Seroprevalence of and risk factors for Leishmania seropositivity in a sample population of Western Sicily (Italy)

Emanuele Amodio[1], Giuseppe Calamusa[1], Enza Sidoti[1], Antonella Migliazzo[2], Fabrizio Vitale[2], Claudio Costantino[1], Francesco Vitale[1]

ABSTRACT

BACKGROUND: ***Leishmania*** **is a vector-borne parasite responsible for significant morbidity and mortality worldwide. The aim of this study was to assess the prevalence of and risk factors for** ***Leishmania infantum*** **seropositivity in a sample of Sicilian population.**

METHODS: A total of 260 subjects were interviewed using a standardized questionnaire and requested for an venous blood sample.

RESULTS: Overall, 36 subjects (13.8%) were seropositive against ***L. infantum*** **with a statistically significant higher prevalence of positivity in older subjects (p=0.04). After adjustment for age, a higher risk for Leishmania seropositivity was found in subjects who had pets living outdoors and untreated with anti-pests, and in those who were current smokers (adj-OR = 2.95 and adj-OR = 3.11, respectively; p < 0.05).**

CONCLUSIONS: Our data confirm that ***Leishmania*** **infections among Sicilian citizens can be considered relatively frequent, suggesting that a percentage of** ***Leishmania*** **seropositivity can be probably attributed to exposure to both old and new risk factors.**

Key words: Leishmania, seropositivity, epidemiology, Sicily

(1) Department of Sciences for Health Promotion "G. D'Alessandro", University of Palermo, Italy

(2) Leishmaniosis National Reference Center of the Istituto Zooprofilattico Sperimentale (IZS) of Sicily (Palermo, Italy)

CORRESPONDING AUTHOR: *Emanuele Amodio, Department of Sciences for Health Promotion "G. D'Alessandro," University of Palermo, Italy, Via Del Vespro n°133, 90127. Tel: +39 338 97 20 919. email: emanuele.amodio@unipa.it*

DOI: ***10.2427/9024***

Published as Online First on June 3, 2014

INTRODUCTION

Leishmania is a vector-borne protozoan parasite responsible for significant morbidity and mortality worldwide, being endemic in 88 countries, with approximately 0.9 to 1.6 million new cases per year [1]. *Leishmania infantum* is the only species present in Sicily and is transmitted through a zoonotic mechanism which involves dogs as the main reservoir of infection [2]. The protozoan is spread through the bite of an infected female phlebotomine sandfly that are widely distributed in Mediterranean Countries with a

peak of activity during summertime [3]. Most human infections by *L. infantum* are subclinical or asymptomatic whereas only a minority, especially in immunosuppressed subjects, can cause obvious disease including cutaneous (CL) and visceral (VL) manifestations [4].

Following World War II, the widespread application of dichlorodiphenyltrichloroethane (DDT) in much of southern Europe was associated with a considerable decrease of several arthropod vectors, including phlebotomine sandflies [5]. The banning of DDT could be responsible for an inversion of this trend as observed in Brazil where the discontinuation of the insecticide has been associated with a marked increase in abundance of the *Anopheles* vector and increased malaria cases [6]. If this association should be confirmed also in Italy, where DDT use has been banned for at least three decades, a variable number of endemic arthropod-borne infections could be insufficiently controlled [7]. According to this hypothesis, an active circulation of Toscana virus, an arthropod-borne virus transmitted by the same vector of *Leishmania*, has been found in the general Sicilian population in 2009 [8]. Therefore, a high number of subjects could be also exposed to *L. infantum* infection in an area as endemic such as Sicily. In this paper, we have assessed the seroprevalence of *L. infantum*-specific antibodies in a sample of the general Sicilian population identifying potential environmental and individual factors associated with *Leishmania* antigen seropositivity.

METHODS

A cross-sectional study was carried out from September 2009 to July 2010 in Calatafimi-Segesta, which is a small city located in the province of Trapani, western Sicily, Italy. Calatafimi-Segesta has a resident population of about 7,144 inhabitants [9] and is situated at an altitude of 338 m above sea level.

A sample of subjects was randomly selected from the National Health roster for Calatafimi, after which, patients were stratified in terms of gender and age according to the whole population structure. Criteria for exclusion of subjects from the study were: residence other than in Calatafimi-Segesta, age <5 years, severe cognitive dysfunction and inability of the general practitioner to reach each subject within the recruitment period. Randomly selected participants who had died before recruitment were also excluded.

As reported in detail elsewhere a random sample of 271 eligible subjects were successfully recruited and enrolled [10]. Each subject provided a venous blood sample and was interviewed using a standardized questionnaire including information on:

- sociodemographics, occupation, educational level, ownership of an extra-urban holiday residence;
- anthropometry;
- lifestyle habits and exposure to animals.

Prior to participation all subjects were asked to give their informed consent. The study was approved by the Institutional Review Board of the AOUP "P. Giaccone", Palermo, Italy. Unfortunately, for 11 subjects the blood sample was not enough to allow laboratory analyses therefore, only the data provided by the 260 individuals was finally included and discussed in our study.

Serum samples were stored at 4 °C for a maximum of 24 h or processed immediately; alternatively they were aliquoted and frozen at -20 °C for future testing.

The presence of anti-*Leishmania* antibodies was measured by an indirect immunofluorescence antibody test (IFAT) performed in conformity of recommendation of the World Organisation for Animal Health using MHOM/TN/80/IPT1 MON 1 as whole parasite antigen fixed on multi-spot slides (BioMerieux) and a fluorescent-labeled, anti-human gamma globulin (SIGMA) as conjugate [11]. Positive sera were serially diluted and tested to establish the maximum reaction titre, starting from dilution at concentration of 1:40. Positive and negative controls were included in each slide. All samples showing positivity at a dilution ≥1:40 were considered to be reactive.

The data was analyzed using the R statistical software package [12]. The significance level for all analyses was chosen to be 0.05, two-tailed. Absolute and relative frequencies were calculated for qualitative variables. Continuous normally distributed variables were summarized as mean ± standard deviation. Categorical and categorized (age and BMI) variables were analyzed using Chi-square test (Mantel-Haenszel), Chi-square for linear trend test or logistic regression analysis as indicated. Odds ratio (OR) and 95%

confidence interval (95% CI) were calculated. Age adjustment was evaluated by performing a logistic regression analysis.

RESULTS

The characteristics of the study of the general population was reported in Table 1. Of the 260 recruited subjects, 141 were females (54.2%) with an average age of 49.2 ± 19.4 years, and 119 were males (45.8%) with an average age of 50.4 ± 21.7 years. Low or intermediate level of education was represented in 64% of respondents, 45.5% of respondents were overweight and 66.5% of participants were married/free union.

Overall, 36 subjects (13.8%) resulted seropositives against *Leishmania* with a statistically significant higher prevalence of positivity in older subjects (p=0.04). A higher, but not statistically significant, percentage of *Leishmania* seropositivity was observed in males (16.8%), overweight (17.2%), widows (20.0%), farmers (33.3%), and subjects with low/intermediate education levels (15.2%).

As reported in table 2, after adjustment for age, subjects having animals living outdoors and not treated with anti-pests or who were current smokers were at higher risk for *Leishmania* seropositivity (adj-OR=2.95, 95% CI=1.2-7.3 and adj-OR=3.11, 95% CI=1.38-7.01, respectively). A higher, but not significant, risk was also found in owners of extra-urban holiday resort (adj-OR=1.38, 95% CI= 0.63-3.01) and, particularly, in subjects using pesticides (indoor, adj-OR= 1.61, 95% CI=0.62-4.11; outdoor adj-OR= 1.86, 95% CI= 0.74-4.65; outdoor/indoor adj-OR= 3.4, 95% CI%=0.91-12.7).

DISCUSSION

The results of the present study confirm that asymptomatic *Leishmania* infections among Sicilian citizens can be considered relatively frequent, showing a prevalence similar to those found in some south-European countries such as Spain (ranging from 4.9% to 24%) and France (ranging from 13% to 32%) [13-16]. Diversely, a lower seroprevalence was observed in Greece (ranging from 0.5% to 2.8%) and other Italian regions as well as Piedmont (7.4%) and Sicily, where other authors have reported very low positivity rates among healthy blood donors (0% in 2005 and 0.75% in 2008) [17-21]. These latter results, carried out in the same regional macroarea, could apparently generate some confusion, however, it should be noted that all of these comparisons should be considered with precautions for two main reasons. Firstly, in absence of gold-standards, the choice of different laboratory methods and cut-off values could affect the prevalence of positive subjects. Considering that the intention of our study was to evaluate the exposure to infection rather than assess a diagnostic significance, in our setting we have adopted a serum antibody titers 1:40 as an indicator of previous *Leishmania* infection as also suggested by other authors [14]. Secondly, some highly selected group as healthy blood donors could not be fully representative of the base population in sex and age distribution, being furthermore of different geographic districts.

Despite this heterogeneity however, the previous comparisons could suggest an active circulation of *L. infantum* in Sicily after a period of relatively good control as a consequence of DDT campaigns. This latter consideration could be supported by about 6% of seropositives found among younger subjects (< 30 years old) born after DDT discontinuation and, intriguingly, our seroprevalence is very close to the prevalence of leishmanin skin test positivity (16.6%) documented in Sicily before DDT discontinuation [22].

Furthermore, a positive association with age has been well documented by others, being probably attributable to a higher cumulative probability of having been infected over the years [13,19].

Of particular interest in terms of public health, the strong association between *Leishmania* seropositivity and daily contact with animals living outdoors and not treated by anti-pests. This observation, confirmed also after adjustment for age, suggests that insecticidal use on animals appears to represent an effective way of reducing *Leishmania* transmission to humans and appears to be consistent with a reduced risk of infection among dogs found by others [23]. *Leishmania* seropositivity has been also significantly associated with current smoking habits. To the best of our knowledge a similar finding is not present in the international literature, probably because it has never been investigated. Although this result needs to be

TABLE 1

CHARACTERISTICS OF THE STUDY POPULATION AND LEISHMANIA SEROPOSITIVITY

	STUDY POPULATION, n*(%)		LEISHMANIA SEROPOSITIVES, n (%)	P-VALUES
TOTAL PARTICIPANTS	260	(100)	36 (13.8)	-
SEX				
FEMALE	141	(54.2)	16 (11.3)	0.20[a]
MALE	119	(45.8)	20 (16.8)	
AGE GROUP (IN YEARS)				
<30	52	(20.0)	3 (5.8)	0.04[b]
30 TO 60	115	(44.2)	16 (13.9)	
> 60	93	(35.8)	17 (18.3)	
BMI				
<18.5	13	(5.1)	0 (0.0)	0.31[c]
18.5 TO 24.9	87	(34.1)	12 (13.8)	
25 TO 29.9	116	(45.5)	20 (17.2)	
>29.9	39	(15.3)	4 (10.3)	
MARITAL STATUS				
SINGLE	69	(26.5)	4 (5.8)	0.11[c]
MARRIED/FREE UNION	173	(66.5)	29 (16.8)	
WIDOW/ER	15	(5.8)	3 (20.0)	
JOB				
STUDENT/UNEMPLOYED	39	(15.1)	1 (2.6)	0.10[c]
HOUSEWIFE	69	(26.6)	8 (11.6)	
SELF-EMPLOYMENT	38	(14.7)	9 (23.7)	
FARMER	9	(3.5)	3 (33.3)	
EMPLOYED	47	(18.1)	6 (12.8)	
RETIRED	49	(18.9)	8 (16.3)	
OTHER	8	(3.1)	1 (12.5)	
HIGHEST EDUCATION LEVEL				
ILLITERATE	16	(6.2)	1 (6.3)	0.59[c]
LOW EDUCATION/INTERMEDIATE EDUCATION	165	(64.0)	25 (15.2)	
HIGH EDUCATION/UNIVERSITY DEGREE	77	(29.8)	10 (13.0)	

* Numbers may not sum to 260 due to missing answers

[a] Chi square

[b] Chi-square for trend

[c] Logistic regression analysis

cautiously interpreted and further investigated, it must be stressed that smokers could be more exposed to sandfly bites due to their habit of smoking in outdoor environments as also requested by the Italian law for public places and, furthermore, smoking could attract sandflies by releasing carbon dioxide, that is recognized to be a nearly universal mosquito activator and attractant [24].

Finally, it should be pointed out that our study may have some limitations due to its relatively small sample size and restriction to the general population of a small city in western Sicily. Moreover, the use of a serodiagnostic

TABLE 2

AGE-ADJUSTED OR FOR LEISHMANIA SEROPOSITIVITY IN DIFFERENT EXPOSURE GROUPS

	LEISHMANIA POSITIVES N, TOTAL BY ROW* (%)	AGE-ADJUSTED OR (95% CI)
HAVING A PET		
NO	22/179 (12.3)	1
YES, LIVING INDOORS	1/18 (5.6)	0.43 (0.05-3.45)
YES, LIVING OUTDOORS AND TREATED WITH ANTI-PESTS	3/24 (12.5)	1.16 (0.31-4.27)
YES, LIVING OUTDOORS AND NOT TREATED WITH ANTI-PESTS	9/32 (28.1)	2.95 (1.2-7.3)
OWNER OF EXTRA-URBAN HOLIDAY RESORT		
NO	25/195 (12.8)	1
YES	11/6.5 (16.9)	1.38 (0.63-3.01)
SMOKING		
NEVER	14/138 (10.1)	1
FORMER	6/55 (10.9)	0.87 (0.31-2.43)
CURRENT	16/67 (23.9)	3.11 (1.38-7.01)
PESTICIDE USE		
NO	12/115 (10.4)	1
YES, ONLY INDOOR	9/62 (14.5)	1.61 (0.62-4.11)
YES, ONLY OUTDOOR	10/55 (18.2)	1.86 (0.74-4.65)
YES, INDOOR AND OUTDOOR	4/14 (28.6)	3.4 (0.91-12.7)

* Numbers may not sum to 260 due to missing answers

method could potentially be limiting, since Pedras et al. reported a specificity of IFAT at a dilution of 1:40 of 83.8% [25]. However, cross-reactivity with *Trypanosoma cruzi* and malaria had a major influence on the reported poor specificity and can be assumed to be absent in our study population.

CONCLUSIONS

Despite these possible limitations, to date this is the first study that tries to estimate *Leishmania* seroprevalence in a randomized sample of the general population in our geographic area, documenting a relatively high *Leishmania* seropositivity prevalence particularly, in older subjects or in those exposed to some risk factors such as current smoking and animals untreated with anti-pests. Further investigations should be carried out to verify the importance of these old and new risk factors.

ACKNOWLEDGEMENTS: *The authors are grateful to the local Municipal Administration of Calatafimi-Segesta (Trapani), Filippo Cangemi, Maria Adelaide D'Anna, Salvatore Gerbino, Vincenzo Malerba, Claudio Minore, Patrizia Parisi, Giovanni Vaiana, Leonardo Vanella and Felicia Coraci for their helpful contribution to the study, and especially to the study participants.*

CONFLICT OF INTEREST: *No competing financial interests exist.*

References

[1] Alvar J, Vélez ID, Bern C, et al. WHO Leishmaniasis Control Team.Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671. doi: 10.1371/journal.pone.0035671.

[2] Torina A, Sole M, Reale S, Vitale F, Caracappa S. Use of phlebotomine sand flies as indicator of leishmania

prevalence in an endemic area. Ann N Y Acad Sci 2008; 1149:355-77.

[3] Bichaud L, Souris M, Mary C, et al. Epidemiologic relationship between Toscana virus infection and Leishmania infantum due to common exposure to Phlebotomus perniciosus sandfly vector. PLoS Negl Trop Dis. 2011;5(9):e1328.

[4] Desjeux P, Alvar J. Leishmania/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol 2003; 97(Suppl 1):3-15.

[5] Maroli MN, Bettini S. Past and present prevalence of Phlebotomus papatasi (Diptera: Psychodidae) in Italy. Parasite 1997; 4: 273-6.

[6] Povoa MM, Conn JE, Schlichting CD, et al. Malaria vectors, epidemiology, and the reemergence of Anopheles darlingi in Bele´m, Parà, Brazil. J Med Entomol 2003; 40(4):379-86.

[7] Amodio E, Turci R, Massenti MF, et al. Serum concentrations of persistent organic pollutants (POPs) in the inhabitants of a Sicilian city.Chemosphere 2012; 89(8):970-4.

[8] Amodio E, Cusi MG, Valenti RM, et al. Immunoglobulin M seropositivity for Toscana virus in a random population sample in Sicily. Int J Infect Dis 2012; 16(8):e633-5.

[9] ISTAT. Istituto Nazionale di Statistica. Available from: http://demo. istat.it/pop2009/index.html

[10] Calamusa G, Valenti RM, Vitale F, et al. Seroprevalence of and risk factors for Toscana and Sicilian virus infection in a sample population of Sicily (Italy). J Infect 2012; 64: 212-7.

[11] World Organisation for Animal Health (OIE). Available from http://www.oie.int/fileadmin/home/eng/health_standards/tahm/2.01.08_leishmaniosis.pdf

[12] R Development Core Team. R statistical software package, version 2.2.0 and 2005.

[13] Garrote JI, Gutierrez MP, Izquierdo RL, et al. Seroepidemiologic study of Leishmania infantum infection in Castilla-Leon, Spain. Am J Trop Med Hyg 2004; 71:403– 6.

[14] Martin-Sanchez J, Pineda JA, Morillas-Marquez F, et al. Detection of Leishmania infantum kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other Leishmania infection markers. Am J Trop Med Hyg 2004; 70:545–8.

[15] Le Fichoux Y, Quaranta JF, Aufeuvre JP, et al. Occurrence of Leishmania infantum parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. J Clin Microbiol 1999; 37:1953–7.

[16] Marty P, Lelievre A, Quaranta JF, et al. Detection by Western blot of four antigens characterizing acute clinical leishmaniasis due to Leishmania infantum. Trans. R Soc Trop Med Hyg 1995; 89:690–1.

[17] Papadopoulou C, Kostoula A, Dimitriou D, Panagiou A, Bobojianni C, Antoniades G. Human and canine leishmaniasis in asymptomatic and symptomatic population in Northwestern Greece. J Infect 2005; 50: 53–60.

[18] Diza E, Kansouzidou A, Gerou S, Vezyri E, Metallidis S, Antoniadis A. Leishmaniases in Northern Greece: seroprevalence of the infection and incidence of the disease during the period 2001–2006. Eur J Clin Microbiol Infect Dis 2008; 27: 997–1003.

[19] Biglino A, Bolla C, Concialdi E, Trisciuoglio A, Romano A, Ferroglio E. Asymptomatic Leishmania infantum infection in an area of northwestern Italy (Piedmont region) where such infections are traditionally nonendemic. J Clin Microbiol 2010; 48:131–6.

[20] Colomba C, Saporito L, Polara VP, Barone T, Corrao A, Titone L. Serological screening for Leishmania infantum in asymptomatic blood donors living in an endemic area (Sicily, Italy). Transfus Apher Sci. 2005; 33:311–4.

[21] Scarlata F, Vitale F, Saporito L, et al. Asymptomatic Leishmania infantum/chagasi infection in blood donors in western Sicily. Trans R Soc Trop Med Hyg 2008; 102:394–6.

[22] Pampiglione S, Manson-Bahr PE, La Placa M, et al. Studies in Mediterranean leishmaniasis. The leishmanin skin test in kala-azar. Trans R Soc Trop Med Hyg 1975; 69(1): 60–8.

[23] Molina R, Espinosa-Góngora C, Gálvez R, et al. Efficacy of 65% permethrin applied to dogs as a spot-on against Phlebotomus perniciosus. Vet Parasitol 2012;187(3-4): 529-33.

[24] Gillies MT. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull Entomol Res 1980; 80:525 -32.

[25] Pedras MJ, de Gouvêa Viana L, de Oliveira EJ, Rabello A. Comparative evaluation of direct agglutination test, rK39 and soluble antigen ELISA and IFAT for the diagnosis of visceral leishmaniasis. Trans R Soc Trop Med Hyg. 2008 Feb;102(2):172-8.