

Leishmaniasis in northern Cyprus: Human cases and their association with risk factors

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ABSTRACT

Background & objectives: Cyprus is located in the eastern part of the Mediterranean Region where leishmaniasis is endemic. The primary objective of this study was to investigate human visceral leishmaniasis (VL) in the northern region of Cyprus where presence of canine leishmaniasis (CanL) and sandflies has been documented in earlier studies. The secondary objective was to assess the association of leishmaniasis with demographic and epidemiological variables.

Methods: Intravenous blood samples were collected from 249 volunteers in Kyrenia district (located in the northern coastal region of Cyprus). Whole blood samples were tested for DNA of *Leishmania* spp by polymerase chain reaction (PCR), while serum samples were analyzed using direct agglutination test (DAT) and rK39 test. For evaluation of possible risk factors, a questionnaire was applied to the participants.

Results: Only three (1.2%) of 249 participants were found seropositive by DAT (n = 2) or rK39 test (n = 1). The remaining samples were negative in serology, and no PCR positivity was detected in any of the 249 participants. Seven individuals, including the seropositive cases, had a history of cutaneous leishmaniasis (CL). Seropositivity and CL were not significantly related with gender (M/F: 40.2/59.8%), age [Mean: 42.85 ± 17.45, Median: 40 (7–86)], occupation (Indoor/Outdoor: 84.7/12.9%), dog ownership (52.6%), and CanL history (5.3%). However, a statistical association was found between seropositivity and past CL infection. Also, a significant relation was observed between participants living in peripheral area (63.1%) and CL infection. Furthermore, leishmaniasis awareness (28.1%) among the study population was statistically correlated with past CL infection and dog ownership.

Interpretation & conclusion: This study demonstrates the presence of leishmaniasis and highlight the need for implementation of efficient control measures on the northern coast of Cyprus.

Key words Diagnosis; epidemiology; leishmaniasis; northern Cyprus; risk factors

INTRODUCTION

Cyprus is an Island located in the eastern part of the Mediterranean Region where leishmaniasis is endemic¹. The island is an important focus for leishmaniasis as various *Phlebotomus* species have been reported in the island, and additionally, presence of canine leishmaniasis (CanL) is also documented¹⁻³. In southern Cyprus, seroprevalence of CanL increased from 1.7 to 14.9% within approximately 10 yr, and *Leishmania infantum* MON-1 strain was identified as the predominant zymodeme, which is commonly encountered in the Mediterranean region^{2,4}. CanL was also reported from northern Cyprus, and the percentages ranged between 1.9 and 13.2%⁵⁻⁶.

Occurrence of human leishmaniasis has been demon-

strated since 1935 in Cyprus². In 1990, Leishmanin skin test revealed 10% positivity in Kyrenia and 35% in Lapithos (both are parts of Kyrenia district, Fig. 1) in northern Cyprus. The disease causing agent was identified as *L. infantum* by DNA hybridization⁴. In 2006, three cutaneous leishmaniasis (CL) and two visceral leishmaniasis (VL) cases were reported in humans (due to *L. donovani* MON-37) in southern Cyprus⁷. In 2014, a familial cluster of CL cases comprising *L. donovani* was reported from southern Cyprus⁸. More recently, a report from northern Cyprus has demonstrated three pediatric VL cases caused by *L. infantum*⁹.

Studies from northern Cyprus indicated that CanL⁶, and sandflies¹ are relatively common in Kyrenia district, which suggests that this region may be at risk for leish-



Fig. 1: Map of Cyprus showing Kyrenia district (indicated by lines) and the capital Nicosia (Adapted from: <http://www.worldatlas.com/webimage/countrys/europe/outline/cy.htm>). The central (Kyrenia municipality▲) and the peripheral locations (L—Lapithos, K—Karavas, D—Dikomo, E—Agios Epiktitos, A—Agios Amvrosios) are indicated in the map.

maniasis. Therefore, this study was designed to investigate primarily the presence of human VL in this specific region of northern Cyprus. Additionally, it was aimed to identify the correlation of leishmaniasis with demographic and epidemiological characteristics of the participants taking into consideration that a number of studies have addressed the association between leishmaniasis and several potential risk factors^{10–13}. This study is the first comprehensive survey conducted on human leishmaniasis in the last two decades in northern Cyprus, and also the first report that analyzes the potential risk factors of leishmaniasis in this region.

MATERIAL & METHODS

Study area

Cyprus is located between the coordinates 35° 5' 42.6912" N and 33° 12' 12.3480" E. The island has Mediterranean climate, which is generally hot and dry in summers, and mild in winters¹. Northern Cyprus (Turkish Cypriot community) is composed of five districts, and has a population of 286,257 people (Census 2011). Kyrenia district is situated on the northern coast of the island, and has a total population¹⁴ of 69,163. Along the northern line of the island, run the Kyrenia mountains (Pentadactylos) which separate Kyrenia district from the inner region. Rainfall occurs more frequently in winters¹⁵, and the average annual rainfall is higher (approximately 500 mm) at the northern side of the mountains than the interior¹⁶. The average annual temperature at the northern coast is 18.8°C. Also, the humidity in Kyrenia and surroundings is higher than the central plain¹⁵. There are six municipalities within the district: Kyrenia, Lapithos, Dikomo, Karavas, Agios Epiktitos, and Agios Amvrosios. Among these, Kyrenia municipality is centrally lo-

cated and relatively more developed. Furthermore, this municipality contains the highest number of inhabitants, which accounts for approximately 50% of the whole district population¹⁴. For this reason, Kyrenia municipality was considered as the central area, while the remaining five were considered as the peripheral region in the study (Fig. 1).

Study design and population

In the present study, Dr Akcicek Hospital that provides health care services for the residents in Kyrenia district was selected as the study setting. The participants were recruited from consecutive admissions to the hospital's routine biochemistry and haematology laboratories between September and December 2014. The possibility of detecting new cases of leishmaniasis could be higher during this period, as the period between April and November is known for sandfly activities in Cyprus. Furthermore, potential transmission of *L. infantum* has been shown to be higher between June and October in the Mediterranean¹⁷. Participants for the study were selected, provided that they were living in Kyrenia district for at least one year and had no underlying immunosuppressive disease. The participants of the study were physically examined and their medical histories were recorded by a general practitioner, having ample experience in leishmaniasis diagnosis. A total of 255 volunteers met the inclusion criteria. Among these, five individuals whose demographic information were missing/invalid and one subject originating from a locality other than Kyrenia district were excluded from the survey. Eventually, 249 healthy subjects from the district were included and analyzed in the study.

Ethical consideration

The ethical approval for the study was obtained from the Near East University Scientific Research Assessment Ethics Committee (Project No: YDU/2014/22-128). Written informed consent was collected from all the participants. Permission was obtained from the parents of individuals younger than 18 yr of age.

Sample collection

Intravenous blood was collected from the study participants. Each sample was collected in two different tubes (ethylene diamine tetra acetic acid [EDTA] and dry tubes). Whole blood samples were kept in the original EDTA collection tubes at –20 °C until used for the molecular tests. Samples in the dry tubes were centrifuged at 4000 rpm for 10 min and sera were separated into 1.5 ml tubes which were stored at –80°C until use for the serological tests.

Data collection

In order to determine the risk factors of leishmaniasis in the study population, a questionnaire was applied to each participant. Individuals younger than 18 yr of age were interviewed in the presence of their parents. Firstly participants' age, gender, residential setting, occupation, and knowledge on leishmaniasis were noted. Students, civil servants, housewives, retirees, and other indoor professionals were categorized as having indoor occupations. Workers such as builders, military personnel, and other outdoor professionals were categorized as having outdoor occupations. The participants who had a history of CL indicated the time elapsed from the diagnosis and treatment.

Declarations of the individuals with past CL infection were also confirmed by the hospital records. Information from dog owners (within the study population) on CanL history of their dogs, and the use of protective collar as a preventive measure for CanL was also recorded. For the individuals with a history of CL, information related with the residential setting, occupation, dog ownership, CanL history, and use of protective collar was based on the year of CL diagnosis. These participants also declared that they were already living in Kyrenia district at least for one year at the time of CL diagnosis and they have never travelled to any *Leishmania* endemic countries.

Direct agglutination test (DAT)

This test was conducted according to the manufacturer's protocol (Academic Medical Centre, The Netherlands)¹⁸. Briefly, the sera were adjusted to final concentration of 1:100; and two-fold serial dilutions were made. Each microplate contained a positive (*Leishmania*-positive serum) and a negative (physiological saline) control. Next, *L. donovani* antigen solution prepared from a freeze-dried antigen preparation was added and microplates were allowed to incubate at room temperature for 18 h. The agglutination results were read by two independent readers. The DAT titre was accepted as the highest serum dilution with an apparent agglutination¹⁸⁻¹⁹. The samples having a titre of $\geq 1:1600$ were considered positive^{18,20}.

The rK39 dipstick test

The test was performed according to the manufacturer's protocol (SD Bioline Leishmania Ab, Republic of Korea)²¹. *Leishmania*-positive and negative control sera were used for the accuracy of the test. Results were considered positive when two distinct red or pink lines, one in the test region and another in the control region, appeared within 15 min.

Molecular analysis

DNA isolation from the blood samples was conducted according to the manufacturer's protocol by using the spin-column method (Gene MATRIX Quick Blood DNA Purification kit, Poland). PCR targeted the internal transcribed spacer 1 (ITS1) which is located between the small subunit ribosomal RNA (SSU rRNA) and 5.8 rRNA genes²². In each run, molecular-grade water was used as the negative control, while DNA from cultured *L. infantum* promastigotes served as the positive control. Five μ l of the amplicons were analyzed by electrophoresis (Major Science, U.S.A.) on 1.5% agarose gels (Bioshop, Canada) and visualized by UV light (Biostep, Germany) after being stained with ethidium bromide (Bioshop, Canada).

Statistical analyses

Descriptive statistics for all variables obtained in the questionnaire were calculated. For categorical variables, frequency and percentage information were given while for the continuous variables arithmetic mean, standard deviation, median, minimum and maximum were calculated. Since data did not meet parametric assumptions, Mann-Whitney U-test was used to compare the distribution of continuous variables between two independent groups. Either Pearson Chi-square or Fisher's exact test was applied for evaluating the associations amongst categorical variables. All statistical calculations were performed with IBM SPSS statistics package for Macintosh (Demo version 22.0; Armonk, NY: IBM Corp.). Level of significance was set at 0.05.

RESULTS

General demographic and epidemiological data of the study population

In total, 249 participants were enrolled in the present study. None of the study participants were associated with underlying immune failure or human immune deficiency virus positivity. In the physical examination of the participants, there was no finding of active CL lesion or hepatosplenomegaly, which is an indicator of VL. Among the participants, 100 (40.2%) were male and 149 (59.8%) were female. The mean age of the volunteers was 42.85 ± 17.45 , and the median age was 40 (7–86). Numbers of the residents in Kyrenia (central) and other five municipalities (Peripheral) were 92 (36.9%) and 157 (63.1%), respectively. Of all the participants, 211 (84.7%) were indoor professionals, while 32 (12.9%) were engaged in outdoor occupations. Six (2.4%) participants did not provide information on their occupation. Seven (2.8%) individuals declared that they were previously

diagnosed with CL. These individuals were clinically diagnosed and their ulcers healed completely after treatment with pentavalent antimonials. Their medical history was checked in and confirmed by the hospital records. A total of 131 (52.6%) participants indicated that they owned dogs. Among these, 7 (5.3%) individuals stated that one or more of their dogs were euthanized due to the severe untreatable CanL.

Diagnostic test results of the study population

In the study, 3 (1.2%) of 249 participants were found seropositive. Two of these were detected positive by DAT (Serum antibody titre—1:1600). The third participant was found positive by rK39 test. No *Leishmania* antibodies were detected by any of the employed serological tests in the remaining 246 volunteers. Furthermore, PCR revealed that blood samples of the entire study population were all negative for *Leishmania* spp.

Correlation of the results with the demographic and epidemiological factors

All the three seropositive individuals were male. Their mean and median age was 46.67 ± 25.79 , and 54 (18–68), respectively. The seropositivity was not significantly related to gender, residential setting, occupation, dog ownership and CanL cases ($p > 0.05$). Due to low number of positive results, no statistical evaluation could be done for the age. All the three seropositive individuals

had a history of CL. Accordingly, a significant association was found between seropositivity and past CL infection ($p < 0.001$) (Table 1).

Among seven participants who had a history of CL, five were male, and two were female. Their mean age was 37.29 ± 23.81 , and the median age was 36 (7–68). CL was not statistically related to age, gender, occupation, dog ownership and CanL ($p > 0.05$). All seven participants originated from peripheral localities, and a significant association was found between CL and residential setting ($p = 0.049$) (Table 1). Detailed test results, and demographic and epidemiological characteristics of seven individuals with past CL infection are summarized in Table 2.

Of the 249 participants, 70 (28.1%) had knowledge about leishmaniasis; however, 179 (71.9%) individuals had no information on the disease. The participants' knowledge on leishmaniasis was not statistically related with their age [Mean: 41.06 ± 17.35 , Median: 38.5 (7–86)], gender, residential setting and occupation ($p > 0.05$). Disease awareness was not significantly correlated with seropositivity ($p > 0.05$), but it was statistically related with past CL infection ($p = 0.020$). No correlation was found between CanL and disease awareness ($p > 0.05$). Nevertheless, dog owners had significantly more knowledge on leishmaniasis, compared to non-owners ($p = 0.001$). Of the 131 dog owners, 36 (27.5%) used protective collar for their dogs, and this preventive measure was significantly more commonly prac-

Table 1. Serology and clinical results of leishmaniasis in the study population according to the demographic and epidemiological factors, Kyrenia district, northern Cyprus, 2014

Variable	Serology result			CL history		
	Positive n (%)	Negative n (%)	<i>p</i> -value	Positive n (%)	Negative n (%)	<i>p</i> -value
<i>Gender</i>						
Male (n = 100)	3 (3)	97 (97)	0.064	5 (5)	95 (95)	0.121
Female (n = 149)	0 (0)	149 (100)		2 (1.3)	147 (98.7)	
<i>Residential setting</i>						
Central (n = 92)	0 (0)	92 (100)	0.298	0 (0)	92 (100)	0.049
Peripheral (n = 157)	3 (1.9)	154 (98.1)		7 (4.5)	150 (95.5)	
<i>Occupation</i>						
Indoor (n = 211)	3 (1.4)	208 (98.6)	1	6 (2.8)	205 (97.2)	1
Outdoor (n = 32)	0 (0)	32 (100)		1 (3.1)	31 (96.9)	
<i>Dog ownership</i>						
Yes (n = 131)	1 (0.8)	130 (99.2)	0.605	1 (0.8)	130 (99.2)	0.055
No (n = 118)	2 (1.7)	116 (98.3)		6 (5.1)	112 (94.9)	
<i>Leishmaniasis in dog(s)</i>						
Yes (n = 7)	0 (0)	7 (100)	1	0 (0)	7 (100)	1
No (n = 124)	1 (0.8)	123 (99.2)		1 (0.8)	123 (99.2)	
<i>CL history</i>						
Yes (n = 7)	3 (42.9)	4 (57.1)	<0.001	NA	NA	NA
No (n = 242)	0 (0)	242 (100)				
<i>Seropositive</i>						
Yes (n = 3)	NA	NA	NA	3 (100)	0 (0)	<0.001
No (n = 246)				4 (1.6)	242 (98.4)	

NA— Not applicable.

Table 2. Detailed diagnostic test results, treatment, and demographic and epidemiological characteristics of seven individuals with a history of CL, Kyrenia district, northern Cyprus, 2014

Characteristics		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Diagnostic tests	DAT	Positive (1/1600)	Positive (1/1600)	Negative	Negative	Negative	Negative	Negative
	rK39	Negative	Negative	Positive	Negative	Negative	Negative	Negative
	PCR	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Time (yr) elapsed after CL diagnosis and treatment		4	1	4	25	5	5	1
Drug used for the treatment		Sb ^v	Sb ^v	Sb ^v	Sb ^v	Sb ^v	Sb ^v	Sb ^v
Gender		Male	Male	Male	Male	Female	Male	Female
Age		68	54	18	60	36	7	18
Residential setting*		Peripheral	Peripheral	Peripheral	Peripheral	Peripheral	Peripheral	Peripheral
Occupation*		Indoor	Indoor	Indoor	Indoor	Outdoor	Indoor	Indoor
Leishmaniasis awareness		Yes	No	No	Yes	Yes	Yes	Yes
Dog ownership*		Yes	No	No	No	No	No	No
Leishmaniasis in dog(s)*		No	–	–	–	–	–	–
Protective collar*		Yes	–	–	–	–	–	–

*Information was based on the year of CL diagnosis; Sb^v—Pentavalent antimonials.

ticed among those who had knowledge on leishmaniasis ($p = 0.008$) (Table 3). Furthermore, the number of dog owners in the peripheral municipalities ($n = 94/157$, 59.9%) was higher than those living in the central ($n = 37/92$, 40.2%), and the difference was statistically significant ($p = 0.003$). On the contrary, use of protective

collar was less frequent in peripheral locations ($n = 21/94$, 22.3%) compared to Kyrenia ($n = 15/37$, 40.5%), and this result was also significant ($p = 0.036$).

DISCUSSION

Although few studies have reported sporadic cases of leishmaniasis in northern Cyprus^{4,9}, there is a lack of comprehensive surveys on human disease in this area. This study was aimed to estimate the prevalence of human leishmaniasis (VL) in Kyrenia district of northern Cyprus, where CanL⁶ and sandflies¹ have been demonstrated to be relatively common.

The present study found three (1.2% of the study population) individuals to be seropositive (case 1 and 2: DAT-positive, case 3: rK39-positive) (Table 2), and these cases were previously diagnosed with CL. The seropositivity is not surprising as both DAT and strip test results can remain positive for a while after the cure due to persisting antibodies²¹. Nevertheless, the positive serology results might also have been caused by asymptomatic infection¹⁹. Moreover, induced immune response in asymptomatic persons can result from repeated exposure to *Leishmania* parasites or release of parasites from the target cells²³, which can also explain the reason of seropositivity. In the remaining four CL cases (4–7) (Table 2), the negative results could be attributed to the absence of immune stimulation²³, as well as low performance of the serological tests in subclinical infections² and CL²¹. Similarly, negative PCR results of the cases with past CL infection could be explained by the low sensitivity of PCR in diagnosis of asymptomatic infections²⁴.

Discrepancies between DAT and rK39 test in our study were presumably caused by their different antigenic

Table 3. Leishmaniasis awareness of the study population and its association with demographic and epidemiological factors, Kyrenia district, northern Cyprus, 2014

Variable	Knowledge on leishmaniasis		
	Yes	No	<i>p</i> -value
<i>Gender</i>			
Male (n = 100)	32 (32)	68 (68)	0.264
Female (n = 149)	38 (25.5)	111 (74.5)	
<i>Residential setting</i>			
Central (n = 92)	26 (28.3)	66 (71.7)	0.968
Peripheral (n = 157)	44 (28)	113 (72)	
<i>Occupation</i>			
Indoor (n = 211)	61 (28.9)	150 (71.1)	0.927
Outdoor (n = 32)	9 (28.1)	23 (71.9)	
<i>Seropositive</i>			
Yes (n = 3)	1 (33.3)	2 (66.7)	1
No (n = 246)	69 (28)	177 (72)	
<i>CL history</i>			
Yes (n = 7)	5 (71.4)	2 (28.6)	0.02
No (n = 242)	65 (26.9)	177 (73.1)	
<i>Dog ownership</i>			
Yes (n = 131)	49 (37.4)	82 (62.6)	0.001
No (n = 118)	21 (17.8)	97 (82.2)	
<i>Leishmaniasis in dog(s)</i>			
Yes (n = 7)	5 (71.4)	2 (28.6)	0.102
No (n = 124)	44 (35.5)	80 (64.5)	
<i>Use of protective collar</i>			
Yes (n = 36)	20 (55.6)	16 (44.4)	0.008
No (n = 95)	29 (30.5)	66 (69.5)	

Figures in parentheses indicate percentages.

nature. DAT utilizes whole promastigotes, while rK39 test contains a recombinant protein as antigen. For this reason, these tests might recognize different antibodies at different levels, or their kinetics might differ in asymptomatic persons and past VL cases²⁰. Furthermore, the polyclonal antibody response against the DAT total antigen could persist for longer period than the monoclonal response against the rK39²³. Hence, these factors might explain the reason of DAT (+) / rK39 (-) (cases 1 and 2) and DAT (-) / rK39 (+) (case 3) results (Table 2).

Although the primary objective of this study was to investigate VL, due to the CL history of seven participants, we also evaluated these cases. Using the data obtained from the serological tests and considering the clinical CL history of seven individuals, we assessed the possible risk factors of leishmaniasis in the study area. For this reason, we analyzed the association of both seropositive and CL positive cases with demographic and epidemiological variables.

In the present study, five of the seven individuals had history of CL and all three seropositive persons were male (Table 2). However, participants' gender was not significantly related with serology result and past CL infection ($p > 0.05$) (Table 1). Ranasinghe *et al*²⁵ also did not find any significance between gender and CL in their study. Yet, CL and VL were documented to be more common in males than females^{11, 26}. Men are exposed to sandflies more frequently than women because of spending more time outside (such as working)¹³. Additionally, Soares *et al*²⁶ suggested that at low levels of exposure women can be more resistant against *Leishmania* due to the hormone related immune protection. For this reason, although the result was not significant, male dominance in both serology results and CL history is remarkable in this study.

Due to the low number of seropositivity, no statistical evaluation could be done for the age. On the other hand, no correlation was found between age and CL ($p > 0.05$). This is consistent with the finding of Ranasinghe *et al*²⁵ where age was not a significant risk for CL. Conversely, Reithinger *et al*¹⁰ found an association between age and anthroponotic CL (ACL), and also documented a greater risk of ACL in younger people. A relation between increasing age and VL has been also demonstrated in another study by Gadisa *et al*¹¹.

Residential setting did not significantly affect the seropositivity ($p > 0.05$), however, living in peripheral locations was statistically related with CL infection ($p = 0.049$) (Table 1). Five of seven individuals with past CL infection were living in Lapithos (Table 2) and this could be due to the presence of sandflies in this region. Demir *et al*¹ reported highest number of sandfly species

diversity in Lapithos (12 species). Furthermore, Ergunay *et al*²⁷ identified *L. infantum* in *P. tobbi* in Lapithos and a nearby village. Together with the previous reports, the present findings suggest that peripheral locations are at higher risk of CL compared to the central municipality.

Participants' occupation was not significantly associated with serology results and CL diagnosis in this study ($p > 0.05$) (Table 1). Six out of seven CL patients and all three seropositive persons had indoor occupations (Table 2). Having an outdoor occupation has been reported as a risk factor for CL. However, Ranasinghe *et al*²⁵ did not find any significant correlation between outdoor and indoor occupation in terms of CL positivity. Considering that some *Phlebotomus* species may be found in peridomestic areas and enter the buildings¹³, both indoor and outdoor occupation could be risk factors for CL.

In this study, it was observed that seropositivity and CL were not significantly affected by dog ownership and CanL ($p > 0.05$) (Table 1). Moreover, only one of the seven CL patients owned dogs that was negative for CanL. In a study carried out by Pérez-Cutillas *et al*¹², *L. infantum* PCR positivity had a significant correlation with dog ownership, however, the positivity among dog owners was not statistically related with present and past CanL cases. Ranasinghe *et al*²⁵ did not find any significant association between having dog and CL. Similarly, Reithinger *et al*¹⁰ detected no statistical correlation between dog ownership and ACL. Given the presence of CanL in Kyrenia district^{3, 5-6}, the findings of this study suggest that dogs could be incidental hosts for leishmaniasis, also indicating the anthroponotic transmission of *Leishmania* in northern Cyprus¹⁰.

In order to determine which factors affect the awareness on leishmaniasis, the disease knowledge of the participants was further analyzed with respect to all of the variables. Leishmaniasis awareness was not statistically associated with age, gender, residential setting and occupation ($p > 0.05$). Also, no correlation was found between leishmaniasis awareness and seropositivity ($p > 0.05$) (Table 3). Likewise, Pérez-Cutillas *et al*¹² have detected no relation between *L. infantum* PCR positivity and leishmaniasis awareness. However, in this study, the rate of leishmaniasis awareness was significantly higher among the individuals who were previously diagnosed with CL ($p = 0.020$). The result is not surprising as these patients became aware of the disease most probably after the clinical diagnosis. Although disease awareness was not significantly associated with CanL cases ($p > 0.05$), the awareness among dog owners was significantly more common than non-owners ($p = 0.001$). Furthermore, the use of a protective collar for their dogs among the dog ow-

ners who had knowledge on leishmaniasis was statistically significant ($p = 0.008$). However, this was restricted within the central residential area. Dog ownership among participants from the peripheral locations was more common ($p = 0.003$), however use of protective collar was less frequent ($p = 0.036$) compared to those from Kyrenia. Poor practice of preventive measures despite of disease awareness has been reported earlier. All of these suggest that attention should be paid to the reasons behind the low implementation of measures¹¹ at the peripheral localities in the study.

Although this study analyzed the impact of person-related risk factors, another point to consider was the favourable environmental conditions of the study area. Occurrence of leishmaniasis cases in Kyrenia district could be attributed to climatic and geographical factors, as these affect the presence of sandflies¹². For example, moisture in Kyrenia and surroundings is higher than the inner cities of northern Cyprus¹⁵, and humidity has been shown to be a requirement for pupation of the vectors¹¹.

Sandfly species have in general a short flight range of approximately 300 m¹¹. The Kyrenia Mountains form a barrier between the northern coast and the central plain, and prevent the sandflies from migrating to the interior. Indeed, *P. neglectus*, the vector of *L. infantum*, has been reported in the north of the Kyrenia Mountains. Due to its weak flying activity, and presence of the mountains in the island, Mazeris *et al*² proposed that this species cannot travel to southern part, and only found in northern Cyprus. Pérez-Cutillas *et al*¹² found that *L. infantum* PCR positivity was higher at the areas of lower temperatures and altitude. The average annual temperature (18.8°C)¹⁵, and altitude of northern coastal regions are lower than the inner cities, suggesting that Kyrenia district is at risk of leishmaniasis. The authors postulated that the wider range of temperature differences in higher areas might decrease the vector survival. The temperature differences are lower in the northern coast¹⁵, and this is also consistent with the findings of Pérez-Cutillas *et al*¹². In the same study, it was also stated that the areas with higher positivity rate were associated with greater rainfall in winter. The authors proposed that more rain in the cold months might cause increased survival of sandfly eggs and larval diapauses¹². Rainfall occurs more frequently in winter¹⁵, and the average annual rainfall in the Kyrenia district is generally higher than the central plain¹⁶, so this was again consistent with the finding of Pérez-Cutillas *et al*¹².

CONCLUSION

The present study primarily aimed to investigate VL in a group of people living in Kyrenia district. Three (1.2%)

of 249 participants were seropositive for leishmaniasis. Furthermore, seven participants (including the seropositive cases) had a history of CL.

Despite the low number of study participants and positive cases, results of the statistical analyses were consistent with earlier reports. In this study, seropositivity for leishmaniasis or history of clinical CL were not significantly associated with gender, age, occupation, dog ownership, and CanL history. However, a statistical correlation was observed between past CL cases and positive serology results. Leishmaniasis awareness was significantly associated with past CL infection and dog ownership. Although number of dog owners was higher, use of protective collar was less common among the participants from peripheral locations. Furthermore, a significant association was observed between participants living in peripheral area and CL infection. These findings suggest that control measures should be implemented more efficiently particularly in the peripheral regions of Kyrenia district.

PCR results were all negative in the present study. Thus, no information on the molecular characteristics of *Leishmania* spp was obtained. This indicates that, future studies are necessary to identify the zymodemes of the parasite strains and analyze their genetic relationship with the isolates in the neighbouring countries.

To represent the whole population of northern Cyprus, more individuals from different localities need to be tested by additional studies. The results of this study would provide a basis for future research to investigate *Leishmania* spp and the disease prevalence in northern Cyprus.

Conflict of interest

The authors report no conflict of interest.

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