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Serological screening for *Leishmania infantum* in asymptomatic blood donors living in an endemic area (Sicily, Italy)

Claudia Colomba [a,*], Laura Saporito [a], Valentina Frasca Polara [a], Teresa Barone [b], Antonino Corrao [a], Lucina Titone [a]

[a] *Istituto di Patologia Infettiva e Virologia, Università di Palermo, piazza Montalto 8, 90134 Palermo, Italy*
[b] *Servizio di Immunoematologia e Medicina trasfusionale, P.O. "G. Giglio", Cefalù, 90015 Palermo, Italy*

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Abstract

The purpose of our study was to assess whether *Leishmania infantum* parasitemia occurs in asymptomatic *Leishmania*-seropositive subjects. Samples from 500 blood donors were tested using an enzyme-linked immunosorbent assay (ELISA). Anti-*Leishmania* antibodies were not found in any sample. Our findings suggest that the risk of *L. infantum* transmission by blood transfusion in Sicily is very low.

Keywords: Blood donors; Italy; Transfusion; Visceral leishmaniasis

1. Introduction

Visceral leishmaniasis (VL) is endemic in areas bordering the Mediterranean sea (Spain, Italy, France, Greece, Morocco, Tunisia, etc.) where it is caused by the protozoa *Leishmania infantum* and is transmitted by the bite of hematophagous sandflies belonging to *Phlebotomus* spp.

Infection is spread throughout the reticuloendothelian system and typically presents as fever, hepatosplenomegaly, pancytopenia, and progressive deterioration of the host. The disease can be fatal if untreated. The clinical features and the outcome of VL depend on a complex interplay between potentially protective cellular immune responses and disease-enhancing ones.

Immunosuppression is one of the factors responsible for increased vulnerability to a primary *leishmania* infection or to reactivation of a latent infection. VL is increasingly reported in human immunodeficiency virus (HIV)-positive

* Corresponding author. Tel.: +39 91 6666071.
E-mail address: claudia.colomba@libero.it (C. Colomba).

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subjects living in the countries of the Mediterranean basin, especially Spain, Italy and France, where over 90% of the published cases have been observed [1,2].

Asymptomatic leishmanial infection may occur in immunocompetent persons. The documentation of positive leishmanin skin tests (evidence of delayed antileishmanial hypersensivity), with no history of VL, is not new [3–5].

Parasite circulation in the peripheral blood has been reported in asymptomatic *Leishmania donovani* and *Leishmania tropica* infection [6–8], in cured *Leishmania brasiliensis* infection [9–11] and in asymptomatic blood donors *L. infantum*-seropositive in an area of southern France and of Balearic Islands [12,13].

Sicily is the largest island in the Mediterranean Sea. It was estimated that approximately 47% of the Sicilian population live in areas at risk for *leishmania* infection, these areas consisting mainly of rural areas, small villages, or peripheral districts of towns where the vector sandfly species are more abundant [14–16]. An epidemiologic survey conducted in Sicily between 1987 and 1995 revealed that the annual incidence of VL in the general population was 6 cases per 1,000,000 residents [17]. In 2002 the incidence reached 9.4 cases per 1,000,000 residents, representing 27% of the national disease burden. Provisional 2003 data confirm this proportion [18].

The purpose of our study was to assess whether *L. infantum* parasitemia occurs in asymptomatic *Leishmania*-seropositive subjects with no history of VL living in a region endemic for VL.

This is the first investigation on asymptomatic leishmaniasis in blood donors living in Sicily, an island endemic for *L. infantum* infection.

2. Patients and methods

The study was performed in Sicily (Italy) over a seven month period (May–December 2002). Blood samples obtained from the Palermo Blood Bank were from 500 blood donors living in Palermo and in the nearby towns of Termini Imerese, Cefalù, Petralia. Donors had no history of VL although they lived in areas where *L. infantum* is endemic. Informed, written consent from all participants was obtained for this study.

On the day of donation sera were stored at 4 °C and the day after they were tested using enzyme-linked immunosorbent assay (ELISA) on microtitration wells sensitized with *L. infantum* soluble promastigote antigens (*Leishmania infantum*-ELISA; Bordier Affinity Products, Switzerland). The presence of parasite specific serum antibodies (IgG) was detected with protein A-alkaline phosphatase conjugate. The lower limit of positivity (cut-off) has been set to discriminate optimally between sera of clinical documented cases of VL and normal human sera (mean $\pm$ 3 standard deviations). The result was considered positive when the absorbance of the analyzed sample was higher than the absorbance of the cut-off. The global sensitivity and specificity of the test were 89.6% and 88.8%. Two hundred and ninety-eight sera were also analyzed by an immunofluorescent antibody test (IFAT) (*Leishmania*-Spot IF; bioMérieux, France). The sensitivity and specificity of the test were 94.4% and 96.5%, respectively.

Seropositive sera would have been examined by polymerase chain reaction (PCR) to assess whether *L. infantum* circulated in peripheral blood of these healthy subjects.

3. Results

Anti-*Leishmania* antibodies, indicative of contact with *L. infantum* in subjects with no history of VL, were not found in any of the 500 samples from blood donors analyzed by ELISA. Successive IFAT performed on 298 sera confirmed the absence of specific antibodies.

4. Discussion

Although Sicily is an endemic area for VL, our findings suggest that the risk of *L. infantum* transmission by blood transfusion is very low or absent. Different reports documented parasitemia in asymptomatic *L. infantum*-seropositive blood donors [12,13]. Several hypotheses may be proposed to explain our different results:

(i) the screened sample is not wide enough although it is similar to that studied by other authors in the Mediterranean area [12,13];
(ii) the antibody titer may be so low that the tests used are not able to detect it; the global sensitivity of the tests used is reported to range from 95% to 100% in an immunocompetent host [19,20], but asymptomatic subjects show a low humoral immunoresponse so that their antibody levels are under the cut-off;
(iii) ELISA and IFAT are rapid and not very expensive techniques, but they are less sensitive than western blotting, which may give positive results even if classical serological tests are negative [13].

On the other hand, it has been demonstrated that the preparation of blood products using leukoreduction by filtration minimizes the potential risk of transmission of several pathogens (e.g. *Leishmania* and cytomegalovirus) [12,21].

A minimal risk could already exist for whole-blood and granulocyte transfusions, because white blood cells are the parasite target.

Finally, assuming a real transmission of *L. infantum* by transfusion, it does not necessarily result in the transmission of the disease. In fact, VL occurring within a few months after a transfusion has not been documented in regions of high prevalence of *Leishmania* seropositivity, and thus of a high density of potentially asymptomatic donors [12].

Nevertheless, we propose to extend our study in other endemic areas of our region using more sensitive techniques to achieve a more complete knowledge of our epidemiologic situation about the existence of asymptomatic *L. infantum* carriers in Sicily.

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