

SEROEPIDEMIOLOGIC STUDY OF *LEISHMANIA INFANTUM* INFECTION IN CASTILLA-LEON, SPAIN

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Abstract. Leishmaniasis has increased in importance in recent years because infection with human immunodeficiency virus (HIV) has emerged as a risk factor for this disease. However, the actual prevalence of leishmaniasis in the general population of Spain is unknown. We present a study of the seroprevalence of infection with *Leishmania infantum* in the general population of Castilla-Leon, Spain. A random sample of individuals presenting to health care clinics (4,825 sera) and of HIV-infected patients in the autonomous community of Castilla-Leon was collected in 1996. The seroprevalence of antibodies to *L. infantum* was determined by an indirect enzyme immunoassay and found to be 4.9% in the general population. There was a significant increase in seroprevalence with age ($P = 0.001$), from 3.96% in those 14–20 years old to 7.2% in those > 70 years old. There were no significant differences between women and men (5.0% versus 4.9%; $P = 0.9534$). Seroprevalence was significantly higher in people from rural areas than in those from cities (6.0% versus 3.4%; $P = 0.001$). Patients infected with HIV had a seroprevalence for *L. infantum* of 64.0%. No differences were observed between women and men, and prevalence did not increase with age.

INTRODUCTION

Leishmaniasis is a parasitic infection caused by protozoa of the genus *Leishmania*, of which at least 20 species have been linked to human infection. In humans, infection with *Leishmania* sp. ranges from asymptomatic forms to severe visceral involvement. The most frequent clinical syndromes are visceral leishmaniasis, which is caused by *L. donovani*, *L. infantum*, and *L. chagasi*, and cutaneous and mucocutaneous leishmaniasis, which are caused by a large number of species, including *L. infantum*. Leishmaniasis can be found in at least 88 countries. In Europe, it is found mostly in the Mediterranean countries, such as Spain.¹ The incidence of this disease has been decreasing in Europe, especially in Spain, since the second half of the 20th century, and it is now considered hypoen-demic.^{1–3}

Leishmania infantum is the only species of *Leishmania* found in Spain. Its principal reservoir is the dog, and it is transmitted to humans by the bites of female diptera of the genus *Phlebotomus*.^{4,5} As in other European countries, the last few years have brought about a radical change in human epidemiology of leishmaniasis in Spain. Transmission by its vectors has decreased, but transmission has increased among intravenous drug users and patients infected with human immunodeficiency virus (HIV).^{1–3} This latter change has caused an increase in the incidence of leishmaniasis since the mid 1980s, in addition to its spread to previously infection-free areas.³ However, the actual prevalence of leishmaniasis in Spain is unknown. The fact that clinical cases represent only a fraction of those infected with *Leishmania* sp. and that there is marked under-reporting of these cases are critical for understanding the epidemiology of this disease.⁶

Leishmaniasis has not been an officially reported disease in Spain since 1997.⁷ In addition, few studies have been conducted on the prevalence of infection with *Leishmania* sp. in the general population, and published reports have almost always used leishmanin (Montenegro) skin tests.^{4,5,8} Most studies on the prevalence of leishmaniasis have been carried out in HIV-infected patients.^{2,9,10} However, the prevalence of

antibodies to *Leishmania* in these patients does not reflect the true situation in general population.

The objective of this study was to determine the prevalence of leishmaniasis in the general population, intravenous drug users positives for antibodies to HIV in Castilla-Leon, Spain. Prevalence was determined by detection of IgG antibodies to *L. infantum* with an enzyme immunoassay (EIA).

MATERIALS AND METHODS

Serum samples from 4,825 healthy individuals more than 14 years old living in the autonomous community of Castilla-Leon were collected to carry out a study on the seroprevalence of several zoonosis (brucellosis, hydatid disease, tularemia, and others). Castilla-Leon is the largest autonomous community in Europe, and is located in the north central region of Spain (Figure 1). Serum samples were taken in public blood sample extraction centers of the Outpatient Health Care Network over a one-year period (1996) and were stratified by province and health area (rural/city) (Table 1). Age and sex were controlled by quotas. Individuals were randomly selected from those who came to health centers for follow-up visits or for medical examinations. All individuals included in the study were given an explanation of its purpose and informed consent was obtained from all participants. Individuals with fevers or suspected of having leishmaniasis were excluded from the study. The study was reviewed and approved by the Ethical Committee of Research of the Faculty of Medicine of the University of Valladolid (Valladolid, Spain).

The risk group for leishmaniasis was composed of 217 sera obtained from 225 HIV-infected intravenous drug users (68 women and 149 men, average age = 33.2 years, range = 16–51) who came to the Hospital Clínico Universitario in Valladolid for medical attention in 1996. They represent 13.7% of all patients in the HIV-infected population registered in the region in 1996 (1,587 individuals). All patients were residents of cities in Castilla-Leon and none presented any clinical symptoms of infection with *L. infantum*.

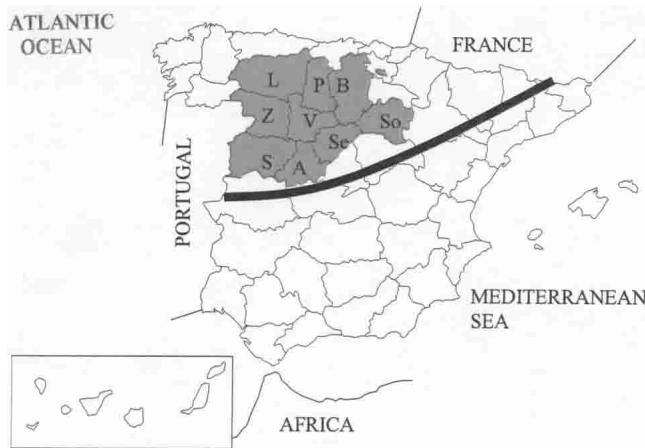


FIGURE 1. Location of the autonomous community of Castilla-Leon, Spain. L = Leon; P = Palencia; B = Burgos; Z = Zamora; V = Valladolid; So = Soria; S = Salamanca; A = Avila; Se = Segovia. The islands in the box are the Canary Islands. The dark horizontal line separates the northern regions of Spain from the Mediterranean regions.

The presence of IgG antibodies against *L. infantum* was studied in each serum by an EIA.⁹ The antigen used was a soluble antigen from *L. infantum* strain LEM-75 (SLA), zymodeme 1, MHOM/FR/78/LEM75, which was isolated according to a modified version⁹ of the method of Scott and others.¹¹ A pool of sera positive for *Leishmania* by an indirect immunofluorescence test (IFT) was used as the positive control. The negative control consisted of the 14 blood donor samples negative for *Leishmania* by the IFT. The EIA positivity threshold was calculated for each plate as the average of the absorbances of the 14 negative control sera plus two standard deviations.

Results were analyzed with SPSS statistical software (SPSS Inc., Chicago, IL) using Pearson's chi-square test and 95% confidence intervals (CIs) for proportion analysis.

RESULTS

IgG antibodies against *L. infantum* were detected in 241 (4.9%) of 4,825 sera studied from the population of Castilla-Leon. The seroprevalence of IgG antibodies to *L. infantum* was very similar in both sexes (5.0% [125 of 2,361] in women and 4.9% [116 of 2,208] in men), with no statistically significant difference ($P = 0.9534$).

Table 2 shows the seroprevalence of *L. infantum* by age

TABLE 1
Sample population characteristics*

Age group (years)	Women	Men	Rural population	City population
14-20	392	413	470	338
21-30	574	407	579	400
31-40	450	418	511	359
41-50	268	309	334	242
51-60	256	217	278	196
61-70	325	357	444	241
>70	221	203	262	161
Total	2,486	2,324	2,878	1,937

* Sex was unknown in 15 cases and habitat was unknown in 10 cases.

TABLE 2

Seroprevalence IgG antibodies to *Leishmania infantum* based on age*

Age group (years)	No. tested	Anti- <i>L. infantum</i> IgG	
		No. (%)	95% CI
14-20	809	32 (3.9)	2.61-5.3
21-30	982	33 (3.3)	2.21-4.5
31-40	870	38 (4.3)	3.0-5.7
41-50	579	31 (5.3)	3.5-7.2
51-60	475	25 (5.2)	3.2-7.2
61-70	685	51 (7.4)	5.4-9.4
>70	425	31 (7.2)	4.8-9.7
Total	4,825	241 (4.9)	4.3-5.6

* CI = confidence interval.

groups. The prevalence of IgG antibodies against *L. infantum* significantly increased with age ($P = 0.001$), ranging from less than 4% in individuals less than 31 years old to more than 7% in those greater than 60 years old.

Seroprevalence based on whether the patients came from rural areas or cities was studied. IgG antibodies against *L. infantum* were significantly more prevalent in those from rural areas (6.1%, 175 of 2,703) than in those from cities (3.4%, 66 of 1,871) ($P < 0.001$).

Determination of the prevalence of IgG antibodies to *L. infantum* in HIV-infected intravenous drug users showed that 139 (64.0%) of the 217 patients studied were antibody positive. This prevalence was significantly higher than that of the general population in Castilla-Leon (64.0% versus 4.9%; 95% CI = 52.58-65.64, $P < 0.001$).

There were no statistically significant differences between the seroprevalences found for women and men (65.2% versus 63.5%; 95% CI = -12.1-15.5, $P = 0.808$). Similarly, there was no significant association between seroprevalence and age.

DISCUSSION

Leishmaniasis is considered endemic in Mediterranean countries, although its prevalence varies from region to region.¹ As determined by the leishmanin skin test, the prevalence varies from 15.3% in Italy,¹² to 32% in France,¹³ to more than 40% in the southern Spain.^{4,5}

Few seroepidemiologic studies on healthy populations have been conducted in Spain. All show serious biases because the number of sera studied have been small and were selected from population groups that do not represent the general population.^{9,14} None of these studies found seropositive cases in healthy people. In contrast, in our study the seroprevalence of *L. infantum* was 5%, ranging from 6.0% in the rural population to 3.4% in the urban population ($P < 0.001$).

Several factors explain the differences between our results and those obtained by the other investigators who used the leishmanin skin test.^{4,5} First, this test is more sensitive (approximately three times greater) in detecting leishmaniasis than serologic testing. Second, when an individual shows a positive result on this test, it remains positive throughout the individual's life.^{15,16}

Conversely, from an epidemiologic point of view, geographic and climatic conditions greatly influence the prevalence of leishmaniasis because they affect the life cycle of the sand fly vector. These conditions in Castilla-Leon are differ-

ent from those in the Mediterranean regions in southern and eastern Spain where the previously mentioned studies were carried out.^{4,5,8} The greater frequency of individuals with positive leishmanin skin test results found in these two regions coincides with a high prevalence of human cases.⁷ Using 1996 as an example (the same year in which Acedo and others⁴ and Morillas and others⁵ conducted their studies), the incidence of human cases in Spain was 0.19 cases/100,000 inhabitants. In southern Spain, Malaga and Granada had more than 0.37 cases/100,000, while in eastern Spain, Cataluña had an incidence of 0.44 cases/100,000.⁷ These data contrast sharply with those from regions in northern and western Spain, where Castilla-Leon had an incidence of 0.04 cases/100,000 (one case) that same year, the Basque region had 0.1/100,000, and Galicia, Asturias, and Cantabria did not report any cases.⁷ The climate of southern and eastern Spain, with an average annual temperature of 17°C (ranging from 1°C to 42.5°C) favors the development of the sand fly vector and constitutes one of the main epidemiologic factors. In contrast, the average annual temperatures are much lower in Castilla-Leon (11.5°C, ranging from -10°C to 38.6°C), which makes vector development difficult and partially explains the much lower incidence of leishmaniasis.

As reported by other investigators,^{4,8} our study shows that there are no differences in the prevalence of leishmaniasis between men and women. The seroprevalence of antibodies to *L. infantum* increased with age, from 3.96% in those less than 21 years old to 7.2% in individuals more than 60 years old. This increased prevalence indicates greater exposure to *Leishmania* in older individuals because seropositivity against *Leishmania* decreases with time, especially during the first year post-exposure.¹⁷ A higher risk of infection in older individuals apparently does not exist in the region we studied. However, it must be remembered that until the 1940s and 1950s leishmaniasis was a severe public health problem in Spain, with an increased incidence of the disease.⁶ Conversely, although seroprevalence was not studied in children less than 14 years old in our study, most reports indicate that seroprevalence in this group is similar or slightly lower than that in those 14–20 years old.^{16,18}

Since 1950, the incidence of leishmaniasis has progressively decreased in Spain. The use of pesticides in agriculture and the campaigns to kill insects carried out in the 1940s and 1950s to eliminate malaria in Spain undoubtedly have played a part.⁶ However, since the mid 1980s, an increase in the incidence of leishmaniasis has been observed. Most studies carried out before 1980 showed that more than 60% of all the cases of leishmaniasis reported in Spain occurred mainly children.^{2,4,5} However since 1990, a decrease incidence in children and an increase incidence in adults has been observed.² In the latter case, most patients presented with various states of immunodeficiency (transplants, cirrhosis, etc.), especially infection with HIV, mostly in intravenous drug users. Our study, which was carried out in a region with low incidence of infection with *Leishmania*, showed that 64.0% of HIV-infected intravenous drug users had IgG antibodies against *L. infantum*. This result was similar to that observed by other investigators in regions with a much higher prevalence of leishmaniasis than in Castilla-Leon.^{10,14} These data confirm the existence of an anthroponotic cycle of leishmaniasis transmission through syringes shared among HIV-infected intravenous drug users.³

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