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Leprosy Associated with Atypical Cutaneous Leishmaniasis in Nicaragua and Honduras

Lucrecia Acosta Soto,[1,2] Nelson Caballero,[3] Lesny Ruth Fuentes,[4] Pedro Torres Muñoz,[1] Jose Ramón Gómez Echevarría,[1] Montserrat Pérez López,[1] Fernando Jorge Bornay Llinares,[2] John L. Stanford,[5,6] Cynthia A. Stanford,[6] and Helen D. Donoghue[5]*

[1]*Sanatorio Fontilles, Alicante, Spain;* [2]*Universidad Miguel Hernández de Elche, Alicante, Spain;* [3]*Asociación para el Desarrollo de los Pueblos (ADP), Nicaragua;* [4]*Head of Leprosy Program, Choluteca, Honduras;* [5]*University College London, London, United Kingdom;* [6]*Catriona Hargreaves Charitable Trust, Mill House, Claygate, Kent, United Kingdom*

Abstract. In Central America, few cases of leprosy have been reported, but the disease may be unrecognized. Diagnosis is based on clinical criteria and histology. Preliminary field work in Nicaragua and Honduras found patients, including many children, with skin lesions clinically suggestive of atypical cutaneous leishmaniasis or indeterminate leprosy. Histology could not distinguish these diseases although acid-fast organisms were visible in a few biopsies. Lesions healed after standard antimicrobial therapy for leprosy. In the present study, patients, family members, and other community members were skin-tested and provided nasal swabs and blood samples. Biopsies were taken from a subgroup of patients with clinical signs of infection. Two laboratories analyzed samples, using local in-house techniques. *Mycobacterium leprae*, *Leishmania* spp. and *Leishmania infantum* were detected using polymerase chain reactions. *Mycobacterium leprae* DNA was detected in blood samples and nasal swabs, including some cases where leprosy was not clinically suspected. *Leishmania* spp. were also detected in blood and nasal swabs. Most biopsies contained *Leishmania* DNA and coinfection of *Leishmania* spp. with *M. leprae* occurred in 33% of cases. *Mycobacterium leprae* DNA was also detected and sequenced from Nicaraguan and Honduran environmental samples. In conclusion, leprosy and leishmaniasis are present in both regions, and leprosy appears to be widespread. The nature of any relationship between these two pathogens and the epidemiology of these infections need to be elucidated.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, a slow-growing intracellular *Mycobacterium*, with tropism for Schwann cell in nerves and macrophages in the skin.[1] It shows a wide range of clinical presentations from tuberculoid (TT) through borderline forms (borderline tuberculoid [BT], mid borderline [BB], and borderline lepromatous [BL]) to lepromatous (LL).[2] Similarly, histopathology of skin lesions varies from compact granulomas to diffuse infiltration of dermis, which largely depends on the immune status of the patient and may not be in agreement with the clinical diagnosis.[3,4] In the absence of a definition defined as a gold standard, various clinical and laboratory criteria are used.[5] The diagnosis of late multibacillary leprosy (LL or BL) is straightforward, whether clinically or in the laboratory, because of the presence of acid-fast bacilli. However, in paucibacillary leprosy (TT and BT), no acid-fast bacilli are observed.[6] A type of leprosy observed in children, known as infantile nodular tuberculoid leprosy, usually characterized by the presence of nodular lesions, is also difficult to diagnose.[7]

Leishmaniasis is estimated to cause the ninth largest disease burden among infectious diseases in terms of morbidity and mortality but is largely neglected in tropical and subtropical countries.[8] Cutaneous leishmaniasis (CL) represents an important public health concern of considerable magnitude in many parts of the world.[9] In Central America, CL is endemic, caused by various *Leishmania* species where it is predominantly a single lesion disease of children.[10] Lesions are mainly on the face at the presumed sites of sand fly bites. Atypical cutaneous leishmaniasis (ACL) is a nonulcerative variant of CL observed in Honduras,[11–13] Nicaragua,[14,15] Costa Rica,[14,16] and Venezuela.[17] The nonulcerating lesions progress slowly and contain few parasites (Figure 1A and B). They occur most frequently on exposed areas of the body, and children are more often affected than adults.[14,15] These atypical lesions are distinct from post-kala-azar dermatitis, classical localized or diffuse CL. Most cases are associated with *Leishmania infantum*[11,12,14,18,19] and a few with *Leishmania mexicana*.[12,19] It is sometimes difficult to visually distinguish tuberculoid leprosy from atypical presentations of CL.[14]

In Nicaragua, at the start of this observational study approximately 13 years ago, there were fewer than 10 leprosy patients being treated at the Dermatology Center in Managua, almost all of them diagnosed several years previously. The latest registered leprosy prevalence rate at the end of 2013 was 0.025 per 10,000 population (15 cases), with 21 new cases reported in 2013. Honduras, immediately to the north of Nicaragua, had a reported prevalence rate of only 0.005 per 10,000 population (four cases), with four new cases registered in 2013.[20]

In Honduras, there are endemic areas where ACL has been clearly identified, in the east, south–central, and west parts of the country.[13] Over the past 15 years, two areas of Nicaragua have been identified where cutaneous lesions of this type are frequently found in children, generally aged between 5 and 14 years. One of these areas is around San Francisco Libre, near Lake Managua in southwest Nicaragua, the other is in a recently populated gold-mining area between the towns of Villa Nueva and Somotillo, Chinandega Province, in northwest Nicaragua, contiguous with the Honduran border. Both districts have high levels of poverty and need of assistance. Subsequently, similar cutaneous lesions were noted in children from the Honduran southwest province of Choluteca, immediately north of Chinandega. Both Nicaragua and Honduras are endemic for this infection, with 2,219 new reported cases in Nicaragua and 2,074 in Honduras during 2013.

Lesions of Central American ACL and paucibacillary leprosy are identical in clinical appearance[14] and a preliminary study

* Address correspondence to Helen D. Donoghue, Centre for Clinical Microbiology, Division of Infection and Immunity, Royal Free Campus, University College London, London NW3 2PF, London, United Kingdom. E-mail: h.donoghue@ucl.ac.uk

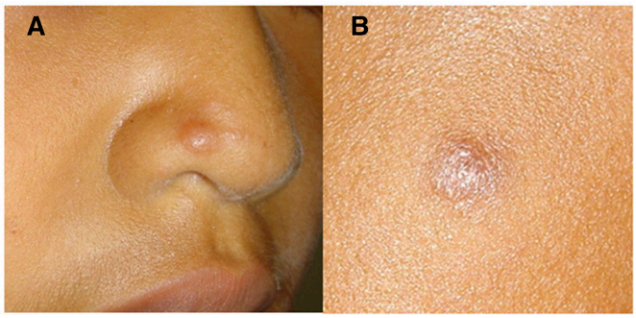

FIGURE 1. Facial skin lesions found to contain both *Leishmania* spp. and *Mycobacterium leprae* from Honduras. (**A**) Nasal lesion. (**B**) Lesion on cheek.

attempted to distinguish the two conditions. Biopsies of lesions were examined, but the histopathology was consistent with either infection. However, the failure to recognize *Leishmania* parasites in any of the lesions and the demonstration of occasional acid-fast bacilli in a few of them strongly suggested indeterminate or tuberculoid leprosy. Therefore, standard treatment of leprosy was given, and many of these patients showed clinical improvement.

A contemporary independent investigation of similar skin lesions in Nicaragua, Honduras, and Guatemala, based on skin tests for *Leishmania* and histology of biopsies, had concluded that *Leishmania* caused the lesions, based on the high level of skin-test positivity, the clinical appearance of the lesions and histopathology, with an aggravated response due to volcanic dust seen in the lesions.[21] However, they found no *Leishmania* parasites in lesions by microscopy or culture.

The aim of the present study was to identify whether *M. leprae* or *Leishmania* spp. were present in skin lesions or other clinical samples from individuals living in Choluteca (Honduras) and Chinandega (Nicaragua), then to consider the potential implications for those living in these communities.

MATERIALS AND METHODS

Patients and study participants. The study areas were located inland from the Central American Pacific coast. The seasons are influenced by height above sea level, and temperatures vary by altitude rather than season, being hot and humid almost all the year. The average high temperature nationwide is 32°C (90°F), and the average low is 20°C (68°F), with a rainy season in spring. In Nicaragua, participants were adults and children living in the northeast gold-mining area between Villa Nueva and Somotillo. In the preliminary study, patients from San Francisco Libre, near Lake Managua, were included. Honduran participants were from the regional hospital in Choluteca, and local village health clinics in El Triumfo, Namasegüe, El Apintal, Santa Ana de Yusguare, and Tablones Arriba. In Nicaragua, case-finding, arranging treatment, and follow-up in the afflicted areas were carried out by the Asociación para el Desarrollo de los Pueblos (ADP), Nicaragua from 2003 until 2011. In Honduras, the earlier leprosy control program had collapsed in 1988, but the appointment of a new leprosy program coordinator led to follow-up of previously registered patients and active case finding from 2007 until 2011.

Ethics statement. The protocol used in Honduras and Nicaragua was reviewed and approved by the local ethics committee (institutional review board) in Manuagua (Nicaragua) and Choloteca (Honduras). In addition, ethical approval was obtained from the Fontilles Scientific Committee that includes independent assessors, and University College London (UCL) Research Ethics Committee. The facilities used belonged to the Ministries of Health of Nicaragua and Honduras. The subsequent planned laboratory work was carried out at UCL and Fontilles according to their procedures for data protection and ethics. The UCL Data Protection guidelines were followed, and all patient data were regarded as confidential medical records.

The local health promoters (paramedical staff) identified any individuals with skin lesions suspicious of leprosy in their communities. These potential cases were advised to come to the local health center when the medical team was visiting their communities and encouraged to also bring along their household members and/or family. In active case finding, a team of doctors visited an area after it had been announced on local radio and television so that people could attend even if not referred by a community health worker. In addition, a small group of previously registered leprosy patients in Choluteca were followed up and were visited at their home if they had difficulty in attending the nearest village clinic. They and their families were also invited to join the study. Oral consent was obtained from each individual as patients waited for their clinical examination and tests. NC (ADP, Nicaragua), LF (Head of leprosy program, Choluteca, Honduras), and the local community nurses or doctors explained the purpose of the project to individual patients and their families. A member of the team acted as an independent witness to observe the verbal consent. These conditions fulfilled both countries regulations on clinical and ethical trials.

Photographs were taken during the clinical examination of patients to assist with diagnosis. Subsequently, selected photographs were deidentified, for use in training sessions. If anyone showed clear clinical symptoms of leprosy, they were offered treatment whether they agreed for samples to be taken (Supplemental Data S1). Consultation was carried out with participants individually or with children and their parents. Children were seen with their parents and a local community nurse, whom they knew, explained the purpose of the study. Irrespective of the views of the parents, if a child was reluctant, no samples were taken.

Groups were defined as household members—living under the same roof and/or were family members of the possible new case; or community members—individuals living in the same community with no known household or family contact with leprosy or with anyone suspected of leprosy.

Field investigations. Children and a few adults with skin lesions resembling those caused by *Leishmania* spp. and/or *M. leprae* were examined for lesions elsewhere on the body, and a note was made of the presence or absence of a Bacillus Calmette–Guérin (BCG) scar. In each district children with lesions, healthy children and adults were subjected to quadruple skin testing with the "new tuberculin reagents" Leprosin A, Tuberculin, Scrofulin, and Vaccin[22] to determine the immune response to *M. leprae*. A flow-chart of the field work can be seen in Figure 2.

Laboratory examination of specimens. *Overview.* The prime purpose of the work was to detect and treat leprosy and/or

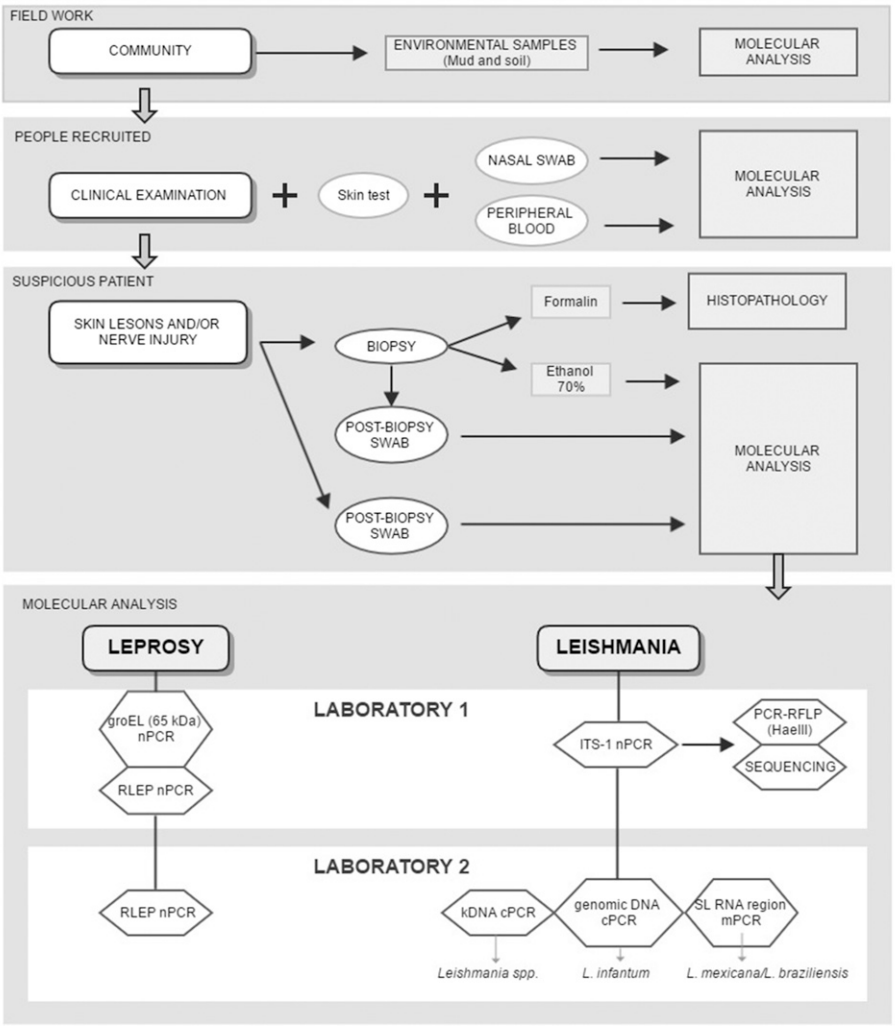

FIGURE 2. Flow chart of work. RLEP: *Mycobacterium leprae*-specific repetitive element; nPCR: nested PCR; PCR-RFLP: PCR-restriction fragment length polymorphism; cPCR: conventional PCR; SL RNA region: spliced leader RNA gene repeats region; mPCR: multiplex PCR.

leishmaniasis in these isolated and rural communities. The molecular investigations were to assist in the diagnosis of the causes of the skin lesions noted in children, and the existing methods in place at each of the participating laboratories were used. Fontilles is the Leprosy National Reference Center in Spain. *Leishmania* samples were examined in the World Health Organization Collaborating Center for Leishmaniasis (WHO-CL) in Madrid. In addition, some *Leishmania* samples were examined at both UCL and WHO-CL. There was close collaboration between the two main laboratories that undertook this study, and the first author worked at both Fontilles and UCL to ensure comparability of findings.

Biopsies. Taken from participants with clinical symptoms of leprosy and/or ACL, based on skin lesion, anesthesia of the skin, and/or a swollen neighboring nerve ganglion. In the preliminary study based in Nicaragua, biopsies were taken at the time of clinical examination and preserved in formalin or 70% ethanol (–20°C) until subsequent molecular analysis. The biopsies were 5 mm in diameter, except in some cases where the location (face) or the age of the patient required a smaller biopsy (3-mm diameter) to be taken (Biopsy punch, KAI Europe GmbH, Germany). In some individuals, swabs were taken from the site after the biopsy and preserved in ethanol 70% at –20°C until later analysis. In the Honduran study, a limited number of small (2 mm) core biopsies were taken. Photographs were taken of all lesions. When feasible, biopsies were split and one half examined by histology, after fixing in 10% formalin and staining. The remaining aliquot was stored in RNALater® (Qiagen, Austin, TX) for subsequent molecular analysis.

Nasal swabs. Taken from children with lesions, from other children and adults living close to them (close contacts) and from adults not known to be in contact with cases, but living in the same region (community contacts).

Peripheral blood samples. Taken from all participants in Honduras. In Nicaragua, venous blood samples were obtained from 50 patients with lesions, 50 close contacts and 50 casual contacts from the gold-mining area. These were immediately distributed, 5 mL into PAXgene® blood RNA Tubes (Becton-Dickinson, Oxford, United Kingdom) for immunological and molecular analyses, and the remainder was allowed to clot for serum extraction. Tubes and swabs were refrigerated until transferred to the laboratory and thereafter stored at –20°C until analysis.

Environmental samples. In both Honduras and Nicaragua, mud and soil samples were taken from roadside verges,

gardens, ditches, riverbanks, and the edges of ponds in the area close to patients' homes. These samples were also refrigerated and stored at –20°C until analysis.

Specimen processing for analysis. Two laboratories carried out sample processing: Laboratory 1 (Sanatorium Fontilles, Alicante, Spain) and Laboratory 2 (UCL). The detailed methods for the examination of each type of specimen in Laboratories 1 and 2 are described in Supplemental Data S2.

DNA amplification. *Working arrangements.* Strict protocols were followed to prevent contamination, with separate rooms for different stages of the process. There were physical barriers with separate areas for extraction, polymerase chain reaction (PCR) set up and product analysis, using a three-laboratory four-workstation method, with separate equipment and protective clothing, with stringent cleaning before each experiment. Filter tips were used routinely. Surfaces and equipment in contact with sample tubes (centrifuges, rotors, mixers, etc.) were cleaned before each assay.

Detection of M. leprae *DNA.* Details of the DNA amplification and detection in Laboratories 1 and 2 are described in Supplemental Data S3. In Laboratory 1, two target loci were used: 1) *groEL* (65 kDa): in a nested PCR the first reaction amplifies a 578-base pair (bp) region, and the second reaction amplifies a 347-bp region, using primers L1 to L4.[23] 2) Nested PCR based on the *M. leprae* repetitive element (RLEP) repetitive sequence was also carried out (described in later sections).

In Laboratory 2, the *M. leprae* PCR targeted the repetitive elements RLEP ($N = 36$). A two-tube nested PCR was used, which gives an outer product of 129 bp and a nested PCR product of 99 bp.[24] An alternative primer pair (111 bp product) was also used: MT2: 5′-CATTTCTGCCGCTGGTATC-3′/ MT4: 5′-ATCATCGATGCACTGTTCAC-3′.[25]

Detection of Leishmania *spp.* In Laboratory 1, the detection of specific genus and/or species DNA of *Leishmania* spp. (ITS-1 gene target) was carried out using nested PCR. The outer primers, LITSR (5′-CTGGATCATTTTCCGATG-3′) and L5.8S (5′-TGATACCACTTATCGCACTT-3′), amplify a 300–350 bp region.[26] The inner primers, SAC (5′-ATTTTCCGATGATTACACC-3′) and VAN2 (5′-GCGACACGTTATGTGAGCCG-3′), amplify an internal region (280–330 bp) of the fragment described by Cruz et al.[27]

In Laboratory 2, *Leishmania* spp. were detected by the genus-specific primers 13A (5′-GTGGGGGAGGGGCGTTCT-3′) and 13B (5′-ATTTTACACCAACCCCCAGTT-3′) that hybridize to a region of the kinetoplast minicircle conserved in all *Leishmania* species, with an amplicon of ~120 bp.[28,29] A specific PCR[30] detected the *Leishmania donovani* complex (*L. infantum*), using outer primers Ext: 5′-AATTCGACGAT-CACGAGGTC-3′/ E2b: 5′-CGACTCGGTTGGCACACTGC-3′, followed by a separate nested reaction, using primers P-1 (5′-ACGAGGTCAGC TCCACTCC-3′) and P-2 (5′-CTGCAACGCCTGTGTCTACG-3′), with an amplicon of 100 bp.[31] The *Leishmania mexicana* complex was detected with primers LU-5A: 5′-TTTATTGGTATGCGAAACTTC-3′ and LM-3A: 5′-GCACC GCACCGG(A/G)CCAC-3′,[32] giving an amplicon range of 218–240 bp. The *Leishmania braziliensis* complex was detected with primers LU-5A and LB-3C: 5′-CGT(C/G) CCGAACCCCGTGTC-3′, with an amplicon of 146–149 bp. Amplicons were detected as previously described, using agarose gel electrophoresis.

Sequencing of amplified DNA. All Laboratory 1 amplicons were sequenced, as were the amplicons from the Laboratory 2 environmental samples (Supplemental Data S3).

RESULTS

Study subjects. *Laboratory 1.* A total of 111 individuals provided data for the study (83 females and 28 males), aged between 6 and 83 years, mean age: 30 years. They all presented skin lesions compatible with leprosy or *Leishmania* or were contacts of these cases. Thirty-seven were from Honduras: 10 from Choluteca and 27 from Marcovia. Seventy-four were from Nicaragua: 59 from Jiñocuabo and 15 from Pacaya. The individuals were classified[2,33] according to the presumptive diagnosis: contact group: 18 from Honduras (Choluteca, Marcovia); household contacts of positive patients: three from Honduras (Choluteca); LL: four from Honduras; TT: one from Honduras; borderline leprosy (BB-BT): one from Honduras (Choluteca); CL: 54 from Nicaragua (nine from Pacaya and 45 from Jiñocuabo); ACL: 10 from Honduras and 20 from Nicaragua (14 from Jiñocuabo and six from Pacaya). In the study, 101 individuals were BCG-vaccinated and 72/74 (Nicaragua) and 18/27 (Honduras) gave a positive skin test.

Laboratory 2. A total of 299 individuals were included in the study carried out at UCL. These comprised 266 individuals with biographical details (134 females and 132 males). In preliminary investigations, an initial cohort of 33 Nicaraguan patients, from San Francisco Libre near Managua and from the northwest region of Chinandega, supplied skin biopsies only. For the main study, in Nicaragua, samples were obtained from 155 individuals, 84 males and 71 females, of whom 91/153 (59.5%) had been vaccinated with BCG. In Honduras, 111 individuals from Choluteca and surrounding villages provided data (63 females and 48 males), aged between 17 months and 94 years, mean age 35 years, of whom 77 had been vaccinated with BCG (69.4%). Of these, 16 were patients, 22 were household contacts and 39 were healthy controls. Of those not vaccinated with BCG, 10 were patients, 10 were household contacts, and 14 were healthy controls.

Biopsies. Laboratory 1: a total of 35 skin biopsies from 33 individuals were analyzed, 19 from Nicaragua and 14 from Honduras (Table 1). Five of the biopsies were positive using primers that amplify a specific fragment of the *groEL* gene and eight for the RLEP repetitive sequence. *Leishmania* spp. DNA was detected in 26 of 33 biopsied individuals (70.3%), and *L. infantum* was identified in these samples by PCR-RFLP (restriction fragment length polymorphism) and/or sequencing. DNA of both species was detected in six of 33 individuals. Post biopsy swabs were taken from 16 individuals. All were negative for the *groEL* gene, but two individuals were positive for the *M. leprae* RLEP target. *Leishmania* spp. DNA was amplified in postbiopsy swabs from 12 of the 16 individuals, and *L. infantum* was detected. DNA of both species was detected in two postbiopsy swabs and was 100% concordant with the biopsy result.

In Laboratory 2, from Nicaragua, 34 separate biopsies were taken for histopathology and revealed 29 clinically identified leprosy patients in the tuberculoid range: TT 10; TT-BT 1; BT 10; BT-BB 3; BB 3; BB-BL 1; IL (indeterminate leprosy) 1; plus no *M. leprae* (2) and dermatitis (3). Thirty-seven other biopsies were examined by PCR (Table 1). All contained *Leishmania* spp. DNA, and six also contained *M. leprae* DNA (16.2%). In Honduras, only 22 biopsies were taken as there were no further supplies of the punch biopsy kits. *Leishmania* spp. DNA was detected in all biopsies, and *M. leprae* DNA was detected in 14/22 biopsies (63.6%). Nine biopsies were clearly positive for *L. infantum* using nested PCR. There

TABLE 1
Confirmation of *Leishmania* spp. and *Mycobacterium leprae* identified in patient samples

Origin/Sample type	PCR *Leishmania* Fontilles	PCR *Leishmania* UCL	ELISA *Leishmania* UCL	Total *Leishmania* infected	*Mycobacterium leprae* PCR (*groEL*) Fontilles	*Mycobacterium leprae* PCR (RLEP) Fontilles	*Mycobacterium leprae* PCR (RLEP) UCL	Total *Mycobacterium leprae* infected
Nicaragua								
Biopsy	16/19	37/37	–	53/56	0/19	2/19	6/37	8/56
Post biopsy swab	11/14	–	–	11/14	0/14	1/14	–	1/14
Blood	–	–	10/108	10/108	–	–	4/32	4/32
Nasal swab	1/73	–	–	1/73	0/73	3/73	13/76	16/149
Total patients	18/74	37/37	10/108	65/219	0/74	4/74	19/119	23/193
Honduras								
Biopsy	10/14	22/22	–	32/36	5/14	6/14	14/22	20/36
Post biopsy swab	1/2	–	–	1/2	0/2	1/2	–	1/2
Blood	–	14/17	–	14/17	–	–	22/98	22/98
Nasal swab	0/26	–	–	0/26	0/26	1/25	22/109	23/134
Total Patients	10/37	35/39	–	45/76	5/37	6/37	51/111	57/148

ELISA = enzyme-linked immunosorbent assay; UCL = University College London; PCR = polymerase chain reactions. Data expressed in positive patients by total samples analyzed by type of sample. PCR *Leishmania* Fontilles: *Leishmania* PCR performed in Fontilles (ITS-1 region); PCR *Leishmania* UCL: *Leishmania* PCR performed at UCL (13A/13B primers); ELISA *Leishmania* UCL; positives ≥ 20 endpoint with ELISA anti-*Leishmania* antibodies made at UCL; Total *Leishmania* infected: Total of ELISA- and PCR-*Leishmania* positives; *M. leprae* PCR (*groEL*) Fontilles: *M. leprae* PCR of *gro-EL* region performed at Fontilles; *M. leprae* PCR (RLEP) Fontilles: *M. leprae* PCR of RLEP region performed at Fontilles; *M. leprae* PCR (RLEP) UCL: *M. leprae* PCR of RLEP region performed at UCL; Total *M. leprae* infected: Total of *M. leprae* PCR positives.

were three possible positives for *Leishmania brasiliensis*, and nine possible positives for *L. mexicana*, but these PCRs yielded several nonspecific bands on agarose gels so require confirmation.

Blood samples. Laboratory 1 did not examine Nicaraguan blood samples, as unfortunately, a consignment of blood samples was lost during civil unrest. In Laboratory 2, 108 Nicaraguan blood samples examined by *Leishmania* enzyme-linked immunosorbent assay (ELISA) showed that 10 patients (9.2%) were positive to anti-*Leishmania* antibodies (Table 1), although only five individuals had a significant skin lesion. Laboratory 2 examined blood samples from Nicaragua using PCR and found 4/32 individuals positive for *M. leprae* DNA (12.5%). These four patients each had positive *M. leprae* histopathology. Honduran blood samples examined by PCR showed that 22 of 98 samples were positive for *M. leprae* DNA (22.4%). A subset of 17 Honduran blood samples was also examined for *Leishmania* spp. DNA and 14 were positive (82.4%).

Nasal swabs. Laboratory 1 found 99 nasal swabs obtained from 98 individuals to be negative for PCR amplification of the gene *groEL*. However, four were positive for the RLEP *M. leprae* target (Table 1), two without any other positive sample and two with previous positive samples (skin biopsy and post biopsy swab). *Leishmania* spp. DNA was detected in one nasal swab that correlated with one of the three positive postbiopsy swabs and skin biopsy negative, and *L. infantum* was identified.

Laboratory 2 found (Table 1) *M. leprae* DNA in 13 nasal swabs from 76 individuals from Nicaragua (17.1%) and in 22 of 109 nasal swabs from Honduras (20.2%). Ninety-seven individuals from Honduras gave both a nasal swab and a blood sample. Only three individuals had both a blood sample and nasal swab positive for *M. leprae*: a patient with indeterminate leprosy, a household contact, and a healthy control.

Environmental samples. *Mycobacterium leprae* DNA was obtained from soil samples taken from a dried riverbed in Chinandega, Nicaragua; and in Honduras, a creek in a village field; a roadside gutter, the base of a tree in the local community center, and garden of a leprosy patient. Figures 2 and 3 show sequencing data from the Honduras (Tablones) roadside gutter and Nicaragua (Chinandega) dried riverbed. Both yielded sequences identical with emb|X17153.1| *M. leprae* repetitive element, RLEP2 bases 396–506 in the NIBI BLAST database. Using both forward and reverse strands, the entire 111 bp target region was sequenced.

Summary of results. Looking at all sample types from the main databases, *M. leprae* DNA was detected in 23/193 individuals from Nicaragua (11.9%), of whom six also had leprosy histopathology. In Honduras, there were 57/148 individuals (38.5%) with *M. leprae* DNA. *Leishmania* spp. DNA was especially widespread in the skin and found in 93.2% of all skin biopsies. Finally, 24 individuals were coinfected with *Leishmania* spp. and *M. leprae*, eight from Nicaragua and 16 from Honduras.

DISCUSSION

Our study confirms that leprosy is present in Northwest Nicaragua and Southwest Honduras, with active infections identified in children. The use of PCR to detect *M. leprae* was found to be much more sensitive than the older system of using quadruple skin tests to detect responses to common (group 1) mycobacterial antigens.[22] The clinical symptoms were more severe in Honduras, which may reflect the lower standard of living in these isolated small communities combined with the limited health care that was available at the time the study was carried out. For example, a small study of 27 ACL patients who attended a dermatology clinic in the Honduran capital city, Tegucigalpa, found that the average age was 9.4 years and most patients came from the south of the country. Over half of the patients (56%) suffered from chronic malnutrition.[19] In the southern part of Honduras (Reitoca), 12.6% (55/438) of schoolchildren evaluated were confirmed with ACL.[13] The presence of unspecific skin lesions in areas where leprosy and ACL are endemic emphasizes the need of a correct diagnosis. Prospective studies that correlate the presence of the etiological agent with the clinical evolution of the lesion are necessary for the implementation of the proper treatment and clinical resolution.

The high proportion of apparently healthy household contacts, and other members of the community with *M. leprae* nasal carriage, shows the efficacy of combining molecular and serological techniques to identify *M. leprae* carriers in the asymptomatic stage of infection, and highlights the importance

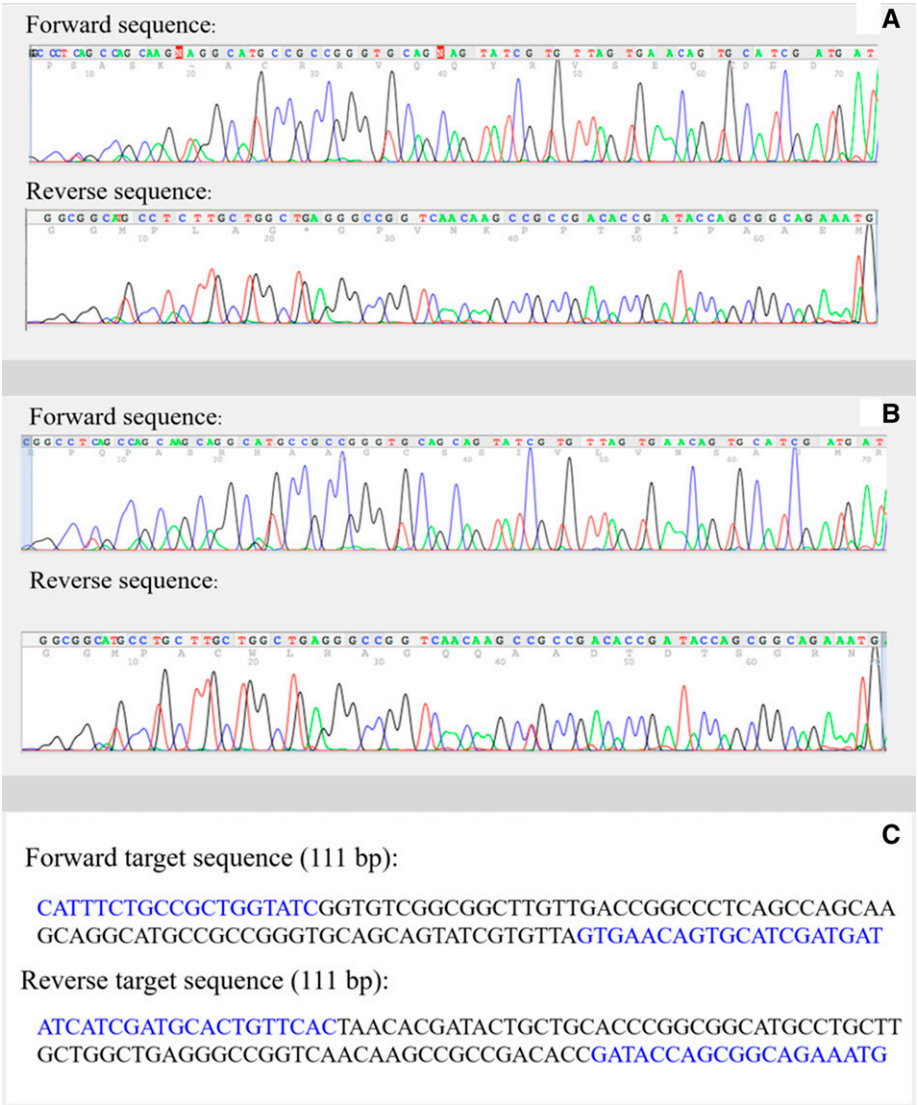

FIGURE 3. DNA sequencing of environmental *Mycobacterium. leprae* PCR products. (**A**) Honduras: Tablones ditch by roadside. (**B**) Nicaragua: Chinandega river bank. (**C**) DNA target sequence and primers (in blue).

of monitoring hyperendemic populations.[34,35] DNA detected in nasal swabs also indicates the presence of bacilli at the site of transmission and possible infection. The concomitant use of both molecular and serological assays enables the detection of subclinical infection in household contacts[35] and *M. leprae* carriers who could transmit and disseminate disease in endemic regions.[36] Chemoprophylaxis of these contacts is suggested.[35] Another significant group included individuals with *M. leprae* in their blood (Table 1). It is not known whether these are healthy carriers or potential future patients. It is noticeable that there was very little overlap between these individuals and the other members of the community with nasal carriage of *M. leprae.* A Brazilian PCR-based study of *M. leprae* in nasal swabs and blood samples of healthy household contacts of leprosy patients[37] reported a much lower level of *M. leprae* carriage (< 2%) and none progressed to clinical signs of the disease during the following year. However, this was clearly a different scenario from that in the present study, where follow-up of healthy household contacts is a good strategy for early diagnosis of leprosy. The identification of *M. leprae* in environmental samples suggests that there is also an environmental reservoir of infection that could be the source of the *M. leprae* in apparently healthy members of the community. Viable *M. leprae* has been reported from Indian soil samples, suggesting that this may provide a possible source of transmission of leprosy.[38,39] It was noted that the villagers were subsistence farmers who lived in locally built huts with earth floors. They were also exposed to dengue fever and Chaga's disease so may have had multiple morbidities.

Using an ELISA assay, *Leishmania* was detected in the blood of many individuals, most being in the *L. donovani* (*infantum*) complex. We concluded that this indicated active infection as the anti-*Leishmania* antibody titers are known to decrease after therapy.[40] Interactions between *M. leprae* and *Leishmania* may occur, as during active leprosy, *M. leprae* antigens can induce an IL-10-mediated regulatory response that controls the immunopathology of *L. braziliensis*-induced mucosal leishmaniasis.[41] In addition, the colocation of *M. leprae* with *Leishmania* spp. in biopsies is especially intriguing. Although a few cases of CL with leprosy have been reported previously,[42,43] in the present study, this was widespread in both Nicaragua and

Honduras. It appears that all skin lesions contained *Leishmania* DNA but in a subset of these, there was also *M. leprae.* The species of sand fly can influence the clinical presentation of ACL because of the immune priming from the *Leishmania* saliva.[15,44] However, it has also been shown in Honduras that different species of *Leishmania* caused an identical pathology.[12] It is well known that ACL is endemic in Central America, but it is of particular interest to try to ascertain whether there is a biological relationship between the *Leishmania* parasite and *M. leprae*. A number of scenarios are possible. The simplest explanation is that activated macrophages engulf nasal *M. leprae* and subsequently migrate to other sites in the body with inflammation, such as ACL. However, the sand fly vector and/or the *Leishmania* parasite may be infected with *M. leprae*. *Mycobacterium leprae* can be cultured in amoebae,[45] and earlier epidemiological studies suggest a role of insects such as mosquitoes in the spread of infection.[46] The geographical environment may also play a role as silicacious minerals can influence the cytokine profiles within the host.[14,21] In conclusion, further studies are required to elucidate the interactions between *M. leprae*, *Leishmania* spp. and the environment.

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Authors' addresses: Lucretia Acosto Soto, Carretera de Orba a Vall de Laguart, Fontilles, Alicante, Spain, and Universidad Miguel Hernández de Elche, Carretera de Valencia, San Juan, Alicante, Spain, E-mails: lacosta@fontilles.org or lacosta@umh.es. Nelson Caballero, Asociación para el Desarrollo de los Pueblos (ADP), Managua, Nicaragua, E-mail: nelcab64@hotmail.com. Lesney Ruth Fuentes, Head of Leprosy Program, Choluteca, Honduras, E-mail: lesnyr@yahoo.com. Pedro Torrez Muñoz, Jose Ramón Gómez-Echevarria, and Montserrat Pérez López, Fontilles Sanatorium, Carretera de Orba a Vall de Laguart, Fontilles, Alicante, Spain, E-mails: ptorres@fontilles.org, jrgomez@fontilles.org, and montse@perezlopez.com.es. Fernando Jorge Bornay-Llinares, Universidad Miguel Hernández de Elche, Carretera de Valencia, San Juan, Alicante, Spain, E-mail: f.bornay@umh.es. John L. Stanford and Cynthia A. Stanford, Catriona Hargreaves Charitable Trust, Mill House, Claygate, Kent, United Kingdom, E-mails: jandcstanford@btinternet.com and stanfordcynthia@gmail.com. Helen D. Donoghue, Centre for Clinical Microbiology, Division of Infection and Immunity, Royal Free Campus, University College London, London, United Kingdom, E-mail: h.donoghue@ucl.ac.uk.

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