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Leishmania infantum cryptic infection in immunosuppressed rheumatologic patients and healthy controls from northern Italy: epidemiological variables that can affect infection risk

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Background: Recent epidemiological data have shown a spread of *Leishmania infantum* in some Italian Northern regions, such as Emilia-Romagna. Although climate changes are influencing the epidemiology of leishmaniasis in Italy, immunosuppression has been identified as a major contributor to disease reemergence in patients with autoimmune rheumatic diseases who live in endemic areas for leishmania.

Material/methods: a qPCR for *L. infantum* (Li) kDNA was tested in 50 patients affected by chronic inflammatory rheumatism (CRI), treated with biologic drugs (BD) for at least 5 years (Group A) and healthy controls (Group B). Demographic and clinical data were collected using questionnaires including residence, housing area (urban or rural context), ownership of a dog. Data analysis was performed using SPSS Statistics (SPSS, Inc., USA). Normality of distribution for continuous variable was checked by Shapiro-Wilk test. Since the variables were not normally distributed, group comparisons between controls and patients affected by CRI were performed using Mann-Whitney U test and Chi-square tests to compare differences in categorical variables. Binary logistic regression analysis was used to assess the independent effects of living in a rural area, having a dog or taking a

steroid in association with a biological drug on the rate of positive PCR results against leishmania. Two-tailed probability values <0.05 were considered statistically significant.

Results: Group A patients resulted to be positive in higher proportion than those from Group B (18/50 - 36% vs 5/50 -10% respectively) ($p<0.001$). Group A patients had also higher parasite loads compared to healthy controls; 14/18 (77,7%) CRI patients lived in rural areas ($p<0.0003$) and Bologna was the province most affected. To have a dog was not found statistically significant. Among Group A, no statistical difference was found in relation to the type of BD although the rate of kDNA positive patients seemed higher when treated with modulators of T lymphocyte activity and anti-IL-6 receptor antibodies, compared to anti-TNF-alpha therapy. Patients that were taking a steroid in association with the BD showed a higher proportion of kDNA positivity than those assuming the BD only ($p<0.05$). Furthermore, living in a rural area or taking a steroid drug in addition to a BD were strongly and independent factors from sex and age associated to an increased risk of being positive to kDNA (O.R.[95%CI]: 8.74 [2.24-34.05] and 5.52 [1.40-21.78], respectively).

Conclusions: Northern Italy, now switched as focally endemic region for Visceral Leishmaniasis, has moderate risk for human disease especially for immunosuppressive patients, more vulnerable to Li. This should be taken into account to promote: i) screening with qPCR in immunosuppressive patients living in rural areas, to facilitate the administration of specific treatment since the first appearance of clinical symptoms, ii) public health interventions targeted to both vector and reservoir control strategies.