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Case Report: Autochthonous Disseminated Dermal and Visceral Leishmaniasis in an AIDS Patient, Southern Thailand, Caused by *Leishmania siamensis*

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Abstract. We report the first establishment of *in vitro* cultivation and genotypic characterization of *Leishmania siamensis* isolated from an autochthonous disseminated dermal and visceral leishmaniasis in a Thai acquired immunodeficiency syndrome (AIDS) patient. The molecular identification has shown that the parasite was identical to *L. siamensis*, a recently described *Leishmania* species reported in the southern provinces of Thailand. The phylogenetic analysis has confirmed *L. siamensis* as closely related to the zoonotic *Leishmania* species *L. enrietti*.

INTRODUCTION

Recently, a few cases of autochthonous leishmaniasis have been reported from Thailand, especially from the southern provinces. However, these cases were diagnosed by detecting amastigotes in clinical specimens without confirmation of the species of *Leishmania*. In 2008, the first case of visceral leishmaniasis (VL) caused by a new species, *L. siamensis*, was described.[1] This parasite was characterized by molecular methods. In 2010, a second case of *L. siamensis* infection was reported in a human immunodeficiency virus (HIV)-infected patient.[2] Meanwhile, a *Leishmania* strain apparently identical to *L. siamensis* was detected as a causative agent of cutaneous leishmaniasis in cows and a horse in Germany and Switzerland.[3,4] However, attempts to isolate the parasite from these human and animal infections by cultivation on axenic media were unsuccessful. This paper is the description of a disseminated dermal and visceral form in a Thai acquired immunodeficiency syndrome (AIDS) patient. For the first time, the parasite was isolated on culture medium and identified as *L. siamensis* (Trang strain) by molecular methods.

CASE REPORT

A 32-year-old Thai female originally living in Trang, southern Thailand, who never traveled to leishmaniasis-endemic areas, was diagnosed with HIV infection in 2007. She was treated by stavudine (D4T)/lamivudine (3TC)/nevirapine (NVP) at a community hospital in Trang. In June of 2009, she developed skin rash and was referred to Trang Provincial Hospital. The treatment was changed to 3TC/D4T/efavirenz (EFV). She was diagnosed with cryptococcal meningitis two times when antifungal treatments, including amphotericin B and fluconazole, were given. During this period, her anemic condition was detected, and a blood transfusion was given. She refused to have bone marrow aspiration for investigating the cause of anemia. Antiretroviral therapy was changed to 3TC/TDF/liponaviar (LPV)/r and then LASTAVIR(D4T + 3TC)/LPV/r. She gained weight, and the skin rash was reduced. CD4 cell count increased from 32 to 107 cells/μL, and she had increased levels of Hb (10.1 g/dL) and Hct (30.7%). In March of 2010, she was diagnosed with cryptococcal meningitis and anemia. 3TC/D4T/LPV/r and fluconazole were given.

In July 2010, she complained of thick and hard skin nodules covering her body for 1 month. Dermatological examination revealed diffused irregular hard subcutaneous nodules varying in size, especially on her face, trunk, and extremities (Figure 1A and B). The nodules at lower extremities showed diffuse brownish scaly plaques. Skin biopsy was performed and sent to the Department of Pathology, Prince of Songkla Hospital, Songkhla. Pathological examination revealed diffuse dermal and subcutaneous infiltration of mixed inflammatory cells composed predominantly of macrophages with an admixture of lymphocytes and plasma cells. The macrophages contained numerous intracellular amastigotes of *Leishmania* (Figure 1C). Physical examination also revealed severe anemia and generalized hepatomegaly. Bone marrow aspiration and blood collection were performed and sent to the Department of Parasitology, Phramongkutklao College of Medicine, Bangkok. *Leishmania* infection was confirmed. In September 2010, the patient experienced seizure and died 2 weeks before amphotericin B was started.

From a bone marrow aspiration, we established an axenic culture of *L. siamensis* in both Scheneider's medium supplemented with 20% fetal bovine serum (FBS) and Novy-MacNeal-Nicolle (NNN) medium incubated at 25°C. Promastigote forms were observed on day 15 to have morphology similar to other *Leishmania* species, and the strain was then cryopreserved in liquid nitrogen. These promastigotes have been continuously maintained in both Scheneider's medium supplemented with 20% FBS and NNN medium. However, this parasite did not grow well in M199. Establishment of amastigotes in BALB/c mice has been ongoing research. A molecular identification was performed by polymerase chain reaction (PCR) amplification of the ssrRNA locus and the conserved part of the minicircle kinetoplastic DNA from both buffy coat and *in vitro* cultivated parasites.[5,6] The PCR products amplified from the ssrRNA and the minicircle kDNA were 540 bp (Genbank accession number JQ280883) and 117 bp, respectively. The ssrRNA sequence was 100% identical to the unpublished *L. siamensis* sequence (accession number JN885899).[1] Lower identity (99%) of ssrRNA sequences was observed between a pair of *Leishmania*

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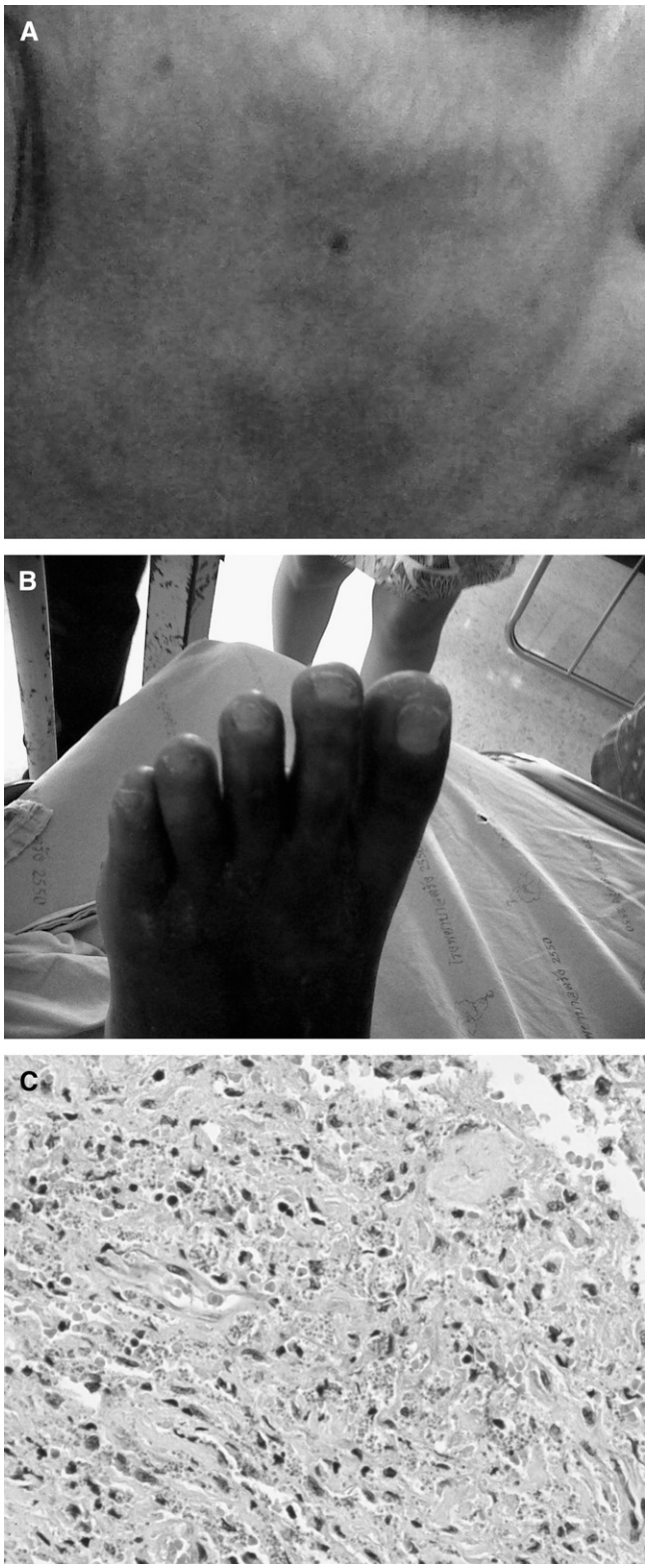

FIGURE 1. Disseminated dermal and visceral leishmaniasis: clinical and histopathological features. (**A**) Diffuse irregular hard surface in the subcutaneous tissue varying in size of large nodules on the face. (**B**) Diffuse brownish scaly plaques at lower extremities. (**C**) Histopathological examination of the skin biopsy revealing numerous intracellular amastigotes of *Leishmania* (hematoxylin–eosin staining).

identified from this study and another 10 *Leishmania* species (*L. amazonensis* [GQ332354], *L. naiffi* [DQ182543], *L. braziliensis* [GQ332355], *L. donovani* [GQ332356], *L. guyanensis* [GQ332358], *L. infantum* [GQ332359], *L. major* [GQ332361], *L. panamensis* [GQ332362], *L. mexicana* [GQ332360], and *L. tropica* [GQ332363]) with different substitution positions.

There were 118 alignable sites of kDNA genes from eight species of studied *Leishmania*. The divergences of kDNA sequences of *Leishmania* isolated in this study were noticeably high. There was about a 74–78% match of kDNA sequences between *Leishmania* from the patient and selected *Leishmania* species, including *L. amazonensis* (EU370875), *L. braziliensis* (EU370882), *L. donovani* (EU370886), *L. infantum* (EU370899), *L. major* (EU370908), *L. tropica* (EU370912), and *L. tarentolae* (AF088235), and only a 63% consensus in the identity of kDNA sequences shown between *Leishmania* identified from the patient and *L. lainsoni* (K01770).

A phylogenetic analysis of the *L. siamensis* (Trang strain) was performed at the French Reference Center for Leishmaniasis (FRCL) by multilocus analysis of three protein coding DNA sequences (locus 03.0980, elongation initiation factor 2 α-subunit [JQ586200]; locus 04.0580, spermidine synthase 1 [JQ586201]; locus 31.2610, RNA polymerase II largest subunit [JQ586202]; they are located on *L. major* chromosomes 3, 4, and 31, respectively). The *L. siamensis* genotype was compared with 17 different *Leishmania* species genotypes representative of the genetic diversity within the *Leishmania* genus (Figure 2). From the 1,680 bp analyzed, 333 informative sites were identified, and the genetic similarity between *L. siamensis* and *L. enrietti*, a zoonotic *Leishmania* species infecting Brazilian guinea pigs, was confirmed. Currently considered as the standard approach for *Leishmania* taxonomy, the isoenzymatic profiling of the Trang strain was performed at FRCL. Unfortunately, some isoenzymes were not detectable, and the others were hardly comparable with the zymodemes described in the other *Leishmania* species, probably because of the genetic divergence of *L. siamensis*.

CONCLUSIONS

This report is the first report of an autochthonous disseminated dermal and visceral leishmaniasis caused by *L. siamensis*. Dermatological manifestations in this patient were similar to those manifestations previously described in AIDS patients who were infected with *L. infantum* and *L. donovani*.[7,8] In this case, the cause of anemia, which might be caused by leishmaniasis, was not properly investigated. Dermal lesions were later developed after amphotericin B was not given. Increased awareness of leishmaniasis, especially for HIV-positive patients, is suggested in the endemic areas. Genetic characterization confirmed that *L. siamensis* is distinct from other reported *Leishmania* spp. Establishment of *in vitro* culture of *L. siamensis* will bring up additional study of important biological parameters of the organism. Our report raises concerns over this emerging disease in Thailand. Epidemiological studies, including disease surveillance, screening of people and animals in the affected areas, and also identification of natural vectors, are urgently needed and essential for prevention and control strategies in Thailand.

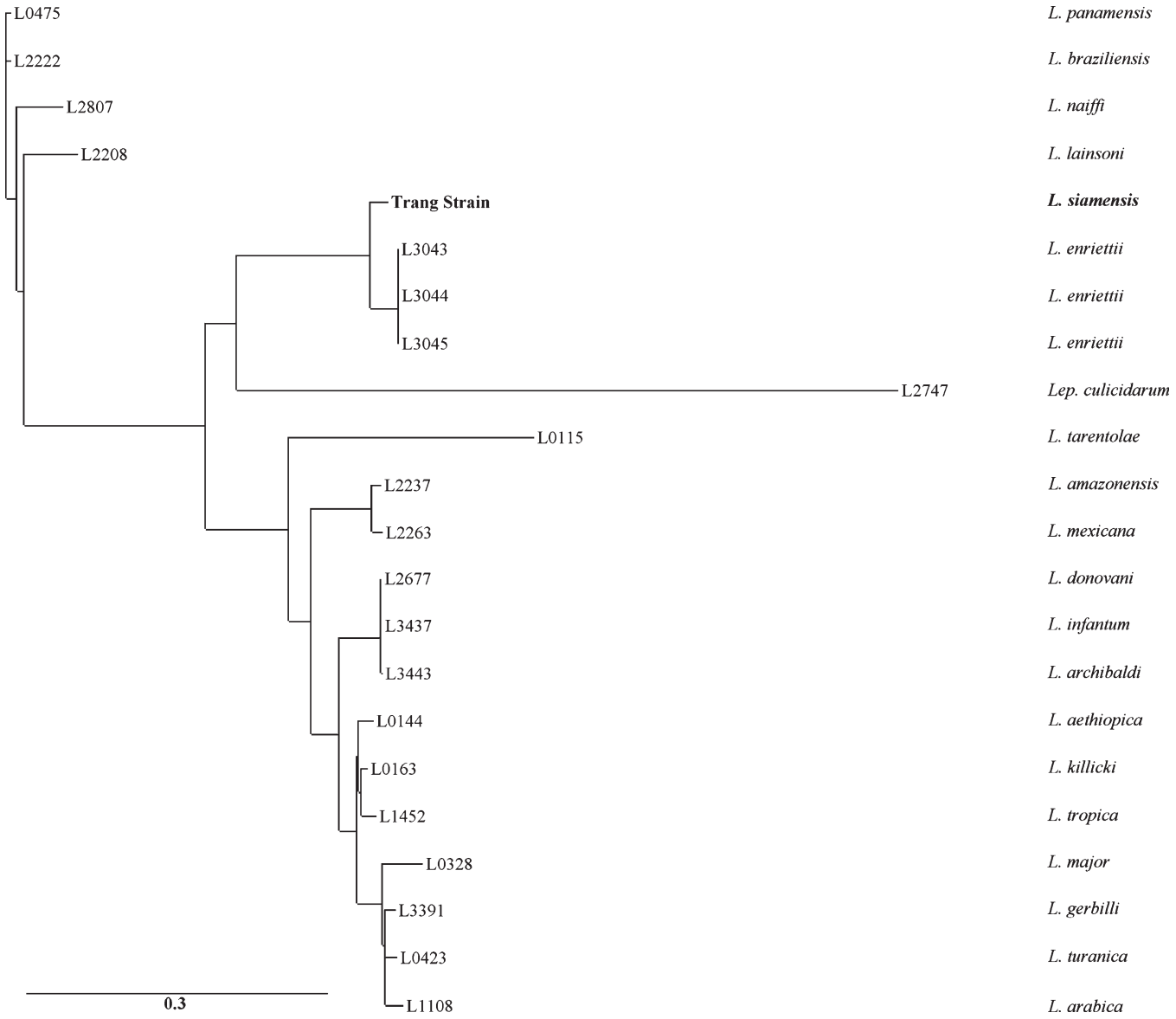

FIGURE 2. Phylogenetic comparison of the Trang strain to 17 other *Leishmania* species. Concatenated sequences of three protein-coding genes (XM_001687340, XM_883450, and XM_001685196; 1,680 nt in all) located on three different chromosomes in the *Leishmania* genome were compared using the maximum likelihood method implemented in PhyML with a GTR + I + G model of nucleotide substitutions selected by the JModelTest. Three distinct and highly supported clusters were distinguishable. Bootstrap values (1,000 replicates) were given at the nodes. A well-individualized cluster included both the Trang strain (*L. siamensis*) and three *L. enrietti* strains, even when the two species were clearly distinct.

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