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A Case–Control Study on the Association Between Intestinal Helminth Infections and Treatment Failure in Patients With Cutaneous Leishmaniasis

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Background. Endemic regions of cutaneous leishmaniasis (CL) and intestinal helminthiasis overlap. CL treatment with systemic pentavalent antimonial drugs (Sb^{5+}) fails in 10%–30% of patients. The study objective was to assess the etiological role of intestinal helminthiasis in CL treatment failure.

Methods. An unmatched case–control study was done in 4 CL treatment sites in Peru in 2012–2015. Cases were CL patients with Sb^{5+} treatment failure; controls were CL patients with Sb^{5+} treatment success. Patients with a parasitologically confirmed CL diagnosis who had received supervised Sb^{5+} treatment and could be classified as cases or controls were eligible. The main exposure variables were intestinal helminthiasis and strongyloidiasis, diagnosed through direct examination, rapid sedimentation, Baermann, Kato-Katz, or agar culture of stool samples. Additional exposure variables were other infections (HIV, human T-lymphotropic virus 1, tuberculosis, hepatitis B, intestinal protozoa) and noninfectious conditions (diabetes, renal insufficiency, and immunosuppressive medication). Age, gender, CL history, probable exposure place, and *Leishmania* species were treated as potential confounders in multiple logistic regression.

Results. There were 94 case and 122 control subjects. Overall, infectious and noninfectious comorbidities were frequent both among cases (64%) and controls (71%). The adjusted odds ratio (OR) for the association between any intestinal helminth infection and CL treatment failure was 0.65 (95% confidence interval [CI], 0.30–1.38), and the adjusted OR for the association between strongyloidiasis and CL treatment failure was 0.34 (95% CI, 0.11–0.92).

Conclusions. In the Peruvian setting, high Sb^{5+} treatment failure rates are not explained by intestinal helminthiasis. On the contrary, strongyloidiasis had a protective effect against treatment failure.

Keywords. case–control study; cutaneous leishmaniasis; intestinal helminthiasis; Peru; treatment failure.

Cutaneous leishmaniasis (CL) is a vector-borne disease caused by the protozoan parasite *Leishmania* [1, 2]. CL is endemic in Central and South America, North Africa, the Middle East, and South Asia. CL is associated with poverty, population displacement, and environmental changes and is considered a neglected tropical disease [3, 4]. About 20% of the estimated 700 000 to 1 million CL cases per year occur in the American region [5, 6].

In the New World, CL can be caused by *Leishmania* species of subgenus *L.* (*Viannia*). In these cases, spontaneous cure is uncommon (estimated in 6% for *L.* [*V.*] *braziliensis*) [7], and a subgroup of patients (<5% but varying from area to area) develop mucosal forms that can be lethal if left untreated [1, 2, 8]. Therefore, systemic treatment with pentavalent antimonial drugs (Sb^{5+}, available as sodium stibogluconate and meglumine antimoniate) is recommended in *Viannia*-endemic regions, despite the toxicity of Sb^{5+} on the heart, liver, and kidneys [2, 9].

Treatment failure occurs in 10%–30% of CL patients receiving Sb^{5+} [2]. It is not entirely clear why treatment fails in some patients and not in others. The parasite species plays a role: In a Peruvian study, treatment failure was 1.6 times more frequent with *L.* (*V.*) *braziliensis* than with other species [10]. Also, if the parasites are themselves infected with *Leishmania* RNA virus, the risk of Sb^{5+} treatment failure may increase [11, 12].

Impaired immune responses to *Leishmania* can also contribute to CL treatment failure. Infections such as HIV [13] and tuberculosis [14, 15], as well as noninfectious conditions such

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as diabetes, post-transplant state, and connective tissue diseases, could modify human immune responses through generalized immune suppression, interference with innate immune mechanisms, and changes in the balance between regulatory and effector T-cell subsets. Except for HIV/AIDS, the association of these diseases with Sb^{5+} treatment outcome has not been firmly established. Although these conditions may explain Sb^{5+} failure in some cases, they are not frequent enough to explain the observed treatment failure proportions of 10% or more.

Intestinal helminth infections are frequent in *Leishmania*-endemic regions and could also affect immune responses, for example, through the associated Th2 polarization [16]. In 2 Brazilian cohort studies, 15% to 88% of patients with cutaneous or mucocutaneous leishmaniasis had positive stool test results for helminths (hookworm, *Trichuris trichiura*, *Ascaris lumbricoides*, *Schistosoma mansoni*, or *Strongyloides stercoralis*) [17, 18]. In these studies, a diagnosis of helminthiasis was associated with mucosal forms of leishmaniasis [18] and poor responses to Sb^{5+} treatment [17, 18]. On the other hand, a randomized controlled trial in Brazil showed that early elimination of helminth coinfection using albendazole, ivermectin, and praziquantel did not lead to faster healing of CL lesions under Sb^{5+} than deferred elimination of helminths [19]. Mouse models of *Leishmania*/helminth coinfection have also produced puzzling findings: Some studies have reported that helminth coinfection makes CL lesions grow faster and larger, while other studies have shown the opposite [20, 21].

To clarify the etiological role of intestinal helminth infection in CL treatment failure, we designed a case–control study including patients with CL in Peru. We hypothesized that helminth infections would be more frequent in cases with CL treatment failure than in control subjects without CL treatment failure. We evaluated the effects of intestinal helminthiasis in general and of strongyloidiasis in particular. Furthermore, we assessed the frequency and possible effects of other infectious diseases (tuberculosis, human T-lymphotropic virus 1 [HTLV-1], HIV, and hepatitis B) and noninfectious comorbidities (diabetes mellitus, renal failure, and intake of immunosuppressive medication) in the same study population.

METHODS

Study Design

This was an unmatched case–control study. Case subjects were defined as CL patients with Sb^{5+} treatment failure, and control subjects were CL patients with Sb^{5+} treatment success. We used the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) recommendations to describe the study methods [22]. The STROBE checklist including detailed study methods is available in the Supplementary Data.

Setting

The study was done in 4 national and regional reference centers for CL: Hospital Cayetano Heredia (Lima Region), Hospital Antonio Lorena and Quellouno-Quillabamba Health Center (Cusco Region), and Moyobamba Hospital (San Martín Region), where national clinical guidelines are followed and where most of the country's CL cases are reported. Patients were recruited between December 21, 2012, and November 30, 2015.

Participants

Patients were eligible if they [1] had a parasitologically confirmed CL diagnosis [2], received a supervised standard treatment course, and [3] could be classified as experiencing treatment failure (case subject) or success (control subject). Parasitological confirmation was defined as a positive microscopic examination of a Giemsa-stained direct smear or *Leishmania* culture or qualitative polymerase chain reaction (PCR) targeting a conserved region of *Leishmania* kDNA minicircles or a histopathological examination revealing amastigotes [23]. Standard treatment for CL in Peru consists of 20 mg Sb^{5+}/Kg/d for 20 days by intravenous or intramuscular injection. For this study, we enrolled patients who had received at least 16 doses of Sb^{5+} in a period of maximum 24 days.

Most of the cases of treatment failure (estimated at 96%) occur within 3 months after the end of Sb^{5+} treatment [24, 25]. Therefore, case ascertainment was based on clinical examination between 0 and 120 days after the end of the Sb^{5+} treatment course. We classified CL patients as cases if they experienced treatment failure, defined as either unresponsiveness (incomplete scarring, persistence of inflammatory signs, worsening of existing lesions, or appearance of new lesions) or relapse (reappearance of lesion or local signs of inflammation after initial cure). Follow-up ascertainment to define the status of control subjects was done between 100 days and 120 days after the end of Sb^{5+} treatment. We classified CL patients as controls if their lesions healed (complete scarring or flattening of lesions and disappearance of inflammatory signs) without relapse.

Variables

The outcome variable was the case or control status. The main exposure variable was intestinal helminth infection, which was assessed as any intestinal helminth infection (yes/no) or as strongyloidiasis (yes/no). Additional exposure variables were the presence of other infections (HIV, HTLV-1, tuberculosis, hepatitis B, and intestinal protozoa) and noninfectious comorbidities (diabetes, renal insufficiency, and use of immunosuppressive medication). We treated the following variables as potential confounders: site of enrollment, age, gender, type of stay in endemic region (residing, regular stays, and occasional travel), probable place of exposure (Amazon rainforest, Andean highlands), history of previous CL, *Leishmania* species, and disease duration.

Data Sources and Measurement

Participants were enrolled at the time of case/control ascertainment, that is, after Sb^{5+} treatment and follow-up. A clinician assessed demographic and clinical characteristics through patient

interview, physical examination, and review of patient files. At the same time, blood, urine, and stool samples were collected from all participants, and a chest x-ray was taken. Skin lesion samples were available in a subset of patients [1]: those who still had lesions at the time of study enrollment (some of the cases with treatment failure) and [2] those for whom a sample had been stored at the time of CL diagnosis (only at Hospital Cayetano Heredia in Lima).

Participants were requested to provide 3 stool samples collected on separate days. Five methods were used to detect infection with intestinal helminths (hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, *Hymenolepis nana*, *Enterobius vermicularis*, *Taenia*, *Fasciola hepatica*, and *Strongyloides stercoralis*) and protozoa (*Blastocystis hominis*, *Entamoeba histolytica*, *Giardia lamblia*, and *Balantidium coli*): direct examination, rapid sedimentation, Baermann test, Kato-Katz, and culture in agar (Table 1). However, the Kato-Katz method to determine parasite burden was not performed in all samples, because this was not feasible in peripheral settings (in Cusco and San Martín). We assumed that patients who had intestinal helminthiasis while receiving Sb^{5+} treatment would still be infected up to 3 months later, because untreated intestinal helminthiasis typically lasts for years [26, 27].

If skin lesion samples were available, *Leishmania* parasite species determination was done at Universidad Peruana Cayetano Heredia according to a previously published algorithm combining PCR assays and restriction fragment length polymorphism analysis [28, 29]. The *Leishmania* parasite load was measured using a SYBR Green-based real-time quantitative PCR assay targeting kDNA minicircles [30].

To determine other coinfections and comorbidities, we used case definitions based on blood, urine, and skin tests (Table 1). Patients in whom a specific coinfection or comorbidity was suspected were referred to the appropriate services for diagnostic confirmation and treatment (HIV or tuberculosis control programs or infectious diseases or internal medicine outpatient clinics).

Study Size

The target sample size was 78 cases and 156 controls. The assumptions were: alpha <5%, power ≥80%, 2 controls per case, intestinal helminth infection in 15% of the control patients, and a minimal detectable odds ratio (OR) of 2.5.

Statistical Methods

The core analysis was the assessment of the association between the main exposure (intestinal helminthiasis) and outcome

Table 1. Diagnostic Tests and Criteria for Coinfections and Comorbidities in Case and Control Subjects With Cutaneous Leishmaniasis

Diagnosis	Diagnostic tests	Criteria
Intestinal helminthiasis	Direct examination, rapid sedimentation, Baermann test, Kato-Katz, and culture in agar of stool samples	Definite: presence of eggs or adult parasites in at least 1 stool sample, detected with at least 1 method
Strongyloidiasis	Direct examination, rapid sedimentation, Baermann test, Kato-Katz, and culture in agar of stool samples	Definite: presence of larvae or adult parasites in at least 1 stool sample, detected with at least 1 method
Intestinal protozoa	Direct examination, rapid sedimentation, and Baermann test of stool samples	Definite: presence of cysts or adult parasites in at least 1 stool sample, detected with at least 1 method
HIV	Rapid test (CTK Biotech HIV Ag/Ab 4th Gen, Khartoum, Sudan)	Definite: positive rapid test followed by 1 positive 4th-generation ELISA result
HTLV-1	HTLV-1 enzyme immunoassay (Bioelisa HTLV, Biokit, Barcelona, Spain)	Probable: 2 positive ELISA results; in case of indeterminate or discordant results, Western blot testing was done in a reference laboratory
Tuberculosis	Tuberculin skin test, chest x-ray, smear microscopy (only in case of cough for >2 weeks)	Definite: microscopic observation of acid-fast bacilli or positive culture or PCR for *Mycobacterium tuberculosis* in any sample; possible: respiratory symptoms + bloody sputum or abnormal chest x-ray or weight loss with night sweats + no improvement with regular antibiotics
Hepatitis B	Rapid test (CTK Biotech HBAg, Khartoum, Sudan)	Definite: positive rapid test followed by 1 positive ELISA hepatitis B surface antigen (HBs-Ag) or a positive total hepatitis B core antibody (anti-HBc) and a negative hepatitis B surface antibody (anti-HBs)
Diabetes mellitus	Glucose in blood and urine	Definite: 2 fasting plasma glucose levels >125 mg/dL on different occasions, followed by increased HbA1c level, or patient with previous diagnosis and on hypoglycemic medication
Renal insufficiency	Creatinine, complete routine urine evaluation	Probable: plasma creatinine level >1.4 mg/dL and abnormal urine test and anemia; definite: creatinine clearance level <60 mL/min
Immunosuppressive medication	Interview, patient file	Definite: intake of an immunosuppressive drug for at least 2 weeks, including but not restricted to corticoids, anti-TNF, or antineoplastic drugs

Abbreviations: anti-TNF, anti–tumor necrosis factor; ELISA, enzyme-linked immunosorbent assay; HTLV-1, human T-lymphotropic virus 1; PCR, polymerase chain reaction.

(treatment failure). We used multiple logistic regression to control for confounding. A priori, all multiple logistic regression models included the main exposure (helminthiasis), place of patient inclusion (Lima, Cusco, or San Martín), and age. Potential confounding variables were only kept in the regression models if their inclusion influenced the strength of association between intestinal helminthiasis and outcome (if the OR varied by at least 10%). Final model selection was based on Akaike's information criterion. All analyses were done for 2 definitions of the main exposure: any helminthiasis vs no helminthiasis and strongyloidiasis vs no strongyloidiasis. *Leishmania* species and parasite load were known for a subgroup of patients only. This information is described separately and not included in the multivariable analysis.

Because the timing of clinical follow-up and study inclusion did not happen for all study participants as originally planned, we did a sensitivity analysis in which we excluded (1) patients who were classified as cases >4 months after the initial treatment because they did not come back to the study center earlier and (2) patients who were classified as controls (disease-free 3 months after the initial treatment) but came back with a recurrence of CL later. The statistical analyses were done using R.

Ethics

The study protocol was approved by the ethics committees of the Universidad Peruana Cayetano Heredia and University Hospital Antwerp and by the Institutional Review Board of the Institute of Tropical Medicine Antwerp. Participants—or in the case of children, their authorized representatives—gave written informed consent.

RESULTS

Participants

Two hundred sixteen CL patients were included: 94 cases (treatment failure) and 122 controls (treatment success). Seventy percent were enrolled in Lima, 20% in Cusco, and 10% in San Martín. The median age (interquartile range [IQR]) was 32 (18–50) years, and the majority (70%) were men. Sixty-eight percent of the participants were born in an endemic region for leishmaniasis, and 46% still lived in such a region. There were no statistically significant differences in demographic and epidemiological characteristics between cases and controls (Table 2).

Skin Lesions

The majority of the participants (185/216; 86%) had a CL diagnosis based on positive smear microscopy results. For the remaining 31 patients (14%), CL diagnosis was based on PCR only (n = 18), culture only (n = 2), histopathology only (n = 2), or a combination of PCR, culture, and/or histopathology (n = 7). At the time of CL diagnosis, the median disease duration (IQR) was 84 (50–148) days (Table 2). Five percent of the patients reported a previous history of CL (Table 2).

When comparing cases with control subjects, most clinical characteristics of CL were similar, except for the number of lesions: 58% of the case subjects had 2, 3, or more lesions compared with 42% among control subjects ($P = .003$) (Table 2). Also, the leishmanin skin test results differed: Case subjects reacted less frequently to leishmanin (frequency of positive reaction, 68%), and their leishmanin response was smaller (median diameter, 9 mm) compared with controls (frequency of positive reaction, 87%; median diameter, 11 mm; $P = .02$) (Table 2).

Coinfection and Comorbidity

The main exposure could be assessed in 201 participants: 123 participants (57%) provided 3 stool samples, 48 (22%) gave 2, and 30 (14%) gave 1 sample. No stool tests could be done for the remaining 15 participants (7%); they are included in the description of the patient population (Table 2) but not in the main analysis (Table 3). For those with more than 1 sample, we looked at all the results, that is, if any sample was positive for a certain helminth, we considered the patient infected. Fifty-two participants out of 201 (26%) had an intestinal helminth infection, including 27/201 patients with strongyloidiasis (13%). The other helminths were hookworm (in 18 patients), *Trichuris trichiura* (n = 17), *Ascaris lumbricoides* (n = 14), *Hymenolepis nana* (n = 9), *Enterobius vermicularis* (n = 2), and *Fasciola hepatica* (n = 1). Twelve percent of the patients (24/201) carried >1 helminth. Strongyloidiasis was significantly less frequent in cases (7%) than in controls (19%; $P = .02$).

Other infections diagnosed in this population were, in order of decreasing frequency: 131 patients (65%) with intestinal protozoa, 6 (3%) with HTLV-1, 4 (2%) with active tuberculosis, 2 (1%) with hepatitis B, and 1 (0.5%) with HIV. There were no significant differences in these proportions between cases and controls (Table 2). Seventeen patients (8%) had a noninfectious comorbidity: diabetes mellitus (n = 5), renal insufficiency (n = 5), or use of immunosuppressive medication (n = 8). Noninfectious comorbidity was less frequent in cases (3%) than in control subjects (11%; $P < .05$). Taken together, infectious and noninfectious comorbidities were frequent among both cases (64%) and controls (71%).

Association Between Intestinal Helminthiasis and Treatment Failure

The crude OR for the association between any intestinal helminth infection and CL treatment failure was 0.57 (95% confidence interval [CI], 0.29–1.08). Exposure was indeed somewhat less frequent in cases (20%) than in controls (31%). When we adjusted the analysis for the variables "place of enrollment," "age in years," and "went to uninhabited rainforest," the adjusted OR came closer to 1 and remained nonsignificant (adjusted OR, 0.65; 95% CI, 0.30–1.38) (Table 3).

Taking strongyloidiasis as the primary exposure variable of interest, the association with CL failure was stronger and reached statistical significance. The crude OR for the

Table 2. Demographic and Clinical Characteristics of 94 Case and 122 Control Subjects Treated With Pentavalent Antimony Drugs for Cutaneous Leishmaniasis: Summary and Comparison

	Cases (n = 94); Treatment Failure, No. (%)	Controls (n = 122); Treatment Success, No. (%)	*P* Value
Demographic characteristics			
Place of enrollment			.2[a]
Lima	72 (77)	80 (66)	
Cusco	15 (16)	28 (23)	
San Martín	7 (7)	14 (11)	
Age, median (IQR), y	30 (18–42)	33 (19–54)	.1[b]
Age groups			.6[a]
<15 y	19 (20)	24 (20)	
15–30 y	27 (29)	28 (23)	
>30	48 (51)	70 (57)	
Male gender	72 (77)	79 (65)	.08[a]
Born in endemic region	59 (63)	88 (72)	.2[a]
Type of stay in endemic region			.06[a]
Lives in endemic region	39 (41)	60 (49)	
Regular stays in endemic region	38 (40)	31 (25)	
Occasional travel	17 (18)	31 (25)	
Went to uninhabited rainforest	58 (62)	60 (49)	.09[a]
Probable place of exposure			.6[a]
Amazon rainforest	59 (63)	82 (67)	
Andean highlands	35 (37)	40 (33)	
Clinical characteristics			
Body mass index, mean (SD), kg/m^2	26 (4)	26 (4)	.6[c]
Noninfectious comorbidity[d]	**3** (3)	**14** (11)	**.05[e]**
History of previous leishmaniasis	4 (4)	5 (4)	1.0
Disease duration, median (IQR), d	79 (44–153)	88 (59–139)	.3[b]
Disease duration <30 d	10 (11)	6 (5)	.2[a]
No. of skin lesions, median (IQR)	2 (1–3)	1 (1–3)	.1[b]
No. of skin lesions			**.003[a]**
1	**40** (43)	**71 (58)**	
2	**30** (32)	**16** (13)	
3 or more	**24** (26)	**35** (29)	
Total lesion surface, median (IQR), mm^2	613 (248–1373)	482 (229–1133)	.4[b]
Logarithm of lesion surface, mean (SD), mm^2	6.4 (1.5)	6.2 (1.3)	.4[c]
Total lesion surface ≥79 mm^2	83 (89)	109 (89)	1.0[a]
Lesion type			.08[a]
Ulcers only	77 (82)	112 (92)	
Nodules, with or without ulcers	10 (11)	5 (4)	
Other	7 (7)	5 (4)	
Lesion location			.09[a]
Ear or nose	18 (19)	11 (9)	
Lower limbs	34 (36)	46 (38)	
Other parts of the body	42 (45)	65 (53)	
Concomitant distant lesion	17 (18)	20 (16)	.9[a]
Regional lymphadenopathy	28 (30)	30 (25)	.5[a]
Positive leishmanin skin test (n = 131)	**43/63 (68)**	**59/68 (87)**	**.02[a]**
Leishmanin response (n = 130), median (IQR), mm	**9 (0–13)**	**11** (7–15)	**.02[b]**
Positive tuberculin skin test (n = 192)	28/83 (34)	32/109 (29)	.6[a]
Tuberculin response (n = 176), median (IQR), mm	0 (0–15)	0 (0–14)	.7[b]
Herpes zoster during/after leishmaniasis treatment	3 (3)	7 (6)	.5[e]
Coinfections			
Any systemic coinfection[f]	6 (6)	7 (6)	1.0[a]
HTLV-1 (n = 205)	2 (2)	4 (3)	.7[e]
HIV (n = 209)	0 (0)	1 (1)	1.0[e]
Active tuberculosis[g]	3 (3)	1 (1)	.3[e]
Hepatitis B (n = 212)	1 (1)	1 (1)	1.0[e]
Intestinal protozoa (n = 201)	55 (61)	76 (68)	.3[a]
Any intestinal helminth[h] (n = 201)	18 (20)	34 (31)	.1[a]
Strongyloidiasis (n = 201)	**6** (7)	**21** (19)	**.02[a]**

Variables in bold font are those with a *P* value <.05 for the comparison between cases and controls.

Abbreviations: CL, cutaneous leishmaniasis; IQR, interquartile range.

[a]Pearson's chi-square test.

[b]Wilcoxon rank sum test with continuity correction.

[c]*T* test.

[d]Diabetes mellitus (in 5 control subjects), renal insufficiency (1 case and 4 controls), or use of immunosuppressive medication (2 cases and 6 controls).

[e]Fisher exact test.

[f]Tuberculosis, hepatitis B, HIV, or HTLV-1.

[g]Chest x-rays were done for 203 (94%) participants. Four study participants had a definite diagnosis of active tuberculosis; these diagnoses were based on a positive sputum smear (n = 2) or culture (n = 1) or on histopathology results (n = 1).

[h]*Strongyloides stercoralis* (6 cases and 21 controls), hookworm (4 cases and 14 controls), *Trichuris trichiura* (6 cases and 11 controls), *Ascaris lumbricoides* (5 cases and 9 controls), *Hymenolepis nana* (6 cases and 3 controls), *Enterobius vermicularis* (1 case and 1 control), and *Fasciola hepatica* (1 case).

association between strongyloidiasis and CL treatment failure was 0.31 (95% CI, 0.11–0.75). Adjusting for "place of enrollment," "age," and "went to uninhabited rainforest" resulted in an adjusted OR of 0.34 (95% CI, 0.11–0.92) (Table 3).

In both multivariable analyses, the variable "went to uninhabited rainforest" was a significant predictor of treatment failure, with an adjusted OR of ~2 (Table 3). Hence, the 2 main predictors of treatment failure identified in this study were strongyloidiasis (less frequent in cases than in controls) and "went to uninhabited rainforest" (more frequent in cases than in controls). We did not identify effect modifiers for these associations, and the sensitivity analysis led to the same conclusions.

Table 3. Association Between Exposure Variables and Treatment Failure in 94 Case and 122 Control Subjects Treated With Pentavalent Antimony Drugs for Cutaneous Leishmaniasis: Simple and Multiple Logistic Regression

Variable	Crude Odds Ratio (95% CI)	Primary Exposure: Any Intestinal Helminth,[a] Adjusted Odds Ratio (95% CI)	Primary Exposure: Strongyloidiasis,[b] Adjusted Odds Ratio (95% CI)
Any intestinal helminth[c]	0.57 (0.29–1.08)	0.65 (0.30–1.38)	-
Strongyloidiasis	**0.31 (0.11–0.75)**	-	**0.34 (0.11–0.92)**
Place of enrollment			
Lima	1	1	1
Cusco	0.60 (0.29–1.19)	0.51 (0.22–1.16)	0.52 (0.23–1.12)
San Martín	0.56 (0.20–1.41)	0.55 (0.19–1.53)	0.62 (0.21–1.74)
Age, y		0.99 (0.97–1.00)	0.99 (0.97–1.00)
Male gender	1.78 (0.98–3.30)	-	-
Type of stay in endemic region		-	-
Lives in endemic region	1		
Regular stays in endemic region	1.89 (1.02–3.54)		
Occasional travel	0.84 (0.41–1.71)		
Went to uninhabited rainforest	**1.66 (0.97–2.89)**	**2.02 (1.12–3.71)**	**2.09 (1.15–3.86)**
Noninfectious comorbidity[d]	0.25 (0.06–0.81)	-	-
Disease duration <30 d	2.30 (0.82–6.99)	-	-
No. of skin lesions		-	-
1	1		
2	1.15 (0.72–1.82)		
3 or more	0.41 (0.23–0.70)		-
Lesion type		-	-
Ulcers only	1		
Nodules only or ulcers and nodules	2.91 (0.99–9.65)		
Other	2.04 (0.63–7.10)		
Lesion location		-	-
Ear or nose	1		
Lower limbs	0.45 (0.18–1.07)		
Other parts of the body	0.39 (0.17–0.91)		

Variables in bold font are those with a *P* value <.05 for the comparison between cases and controls on multivariable analysis.

Abbreviation: CI, confidence interval.

[a]The following variables were included in the final model: any soil-transmitted helminth, place of enrollment, age, and went to uninhabited rainforest.

[b]The following variables were included in the final model: strongyloidiasis, place of enrollment, age, and went to uninhabited rainforest.

[c]*Strongyloides stercoralis* (n = 27), hookworm (n = 18), *Trichuris trichiura* (n = 17), *Ascaris lumbricoides* (n = 14), *Hymenolepis nana* (n = 9), *Enterobius vermicularis* (n = 2), or *Fasciola hepatica* (n = 1).

[d]Diabetes mellitus (n = 5), renal insufficiency (n = 5), or use of immunosuppressive medication (n = 8).

Leishmania Species Determination and Parasite Load Measurement

Leishmania species determination was done for 136 out of 216 study participants (63%, 73 cases and 63 controls). For the other 80 participants, no samples had been stored (n = 57) or the available samples did not provide a sufficient amount of DNA (n = 23). Furthermore, in 21 of 136 patients who were tested (15%), the results were inconclusive (Table 4).

In the remaining 115 patients, the detected *Leishmania* species were *L.* (*V.*) *braziliensis* (n = 42), *L.* (*V.*) *peruviana* (n = 41), *L.* (*V.*) *guyanensis* (n = 22), *L.* (*V.*) *lainsoni* (n = 6), and mixed/hybrid forms of *L.* (*V.*) *braziliensis*/*L.* (*V.*) *peruviana* (n = 4) (Table 4). The species distribution differed between cases and controls (*P* = .01): *L.* (*V.*) *braziliensis* was more frequent in cases (38%) than in controls (22%), and *L.* (*V.*) *peruviana* was less frequent in cases (26%) than in controls (35%).

There was a strong and significant association between the variable "went to uninhabited rainforest" and *Leishmania* species: *L.* (*V.*) *braziliensis* was diagnosed in 56% (35 of 62 tested) of those who went to uninhabited rainforest compared with 13% (7 of 53 tested) among the patients who did not report contact with uninhabited rainforest (OR, 8.3; 95% CI, 3.4–22.9; *P* < .00001).

Parasite load was determined for 45 cases and 42 controls, who had sufficient amounts of parasite DNA in samples to be quantified. There was no significant difference between them: The mean logarithm of the parasite load of the cases (SD) was 10.0 (4.3), and that of controls was 10.5 (3.5; *P* = .3).

Table 4. *Leishmania* Species and Parasite Load in a Subset of Case and Control Subjects With Available Skin Lesion Samples

	Cases; Treatment Failure, No. (%)	Controls; Treatment Success, No. (%)	*P* Value
Leishmania species determined[a]	n = 73	n = 63	
Leishmania (*Viannia*) *braziliensis*	28 (38)	14 (22)	.01[b]
Leishmania (*Viannia*) *peruviana*	19 (26)	22 (35)	
Leishmania (*Viannia*) *guyanensis*	7 (10)	15 (24)	
Leishmania (*Viannia*) *lainsoni*	1 (1)	5 (8)	
Mixed/hybrid *L.* (*V.*) *braziliensis*/*L.* (*V.*) *peruviana*[c]	3 (4)	1 (2)	
Nonconclusive result[d]	15 (21)	6 (10)	
Leishmania parasite load[e]	n = 45	n = 42	
Median parasite load (interquartile range)	77 813 (394–350 000)	51 812 (4260–404 688)	.6[f]
Mean log of parasite load (SD)	10.0 (4.3)	10.5 (3.5)	.3[g]

[a]Based on an algorithm combining PCR assays and restriction fragment length polymorphism analysis targeting mannose phosphate isomerase, cysteine proteinase b, and heat shock protein–70 genes [28, 29].

[b]Fisher exact test for 6 × 2 table. The *P* value for the comparison of *L.* (*V.*) *braziliensis* against all other species was also .01.

[c]Samples displaying banding characteristics of both *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis*.

[d]If there was insufficient amplification of genomic DNA to perform the RFLP analysis or if the RFLP pattern did not allow distinguishing between species.

[e]Parasite load was calculated as [parasite DNA equivalents per reaction/amount of tissue DNA per reaction] × 10^3 and expressed as number of *Leishmania* parasites per microgram of tissue DNA.

[f]Mann-Whitney test.

[g]*T* test.

DISCUSSION

Coinfections were frequent among patients with CL: Infections with intestinal protozoa, *Strongyloides*, and other intestinal helminths were common both in case and control subjects. Nevertheless, the main findings of this study were not in line with our original hypothesis: Intestinal helminthiasis was not more frequent in cases with CL treatment failure than in control subjects with treatment success. On the contrary, strongyloidiasis came out of the analysis as a protective factor against treatment failure in CL. Furthermore, having traveled to uninhabited rainforest was 2 times more frequent in cases than in controls, which may be explained by the infecting *Leishmania* species. Indeed, we found that having traveled to uninhabited rainforest was linked to *L.* (*V.*) *braziliensis*, a species with a predominantly sylvatic transmission cycle, and that, in line with previous studies, *L.* (*V.*) *braziliensis* was linked to treatment failure [10, 24].

The major strength of this study is that the patients were well characterized: Most of the risk factors for treatment failure described in the literature were included in data collection and analysis. In addition, we could evaluate several potentially relevant coinfections and comorbidities at the same time. Hence, an extensive assessment of confounding was possible, and it appeared that confounding factors did not affect the main findings in any major way. In addition, this study population included patients with a variety of coinfections and *Leishmania* species, which allowed for comparisons that were difficult to make in previous studies from Brazil, where the whole study population carried *L. braziliensis*, or where a large majority had helminthiasis [17, 18].

This study also has several limitations. First, the inclusion of control patients was more difficult than anticipated (the planned 1:2 ratio of cases to controls was not reached). In the 4 participating centers, all patients with CL were invited for a follow-up visit 3 months after treatment, but many did not show up. Although we think that patients who came for follow-up were similar in terms of exposure compared with those who did not come for follow-up, we cannot rule out that this self-selection among controls influenced the results somewhat.

Second, *Leishmania* species determination could not be done for all participants and was therefore not included in multivariable analyses. However, we found a strong link between *L. braziliensis* and the variable "went to uninhabited rainforest," for which we did adjust. Also, the *Leishmania* parasite load estimation was only done in a subset of patients, and the proportion of missing data was higher in controls than in case subjects.

Finally, because our study did not include formal immunological evaluations, it yielded little evidence explaining the biological pathways behind the findings. We did find stronger reactions to the leishmanin skin test (a delayed-type hypersensitivity test and indicator of cellular immune response) in control subjects than in cases, which is consistent with a previous study [31], but we did not find a significant link between strongyloidiasis infection and leishmanin skin test results. Other elements that may affect the modulation of the immune response and that were not addressed in this study are the helminth species and infection load, as well as the possible role of *Leishmaniavirus* [11].

The findings of this study are not in line with previous reports from Brazil [17–19]. Although we cannot rule out unobserved confounding, such differences may also be due to the complexity of interactions between infections. Both tegumentary leishmaniasis and strongyloidiasis are polar diseases with a broad spectrum of clinical manifestations. As this study enrolled consecutive patients with cutaneous leishmaniasis, it mainly

describes the middle of the spectrum, and extrapolation to the extremes (eg, disseminated leishmaniasis and *Strongyloides* hyperinfection) may not be appropriate. Mild infections with intestinal helminths may have a different impact on leishmaniasis than severe infections, and none of the studies assessed intestinal parasite load. The frequency of specific intestinal helminths species also varied across studies: Strongyloidiasis was more frequent in our study.

Because of the complex life cycle of *Strongyloides* in humans (affecting skin, blood, lung, and gut) and the fact that it can cause longstanding infections, we decided a priori to analyze the effect of strongyloidiasis separately. In patients with tuberculosis, strongyloidiasis modulates T-cell and B-cell responses, leading to increased mycobacterial burden [32, 33]. How strongyloidiasis affects the immune system in the skin is not well known, but it may well be that while *Strongyloides* induces a strong Th2 immune response at the systemic level, it affects the skin (and granuloma conformation) in a different way [34, 35]. Future studies should confirm this.

In conclusion, the high failure rates of Sb^{5+} treatment in the Peruvian setting cannot be explained by an effect of intestinal helminthiasis. Hence, testing and treating CL patients for intestinal helminthiasis will probably not improve the overall success rate of CL treatment. It is also unlikely that the set of other infectious and noninfectious conditions evaluated in this study plays an important role in Sb^{5+} failure. The unexpected link between the presence of strongyloidiasis and CL treatment success requires further confirmation and explanation.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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