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SHORT COMMUNICATION

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Seroprevalence of *Leishmania infantum* in outdoor workers from southern Italian endemic regions

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Abstract

Visceral leishmaniasis is a zoonotic vector-borne disease caused by *Leishmania infantum*. The infection often remains asymptomatic, though clinical forms may occur in immunosuppressed individuals. Although data on leishmaniasis in humans are available worldwide, the exposure to *L. infantum* of workers conducting outdoor activities has been scantly investigated, and it is limited to military personnel operating in endemic regions. This study aimed to assess the seroprevalence of *L. infantum* in different groups of outdoor workers and the occupational risk factors. The cross-sectional study was performed on 229 workers including forestry guards, farmers, veterinarians, geologists and agronomists from three regions of southern Italy (i.e., Apulia, Basilicata and Campania). All serum samples were screened for *L. infantum*-specific IgG/IgM by using automated indirect chemiluminescent immunoassays. Overall, 5.7% (13/229) of workers was positive to anti-*L. infantum* antibodies, with the highest seroprevalence in veterinarians (13.6%). An increased occupational health surveillance for *L. infantum* infection in outdoor workers is essential to better understand the risk of exposure in specific jobs. Furthermore, guidelines and education along with a One Health collaboration among veterinarians, physicians, parasitologists and occupational health care professionals are crucial for the prevention of this disease.

KEYWORDS
exposure risk prevention, leishmaniasis, occupational categories, phlebotomine sand fly, seroprevalence, vector-borne disease, zoonosis

1 | INTRODUCTION

Leishmaniases are diseases caused by more than 20 *Leishmania* species, causing clinical manifestations broadly grouped in cutaneous (CL), mucocutaneous (MCL) and visceral (VL) forms (WHO, 2022). The epidemiology and clinical presentation of the diseases vary according to a range of factors linked to the host availability, human immune system, presence and seasonality of vectors in different ecological contexts, making these diseases a major problem in developing countries (WHO, 2022). These intracellular protozoa

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are transmitted through the bite of infected female phlebotomine sand flies belonging to the genera *Phlebotomus*, in the Old World, and *Lutzomyia* in the New World (Maroli et al., 2013). Dogs play a pivotal role as reservoirs of zoonotic *Leishmania infantum*, which is increasingly spreading to previously non-endemic regions, due to several factors such as the increase in pet travelling along with owners, relocation of sheltered animals as well as to the modification of the ecology of arthropod vectors, as recorded in Italy (Bouattour et al., 2021; Le Rutte et al., 2018; Mendoza-Roldan et al., 2020; Müller et al., 2022; Otranto et al., 2009).

The clinical spectrum of this disease depends on the parasite species and the host immune response in both animals and humans, varying in the latters from asymptomatic infection (i.e., 80%–95%) to different clinical and subclinical manifestations (i.e., 5%–20% of cases) (Ávila et al., 2023; Ibarra-Meneses et al., 2022; Paltrinieri et al., 2010). In humans, immunosuppressive conditions are considered risk factors for VL (Saini et al., 2022), though asymptomatic infections are probably underestimated (Iatta et al., 2021; Michel et al., 2011; Šiško-Kraljević et al., 2013). Although notification of human leishmaniasis is compulsory in most regions of southern Europe, including Italy, the low number of cases recorded annually (i.e., about 100) suggests that this disease is underreported (Di Muccio et al., 2015). In this context, in endemic areas where up to 50% of dogs are infected with *L. infantum* (Ramos et al., 2022), most of humans may be asymptomatic (Molina et al., 2020). For example, up to 6.6% of immunocompetent inhabitants of Linosa and Lampedusa (Sicily, Italy) were seropositive for *L. infantum* (Abbate et al., 2020; Iatta et al., 2021), demonstrating that the parasite circulates in human population in presence of canine infection. Data concerning the human seroprevalence of *L. infantum* in Europe demonstrate that it may range from 0.5% to 15% (Aliaga et al., 2019; Papadopoulou et al., 2005; Theocharidou et al., 2019), whereas studies in occupational categories have been conducted only in military personnel operating in endemic regions (Kniha et al., 2020; Obwaller et al., 2018). Aware of the difficulties in correlating occupational risks with human leishmaniasis, this study assessed the seroprevalence of *L. infantum* in different groups of outdoor workers and the occupational risk factors associated with this infection.

Impacts

- Seroprevalence investigation of *Leishmania infantum* in outdoor workers at occupational risk.
- 5.7% (13/229) of workers was seropositive to *L. infantum*, with the highest prevalence in veterinarians (13.6%).
- An increased health surveillance in exposed workers should be recommended.

2 | MATERIALS AND METHODS

2.1 | Study population and sample collection

From February to December 2021, a cross-sectional study was conducted on workers ($n=229$) from three southern Italian regions (i.e., Apulia, Basilicata and Campania) characterized by a typical Mediterranean temperate climate. Enrolled individuals were employed to perform different outdoor jobs (i.e., forestry guards, farmers, veterinarians, geologists and agronomists) being workers from Campania and Basilicata recruited on volunteer basis during educational meeting. Whereas those from Apulia including administrative employees, not performing any outdoor activities as occupational tasks, employed at the University of Bari (Italy), were examined and asked for consent during the occupational health surveillance. Geologists and agronomists were considered as a single group for their similar risk of exposure to phlebotomine sand flies. All the participants were older than 18 years, with a working seniority in their current position longer than 1 year and reporting no prior history of immunodeficiency.

All the participants filled out a questionnaire administered by medical personnel, enquiring about socio-demographic characteristics, pathological anamnesis, particularly chronic immune system disorders, outdoor working features and recreational activities (i.e., hiking, hunting, camping, gardening and farming), occupational and non-occupational contact with animals, working activity in lowland (<300 m above sea level), housing in a rural area, time of day when outdoor working activities were performed, and potential risk factors associated with an increased likelihood of exposure to *L. infantum* infection in the work environment and during leisure time.

This study was conducted in accordance with ethical principles of the Declaration of Helsinki. Patients provided written informed consent after they were fully informed about the research aims and features. The research was approved by the ethics committee of the University Hospital of Bari (Italy) (approval no. 6394, protocol no. 0044469–23,062,020).

2.2 | Serological testing

Blood samples were collected from enrolled subjects by using 5-mL BD Vacutainer Serum separating Tubes (Beckton Dickenson) and kept at 4°C before being processed. Serum samples were centrifuged at 2000 × g for 10 min and stored at −20°C until further analysis.

All serum samples were diluted 1:20 and analysed for *L. infantum*-specific IgG/IgM by using automated indirect chemiluminescent immunoassays (CLIA) (Leishmania Virclia® IgG + IgM monotest, Vircell) based on the Thunderbolt platform (Gold Standard Diagnostics). The sensitivity and specificity of the test declared by the supplier were 92% and 99%, respectively.

The CLIA works with primary sample tubes and requires only a minimum of 5 μL serum. Each monodose test includes reaction wells coated with *L. infantum* antigen, a calibrator and a negative control that enables the validation and interpretation of results for each individual sample and is not based on stored curves. Semi-quantitative

TABLE 1 General and occupational characteristics of the studied population.

Job	Age (years) median (range)	Working seniority (years) median (range)	Female gender (%)	Immune system disorders (%)	Work area: Lowland (%)	Occupational contact with animals (%)	Outdoor work 5–10 AM. (%)	Outdoor work 6–8 PM. (%)	Outdoor hobbies (%)	Domestic contact with pets (%)	Worker from A/B/C
Forestry workers ($n=81$)	50 (22–75)	14 (1–45)	13.5	1.2	3.7	16.9	17.2	16	87.6	69.1	3/28/50
Farmers ($n=32$)	48 (24–72)	15 (1–30)	9.4^{a}	6.2	3.1	96.8^{a}	28.1	6.2	71.8	62.5	2/30/0
Veterinarians ($n=44$)	45 (26–70)	12 (1–30)	41	6.8	31.1^{b}	100^{a}	13.6	13.6	63.6	59	39/1/4
Geologists/Agronomists ($n=27$)	54 (27–65)	20 (1–32)	33	7.4	40^{b}	0.0	18.5	55.5^{c}	77.7	51.8	27/0/0
Administrative employees ($n=45$)	50 (28–67)	15 (1–32)	53	9^{b}	100	0.0	0.0	0.0	26.6^{a}	33.3^{a}	38/2/5
Total ($n=229$)	50 (22–75)	(1–45)	28.4	5.2	25.2	30	19.2	21.3	67.6	58.5	109/61/59

Note: $^{a}p<0.001$; $^{b}p<0.01$; $^{c}p<0.05$.

Abbreviations: A, Apulia; B,Basilicata; C, Campania.

IgG/IgM results are presented as relative light units (RLU) index which was calculated as the ratio between the sample and calibrator relative light units and interpreted according to the manufacturer's instructions.

2.3 | Statistical analyses

Shapiro–Wilk test and graphical evaluations of each variable were performed to demonstrate the correspondence with the normal distribution. Student's *t*-test or Mann–Whitney *U* test was performed to assess comparison between two groups in terms of continuous variables, while differences among more than two groups were studied through one-way analysis of variance (ANOVA) followed by Tukey post hoc test, where necessary. Pearson χ^2 test was used for comparison in terms of categorical variables. Confidence interval values at 95% were calculated for infection proportions herein found.

Statistical analyses were performed using R software version 3.5.2 (The R Foundation for Statistical Computing) with p lower than 0.05 considered statistically significant.

3 | RESULTS

The demographic and occupational characteristics of the recruited population according to their job are described in Table 1.

Overall, the recruited workers had a median age of 50 years (range 22–75) and an median working seniority of 15 years (range 1–45). No statistically significant differences in the exposure risk to the *L.infantum* were found between the different jobs for age and working seniority. The percentage of female workers was different among the job groups, being significantly lower in farmers (9.4%, $p<0.001$).

Considering the potential risk factors for the development of clinical leishmaniasis, the percentage of subjects with immune system disorders was significantly higher in the group of administrative employees (9%, $p<0.05$), whereas no significant differences were found for other chronic illnesses, smoking habits and alcohol consumption (data not shown). Furthermore, all workers recruited referred no current acute illnesses or other symptoms at the time of blood collection. Considering the work activity, a significantly higher percentage of geologists/agronomists and veterinarians worked in lowland areas (40% and 31.1%, respectively, $p<0.05$). In addition, all veterinarians (100%) and nearly all farmers (96.8%) reported work activities involving contact with animals ($p<0.001$) compared to the other job categories. According to the time of day when phlebotomine sand flies were more active, a significantly higher percentage of subjects working in the 6–8 PM, time slot was observed in the geologists/agronomist group (55%, $p<0.01$). Finally, a significantly lower percentage of subjects reporting outdoor hobbies and the presence of pets in their homes was found in the administrative employee group (26.6% and 33.3%, respectively, $p<0.001$).

The number and prevalence of workers seropositive for *L. infantum* are reported in Table 2. Overall, 13 out of 229 workers (5.7%, 95% CI 3%–9%) were seropositive to *L. infantum*, with no significant difference for age and working seniority. Among the personal and occupational characteristics investigated by the questionnaire, a significantly higher seropositivity was found in veterinarians (13.6%; 95% CI 5%–20%, $p < 0.05$), workers from Apulia (9.2%; 95% CI 4%–16%, $p < 0.01$), female (9.2%; 95% CI 3%–19%, $p < 0.05$), workers with activities in lowland (8.6%, 95% CI 3.7%–18.6%, $p < 0.05$) and in contact with animals (6.1%, 95% CI 3%–11%, $p < 0.01$).

4 | DISCUSSION

In a descriptive way, data herein obtained demonstrated a *L. infantum* seroprevalence of 5.7% in asymptomatic workers conducting different outdoor activities, being veterinarians, herein firstly reported, the occupational category with higher prevalence (13.6%). In particular, the seroprevalence herein recorded in southern Italy is consistent with that reported in healthy subjects (6.6%) from the Pelagie archipelago in Sicily, which is a highly endemic region for VL (Cascio et al., 2002; Iatta et al., 2021). Indeed, although *L. infantum* has spread throughout Italy, the southern and insular regions are considered highly endemic areas for leishmaniasis given the favourable environmental conditions for the presence of *Phlebotomus perniciosus*, *Phlebotomus perfiliewi* and *Phlebotomus neglectus*, which are the most common species of competent vectors in Italy (Latrofa et al., 2018; Mendoza-Roldan et al., 2021; Moirano et al., 2022; Tarallo et al., 2010).

TABLE 2 Characteristics of seropositive workers to *Leishmania infantum*.

Characteristics of seropositive workers	N. Positive/n. tested	Seropositive (%)
Age (years)		
22–41	6/229	2.6
50–74	7/229	3.1
Working seniority (years)		
2–10	6/229	2.6
15–30	7/22	3.1
Sex		
Male	7/164	4.3
Female[a]	6/65	9.2
Jobs		
Forestry workers	1/81	1.2
Farmers	2/32	6.2
Veterinarians	6/44	13.6[a]
Geologists/Agronomists	2/27	7.4
Administrative employees	2/45	4.4
Work area: lowland	5/58	8.6[a]
Workers from regions		
Apulia	10/109	9.2
Basilicata	3/61	4.9
Occupational contact with animals	9/147	6.1[b]
Total	13/229	5.7

Note: [a]$p \leq 0.05$; [b]$p < 0.01$.

Recently outdoor working activities such as veterinarians and workers in contact with animals have been reported as job categories at risk for vector-borne infections (Stufano et al., 2022). Indeed, the close relationship with dogs, the primary reservoirs of the parasite, and the work attitude in conducting field activities in peri-urban and rural areas may represent an occupational risk factor for vector's exposure (Davitt et al., 2022). Sand flies usually do not fly long distances, being their breeding sites mainly present in areas where animals are available for their blood meal. For example, a high prevalence of vector-borne pathogens (VBP) of zoonotic concern, including *L. infantum*, has been recorded in dogs living in a shelter in southern Italy, suggesting the potential risk of infection for workers and humans in the neighbourhoods (Panarese et al., 2022). Recent data reported a high seropositivity of *L. infantum* in dogs, reptiles and sand flies in an area of the Apulia region, about 4.2 km away from the site where most of the positive veterinarians, enrolled in this study, work (Mendoza-Roldan et al., 2022). Indeed, the close relationship of dogs to their owners in domestic environment has been also described as a possible source of infection (Ferroglio et al., 2006). Furthermore, clinical cases of CL and VL were reported in farmers (Dalal et al., 2021; Poulaki et al., 2020). As expected, workers conducting outdoor activity in lowland where the environmental conditions are more favourable to sand fly ecology are more prone to their bites, thus at higher risk of *L. infantum* infection (Medlock et al., 2014; Moirano et al., 2022).

Although the percentage of female was significantly lower in the overall study population, a higher seroprevalence was observed in females (9.2%) than in males (4.2%). Therefore, in occupational settings, asymptomatic infections seem to follow a different trend than clinical leishmaniasis, being more frequent in males (Cloots et al., 2020; Travi et al., 2002). The higher infection rate observed in female workers may be related to different factors including the work environment and the fact that the highest percentage of females was recorded among the veterinarians (41%).

Potential limitations of the study should be considered, including the low number of individuals herein enrolled for each job investigated, and the different proportion in job categories analysed for each region that may have affected the prevalence of *L. infantum* infection. Further surveys in wider exposed populations, such as shelter workers and hunters, are needed for better evaluating the occupational infection risk and the adoption of preventative measures for reducing the risk of VBP transmission. Finally, a One Health collaboration approach among veterinarians, physicians, parasitologists and occupational health professionals is crucial in taking care of the worker's and public health.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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