal activity indicated that the highest number of sand flies were trapped during dusk at the beginning of the night, no significant hourly pattern was determined. The activity of sand flies was strongly associated with relative humidity rather than with temperature. Based on our data we can speculate that the most appropriate transmission period of CL is during the hot humid nights from July to September.

That children (5–13 years old) and adolescents (14–19 years old) represent a substantial proportion of all leishmania cases was not surprising, since these age groups are the most numerous. Unfortunately we have no detailed information about the overall proportion of infected individuals in the different age groups or information about the age structure of the population. Furthermore, the fact that the presence of a dog is one of the most important risk factor for CL in our analysis suggests that the transmission occurs in the peri-domiciliary space and in such case the difference of disease prevalence among various age groups is not expected. Nevertheless, the higher presence of CL in children and adolescents could be also related to the lowered immunity of young people and/or to their higher exposure to sand fly vectors. There was no significant difference in the age of persons with lesions and with scars. Unexpectedly, however, in nine persons (10.8% of all infected) both lesions (demonstrating a recent infection) and scars (persisting after a past infection) were detected. Cutaneous leishmaniasis is known to induce a lifelong protective immunity that is capable of preventing re-infection.[17,18] Our results suggest that either cutaneous *L. donovani/L. infantum* can infect humans repeatedly, or that patients are infected for a prolonged period and the infection may reactivate due to immunosuppression or a failure of protective immunity. Further research is required to elucidate the reasons for this unexpected finding.

In summary, the epidemiology of CL in the Cukurova region is complex, and even though the etiological agent (*L. donovani* complex) and sand fly vector (*P. tobbi*) were identified,[3] a number of key questions remain unanswered. Our data have several implications for the design of a control program. On the basis of the risk factor analysis, dogs appear to play a role in the transmission cycle, in spite of the fact that other findings indicate an anthroponotic mode of transmission.[3] Further research on dogs will be essential for clarifying the transmission mode and the role of dogs in the epidemiology of cutaneous leishmaniases due to the *L. donovani* complex. Local people are highly exposed to sand flies while sleeping outside during summer months. We suggest that the use of insecticide impregnated bed nets would improve their personal protection against *P. tobbi* and may reduce the transmission of CL in this region of South Anatolia. Therefore, a medium-scale field trial with long-lasting insecticide-treated bed nets is planned in three villages in the area.

Authors' contributions: JV and BA contributed equally to the study. BA conceived the study; BA and OEK designed the study and performed field data collection; JV participated in the field data collection and prepared the first draft of the manuscript; PK analyzed the data; PV validated and interpreted the data. All authors contributed to revision of the manuscript and read and approved the final version. JV and BA are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: The National Ethics Committee for Health Research of Turkey approved the study and

the Turkish Ministry of Health gave administrative authorization.

Agreement to carry out the study was also obtained from the local health authorities, the village committees and the adults who participated in the study. For children, informed consent was obtained from their parents.

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