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

Dalila Y. Martínez,1,2,# Alejandro Llanos-Cuentas,2,3 Jean-Claude Dujardin,4 Katja Polman,5,6 Vanessa Adaui,4,8 Marleen Boelaert,6 and Kristien Verdonck1,6

1Facultad de Medicina, Universidad Peruana Cayetano Heredia, Lima, Peru, 2Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Cayetano Heredia, Lima, Peru, 3Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium, 4Instituto de Medicina Tropical “Alexander von Humboldt,” Universidad Peruana Cayetano Heredia, Lima, Peru, 5Faculty of Pharmaceutical, Biomedical, and Veterinary Sciences, University of Antwerp, Antwerp, Belgium, 6Institute of Tropical Medicine, Antwerp, Belgium, 7Department of Health Sciences, VU University, Amsterdam, the Netherlands, and 8Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Background. Endemic regions of cutaneous leishmaniasis (CL) and intestinal helminthiasis overlap. CL treatment with systemic pentavalent antimonial drugs (Sb5+) 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 Sb5+ treatment failure; controls were CL patients with Sb5+ treatment success. Patients with a parasitologically confirmed CL diagnosis who had received supervised Sb5+ 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 Sb5+ 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 (Sb5+), available as sodium stibogluconate and meglumine antimoniate) is recommended in Viannia-endemic regions, despite the toxicity of Sb5+ on the heart, liver, and kidneys [2, 9].

Treatment failure occurs in 10%–30% of CL patients receiving Sb5+ [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 Sb5+ 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
as diabetes, post-transplant state, and connective tissue diseas
es, could modify human immune responses through genera
ilized immune suppression, interference with innate immune
mechanisms, and changes in the balance between regulatory
and effector T-cell subsets. Except for HIV/AIDS, the associa
tion of these diseases with Sb5+ treatment outcome has not been
firmly established. Although these conditions may explain Sb5+
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 Sb5+ treatment
[17, 18]. On the other hand, a randomized controlled trial in
Brazil showed that early elimination of helminth infection using
albendazole, ivermectin, and praziquantel did not lead to faster
healing of CL lesions under Sb5+ than deferred elimination of hel-
minths [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 infec-
tions 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 strongylo-
diasis 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 non-
infectious 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 Sb5+ treatment failure, and control
subjects were CL patients with Sb5+ 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 cen-
ters 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 con-
ﬁrmed CL diagnosis [2], received a supervised standard
treatment course, and [3] could be classiﬁed as experienc-
ing treatment failure (case subject) or success (control sub-
ject). Parasitological conﬁrmation was deﬁned as a positive
microscopic examination of a Giemsa-stained direct smear
or Leishmania culture or qualitative polymerase chain reac-
tion (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 Sb5+/Kg/d for 20 days by intravenous or intramuscular
injection. For this study, we enrolled patients who had received
at least 16 doses of Sb5+ in a period of maximum 24 days.

Most of the cases of treatment failure (estimated at 96%)
curr within 3 months after the end of Sb5+ treatment [24, 25].
Therefore, case ascertainment was based on clinical examina-
tion between 0 and 120 days after the end of the Sb5+ treatment
course. We classiﬁed CL patients as cases if they experienc-
ted treatment failure, deﬁned as either unresponsiveness (incom-
plete scarring, persistence of inﬂammatory signs, worsening of
existing lesions, or appearance of new lesions) or relapse (re-
appearance 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 Sb5+ 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 strongyloidiatis (yes/no). Additional exposure variables
were the presence of other infections (HIV, HTLV-1, tubercu-
losis, hepatitis B, and intestinal protozoa) and noninfectious
comorbidities (diabetes, renal insufﬁciency, and use of immu-
nosuppressive 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 dis-
ease duration.

Data Sources and Measurement

Participants were enrolled at the time of case/control ascen-
tment, that is, after Sb5+ treatment and follow-up. A clinician as-
sessed 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, Enteroabius 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 Martin). We assumed that patients who had intestinal helminthiasis while receiving Sb5+ 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

| 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 |
| Strongyloidesis                    | 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, Baermann test, Kato-Katz, and culture in agar 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 (ICTK 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 (ICTK Biotech HBsAg, 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.
ported a previous history of CL (Table 2).

The time of CL diagnosis, the median disease duration (IQR) was 84 (50–148) days (Table 2). Five percent of the patients remained 31 patients (14%) with 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).

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

| Variable                                      | Cases (n = 94) | Controls (n = 122) | PValue |
|-----------------------------------------------|----------------|-------------------|--------|
| Place of enrollment                           |                |                   |        |
| Lima                                          | 72 (77)        | 80 (66)           |        |
| Cusco                                         | 15 (16)        | 28 (23)           |        |
| San Martin                                    | 7 (7)          | 14 (11)           |        |
| Age, median (IQR), y                          | 30 (18–42)     | 33 (19–54)        | .1b    |
| Male gender                                   | 72 (77)        | 79 (72)           |        |
| Born in endemic region                        | 72 (77)        | 79 (65)           | .08a   |
| Occasional travel                             | 17 (18)        | 31 (25)           |        |
| Went to uninhabited rainforest                | 58 (62)        | 60 (49)           | .09a   |
| Probable place of exposure                    |                |                   | .6a    |
| Clinical characteristics                      |                |                   |        |
| Body mass index, mean (SD), kg/m²             | 26 (4)         | 26 (4)            | .6c    |
| History of previous leishmaniasis             | 4 (4)          | 5 (4)             |        |
| Disease duration, median (IQR), d             | 79 (44–153)    | 88 (59–139)       | .3c    |
| Disease duration <30 d                        | 10 (11)        | 6 (5)             | .2c    |
| No. of skin lesions, median (IQR)             | 2 (1–3)        | 1 (1–3)           |        |
| No. of skin lesions                           |                |                   | .003b  |
| Total lesion surface, median (IQR), mm²       | 613 (248–1373) | 482 (229–1133)    | .4b    |
| Logarithm of lesion surface, mean (SD), mm²   | 6.4 (1.5)      | 6.2 (1.3)         | .4c    |
| Total lesion surface ≥79 mm²                  | 83 (89)        | 109 (89)          | 1.0a   |
| Lesion type                                   |                |                   |        |
| Ulcers only                                   | 77 (82)        | 112 (92)          |        |
| Nodules, with or without ulcers               | 10 (11)        | 5 (4)             |        |
| Other                                         | 7 (7)          | 5 (4)             |        |
| Lesion location                               |                |                   | .09c   |
| Ear or nose                                   | 18 (19)        | 11 (9)            |        |
| Lower limbs                                   | 34 (36)        | 46 (38)           |        |
| Other parts of the body                       | 42 (45)        | 65 (53)           |        |

Table 2. Continued

| Variable                                      | Cases (n = 94) | Controls (n = 122) | PValue |
|-----------------------------------------------|----------------|-------------------|--------|
| Concomitant distant lesion                    |                |                   |        |
| Regional lymphadenopathy                      | 28 (30)        | 30 (25)           | .5a    |
| Positive leishman skin test (n = 131)         | 43/68 (68)     | 59/68 (87)        | .02a   |
| Leishman response (n = 130), median (IQR), mm | 9 (0–13)       | 11 (7–15)         | .02b   |
| Tuberculosis response (n = 192), median (IQR), mm | 28/83 (34)    | 32/109 (29)       | .6a    |
| Interstitial protozoa (n = 201), median (IQR), mm | 55 (61)       | 76 (68)           | .8a    |
| Any intestinal helminth (n = 201)              | 18 (20)        | 34 (31)           | .1a    |
| Strongyloides (n = 201)                        | 6 (7)          | 21 (19)           | .02c   |

Variables in bold font are those with a P value <0.05 for the comparison between cases and controls.

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

*Pearson's chi-square test.

Wilcoxon rank sum test with continuity correction.

'T test.

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

'Fisher exact test.

'Tuberculosis, hepatitis B, HIV, or HTLV-1.

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

Association between strongyloides 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 strongyloides (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.
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 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, * Adjusted Odds Ratio (95% CI) | Primary Exposure: Strongyloidesis, * Adjusted Odds Ratio (95% CI) |
|---------------------------------------------|---------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------|
| Any intestinal helminth                     | 0.57 (0.29–1.08)          | 0.65 (0.30–1.38)                                                          | -                                                                |
| Strongyloidesis                             | 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 Martin                                  | 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                     |                           |                                                                           |                                                                  |
| 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                  | 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                                 |                           |                                                                           |                                                                  |
| 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.

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

*bThe following variables were included in the final model: strongyloidesis, place of enrollment, age, and went to uninhabited rainforest.

*cStrongyloides 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).

*dDiabetes mellitus (n = 5), renal insufficiency (n = 5), or use of immunosuppressive medication (n = 8).
**Table 4. Leishmania Species and Parasite Load in a Subset of Case and Control Subjects With Available Skin Lesion Samples**

| Leishmania species determined | Cases; Treatment Failure, No. (%) | Controls; Treatment Success, No. (%) | PValue |
|-----------------------------|-----------------------------------|--------------------------------------|--------|
| Leishmania (Vianna braziliensis) | n = 73                             | n = 63                               |        |
| 28 (38)                      | 14 (22)                            | .01b                                |
| Leishmania (Vianna peruviana) | 19 (26)                            | 22 (35)                              |        |
| Leishmania (Vianna guyanensis) | 7 (10)                             | 15 (24)                              |        |
| Mixed/hybrid L. (V) braziliensis/L. (V) peruviana | 3 (4)                              | 1 (2)                                |        |
| Nonconclusive result | 15 (21)                            | 6 (10)                               |        |
| Leishmania parasite load | n = 45                             | n = 42                               |        |
| Mean log of parasite load (interquartile range) | 77813 (394–350 000) 51812 (4260–404 688) |        |
| Mean log of parasite load (SD) | 10.0 (4.3)                         | 10.5 (3.5)                           | .3g    |

*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].

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

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

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

*Parasite load was calculated as [parasite DNA equivalents per reaction/amount of tissue DNA per reaction] × 10^2 and expressed as number of Leishmania parasites per microgram of tissue DNA.

fMann-Whitney test.

/ 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: Strongyloides 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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