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Case Report: Autochthonous Visceral Leishmaniasis in a Human Immunodeficiency Virus (HIV)-Infected Patient: The First in Thailand and Review of the Literature

Chusana Suankratay,* Gompol Suwanpimolkul, Henry Wilde, and Padet Siriyasatien

Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; Department of Medicine and Division of Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract. We report a case of visceral leishmaniasis in a human immunodeficiency virus (HIV)-infected 37-year-old Thai fisherman who presented with nephritonephrotic syndrome, fever, anemia, and thrombocytopenia. Bone marrow biopsy revealed many amastigotes within macrophages. Kidney biopsy showed membranoproliferative glomerulonephritis. Polymerase chain reaction (PCR) and nucleotide sequence analysis of the internal transcribed spacer 1 of the small subunit ribosomal RNA gene in blood and kidney biopsy specimens showed *Leishmania* species previously described in a Thai patient with visceral leishmaniasis. Only four autochthonous cases of leishmaniasis have been reported in Thailand since 1996. To the best of our knowledge, this is the first report of autochthonous visceral leishmaniasis in an HIV-infected Thai. With an increasing number of patients with autochthonous leishmaniasis in association with the presence of potential vector, it remains to be determined whether this vector-borne disease will become an emerging infectious disease in Thailand.

Leishmaniasis is a vector-borne infection caused by an obligate intracellular protozoon, *Leishmania* sp., which is transmitted by phlebotomine sandflies.[1–3] It occurs worldwide in tropical and subtropical regions including the Middle East, India, China, Africa, and southern and central America. Thailand is not a known endemic area for leishmaniasis. Most imported cases were reported between 1960 and 1986 in Thai workers returning from the Middle East.[4,5] The first reported indigenous patient with leishmaniasis was a 3-year-old girl living at Suratthani Province of southern Thailand in 1996.[6] Several autochthonous cases with leishmaniasis were recently seen in northern, central, and southern Thailand.[7–9] Interestingly, these patients were from provinces where a potential sandfly vector has never been reported.[10–12] We describe the first report of visceral leishmaniasis in a human immunodeficiency virus (HIV)-infected patient and review all previous reports of autochthonous cases of leishmaniasis in Thailand.

CASE REPORT

A 37-year-old Thai fisherman with known HIV infection presented with progressive leg edema, ascites, and low-grade fever of 8 weeks duration. Seven weeks prior to admission (PTA), he was hospitalized at Chantaburi Provincial Hospital with a diagnosis of nephritonephrotic syndrome (hypertension, edema, heavy proteinuria, microscopic hematuria, azotemia, hypoalbuminemia, and hypercholesterolemia). He was treated with prednisolone 50 mg/day. Two weeks PTA, he had not improved and developed thrombocytopenia (platelet count of 85,000/μL) and anemia (hematocrit decreased from 29% to 23.4%). Bone marrow aspiration and biopsy were performed and revealed decreased cellularity and many "yeast-like" organisms 1–2 μM in size. Fungal cultures of both specimens did not grow any fungi. He was then transferred to King Chulalonkorn Memorial Hospital in Bangkok for further evaluation. The patient was born at Chantaburi, eastern Thailand. He had never been outside Thailand except for having worked as a fisherman in the Indian Ocean and northern Indonesian sea from 1999 to 2001. The HIV was diagnosed at 33 years of age presenting with active tuberculosis. He received a standard 6-month course of therapy comprised of isoniazid, rifampin, pyrazinamide, and ethambutol and also started on stavudine, lamivudine, nevirapine, and cotrimoxazole. His CD4 cell counts increased from 40 to 129 cells/μL and plasma HIV RNA levels became undetectable at 8 weeks PTA. He was also found to have chronic hepatitis C infection and a history of intravenous drug use. He smoked and drank alcohol daily. Physical examination showed a temperature of 38.5°C, moderate pallor, mild hepatomegaly, and moderate pedal edema. Blood complement levels of C3 and C4 were decreased. A kidney biopsy was performed and revealed membranoproliferative glomerulonephritis with focal segmental glomerulosclerosis. However, no organisms were demonstrated on Giemsa's staining in the renal biopsy slides. A review of the histopathology of the bone marrow revealed, amastigotes of *Leishmania* sp. within macrophages and bar-shaped kinetoplast were also seen (Figure 1). The blood and kidney biopsy specimens were sent to the Department of Parasitology, Chulalongkorn University, for identification of the species of *Leishmania*. The samples were then tested for *Leishmania* using the primers specific for 18S ribosomal RNA (rRNA) genes as described by Le Fichoux and others[13] and for species differentiation using the primers specific for internal transcribed spacer1 (ITS1) regions of the rRNA as described by Uliana and others.[14] Both specimens obtained from our patient were positive for *Leishmania*. Nucleotide sequences of the amplified PCR products for the 18S rRNA gene were identical to the sequence of *Leishmania* sp. previously reported in GenBank (GenBank accession no. AF303938). The species of *Leishmania* identified by nucleotide sequences of the amplified PCR products of the ITS1 region of the rRNA gene was identical to the new species of *Leishmania* (GenBank accession no. EF200011) (Figure 2), previously reported from a Thai patient with visceral leishmaniasis by Sukmee and others.[8] Nucleotide sequences of the 18S rRNA gene and the ITS1 region of the rRNA gene of this report were submitted to GenBank under accession nos. GQ226033 and GQ226034. The patient gradually improved clinically along with his bone marrow and renal status after treatment with amphotericin B deoxycholate (2 mg/kg every other day) for 2 weeks. He was discharged on oral itraconazole (400 mg/day). He

*Address correspondence to Chusana Suankratay, Division of Infectious Diseases, Department of Medicine, Chulalongkorn University Hospital, Bangkok, 10330, Thailand. E-mail: chusana.s@chula.ac.th

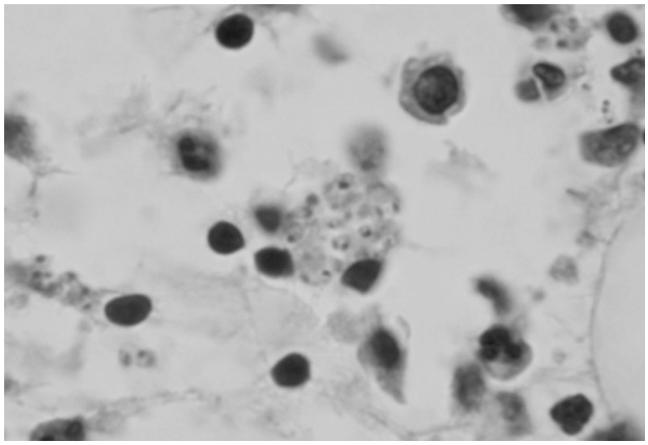

FIGURE 1. *Leishmania* sp. amastigotes are observed within a macrophage in a bone marrow aspirate. Both nucleus and kinetoplast can be seen within some amastigotes.

was doing well when last seen 3 months after diagnosis of leishmaniasis.

CONCLUSIONS

The clinical features of leishmaniasis can be categorized into visceral, cutaneous, and mucocutaneous forms depending on the species of *Leishmania*. Our patient had visceral leishmaniasis characterized by prolonged fever, anemia, thrombocytopenia, hepatomegaly, and nephritonephrotic syndrome. Glomerular involvement is rare in human visceral leishmaniasis.[15–17] It can be associated with or without immune complex-mediated glomerulonephritis including proliferative glomerulonephritis[17] and collapsing focal segmental glomerulosclerosis.[16] Our patient presented with nephritonephrotic syndrome associated with immune-complex-mediated membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis. He gradually improved after treatment with amphotericin B deoxycholate followed by itraconazole. Nephrotic syndrome complicating *Leishmania*/HIV coinfection has been reported in previously.[15]

We believe that our patient acquired the disease in Chantaburi province of Thailand even though he had been in northern Indonesia from 1999 to 2001. The incubation period of visceral leishmaniasis generally varies from 3 to 8 months, but it could be as short as 2 weeks or longer than 1 year.[18] However, the infection may remain asymptomatic in some patients. However, there has never been a report of leishmaniasis from Indonesia. Therefore, ours is the first report of autochthonous visceral leishmaniasis in an HIV-infected patient from Thailand. To the best of our knowledge, there have seen five autochthonous cases of leishmaniasis (including our patient) in Thailand (Table 1).[6–9] However, there are

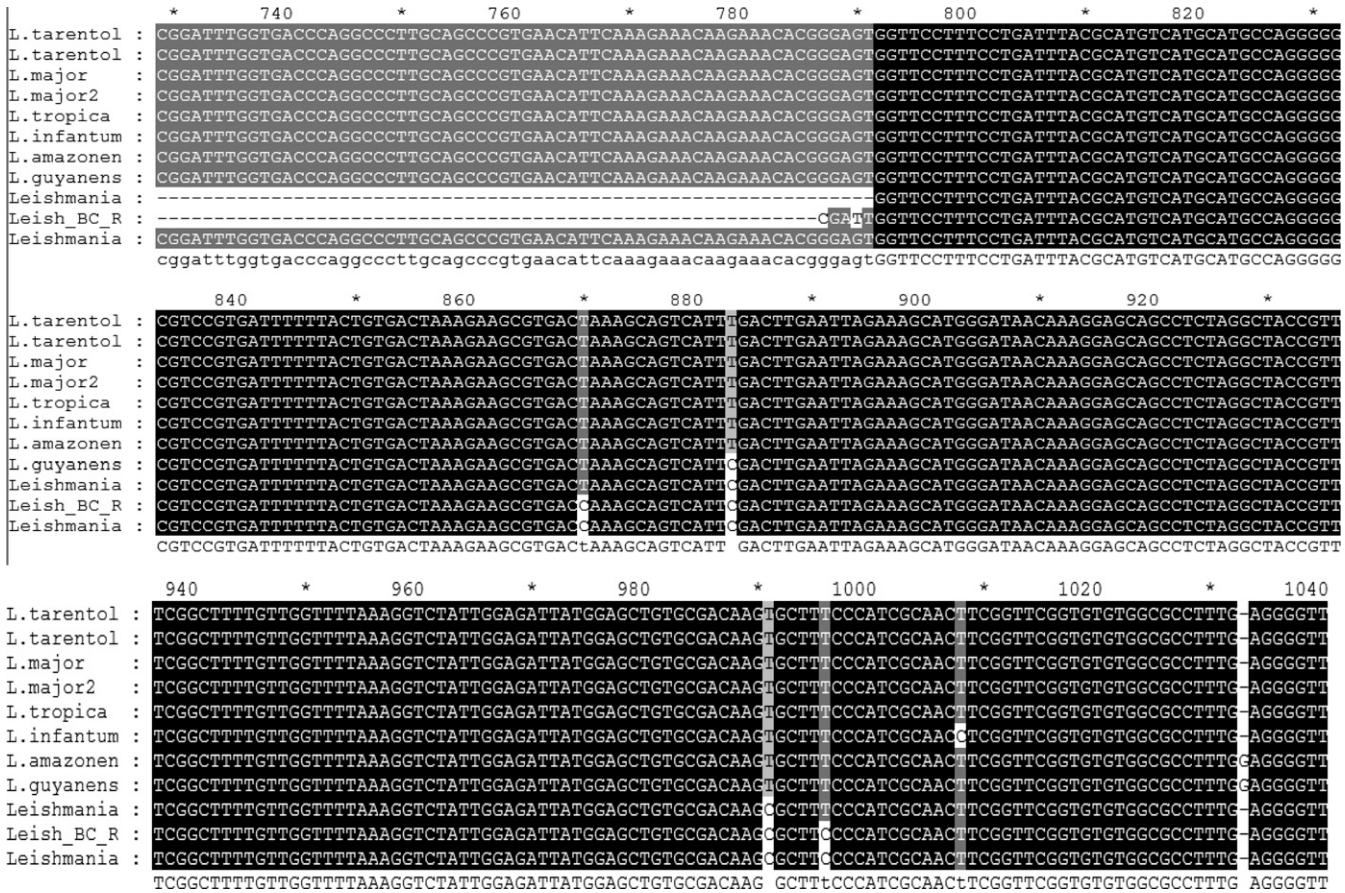

FIGURE 2. The species of *Leishmania* of our patient (Leish_BC_R) identified by nucleotide sequences of the amplified polymerase chain reaction (PCR) products of the ITS1 region of the ribosomal RNA (rRNA) gene, was identical to the new species of *Leishmania* (GenBank accession no. EF200011). Nucleotide sequences of the 18S rRNA gene and the ITS1 region of the rRNA gene were then submitted to GenBank under accession nos. GQ226033 and GQ226034.

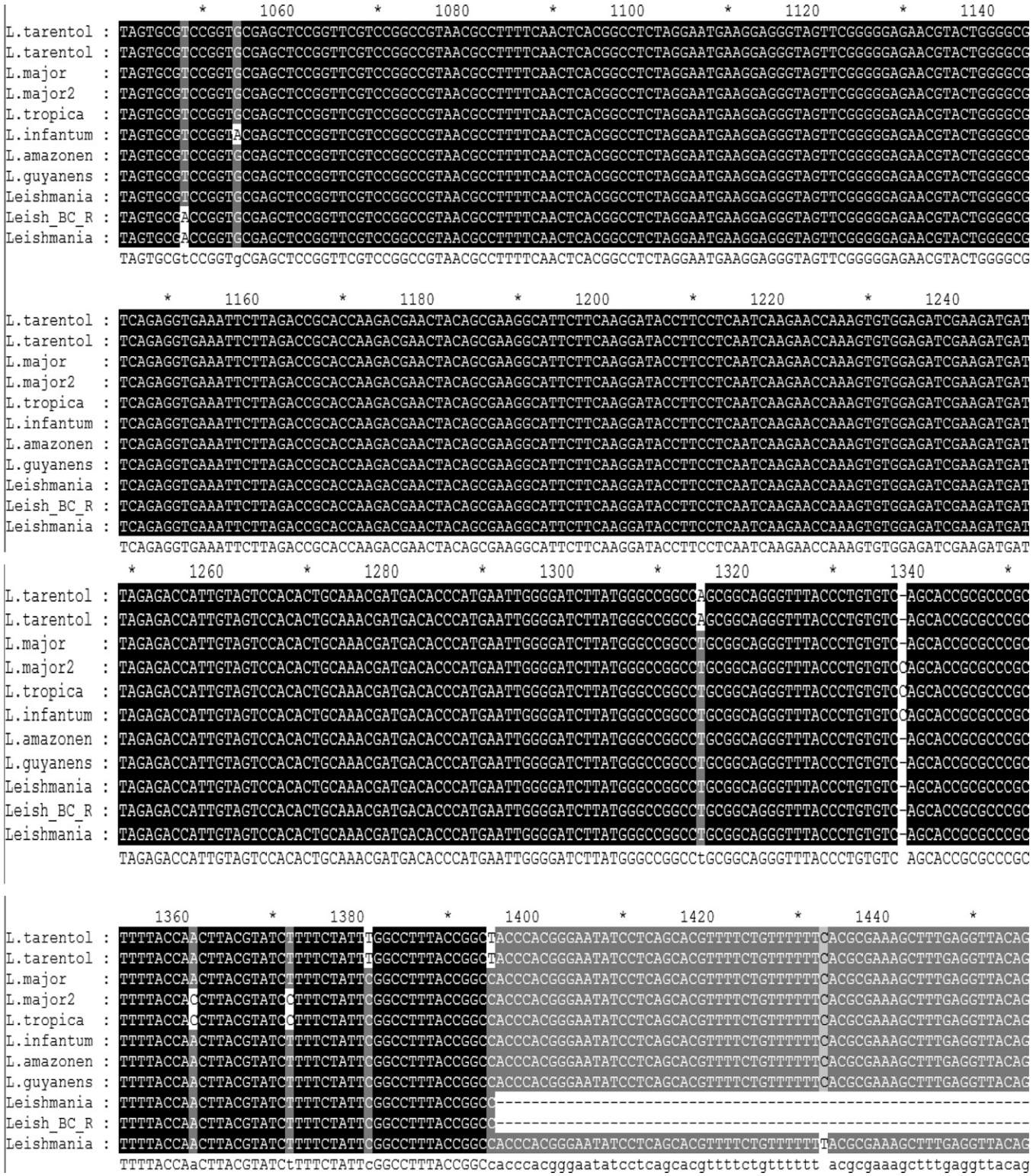

FIGURE 2. Continued

two additional non-reported autochthonous cases with visceral and cutaneous leishmaniasis from Nakorn Sri Thammarat and Chian Rai, respectively (Sukmee T, personal communication). All patients had visceral leishmaniasis, and lived in all parts of the country except for Northeast Thailand. All were males except one 2-year-old girl, and had no HIV infection. The species of *Leishmania* was identified in only three patients as *Leishmania infantum* (one patient from Bangkok) and a new species (one from Nan and one from Chantaburi [our patient]). The nucleotide sequence of the ITS1 region of the rRNA gene of *Leishmania* in our patient is identical to the suspected new species of *Leishmania* previously reported from a

TABLE 1
Summary of five case reports of autochthonous leishmaniasis in Thailand*

Year, province, part of country	Age (years), sex	Occupation	Underlying disease	Clinical features; duration	Investigations			Treatment	Outcome
					Form of leishmaniasis, species of *Leishmania*	Sandfly vectors	Animal reservoirs (DAT)		
1996, Suratthani, South[6]	2, Female	NA	No	Fever, hepatospleno- megaly, anemia, thrombocy topenia; 2 months	VL, no species identified	No study	No study	Pentamidine isethionate for 15 doses	Cured after 2 years of follow-up
2005, Nan, North[7]	40, male	Construction worker in several provinces	Ampheta-mine and opium addiction	Fever, hepatospleno-megaly, pantypope- nia, mediastinal mass; 31 months	VL, no species identified	No potential vectors	Positive in 3 cows and 1 cat	2 courses of ABd for 30 days ABd (100 mg)	Remission
2006, Phangnga, South[8]	55, male	Worker in rubber plantation	No	Fever, hepatospleno-megaly, pancytopenia; 3 years	VL, new species	No potential vectors	Positive in 9 cats	mixed with 1 mg lipid for 14 days	Relapse 2 months after treatment
2007, Bangkok, Center[9]	66, male	Lumber truck driver	Diabetes, hyper-tension	Fever, weight loss, hepatosplenomegaly pancytopenia; 6 months	VL, *L. infantum*	Inability to obtain vectors due to raining	Negative in 9 dogs, 1 cat, 3 rats	ABd every other day for 30 days	Remission
2009, Chantaburi, East (present report)	37, male	Fisherman, a history of travel to North Indonesia	AIDS, chronic HCV infection	Fever, nephritonephrotic syndrome, hepatomegaly, anemia, thrombocytopenia; 8 weeks	VL, new species	No potential vectors	Negative	ABd every other day for 14 days, and itraconazole (400 mg/day)	Remission

*NA = not applicable, ABd = amphotericin B deoxycholate, VL = visceral leishmaniasis, DAT = direct agglutination test for *Leishmania* antibody, AIDS = acquired immunodeficiency syndrome; HCV = hepatitis C virus.

40-year-old construction worker from Nan province in 2005.[8] The phylogenetic tree of the ITS1 sequence of the rRNA gene from our patient is situated as a sister taxon of the clade of *Leishmania brasiliensis* and *Leishmania guyanensis*, which are the causative agents of New World visceral and cutaneous leishmaniasis.[19]

There were no known potential vector sandfly species of *Leishmania* during surveys of sandflies collected in the village of our patients (Table 1), even though phlebotomine sandflies are widely distributed in Thailand.[10–12] Previous studies in Thailand showed the presence of cow-biting sandflies, *Phlebotomus major major* and cow- and bat-biting cave-dwelling sandflies, *Psilocybe argentipes*, which have been shown to be vectors of Old World visceral leishmaniasis.[10–12]

A serologic study for *Leishmania* antibody using the direct agglutination test (DAT) was carried out to identify the animal reservoirs of the disease and published in four reports (Table 1). Positive DAT results in cats and cows were reported in two studies from Nan and Phangnga. The specificity and sensitivity of DAT to detect serum antibody for *Leishmania* have been shown to be very high.[20] Domestic animals are one of the most important reservoirs of leishmaniasis.[21] On the basis of the presence of potential sandfly vectors and animal reservoirs of leishmaniasis in Thailand, it is possible that the disease is transmitted from cows or cats with asymptomatic, subclinical, or viscerocutaneous infection to humans by these vectors.

In conclusion, to the best of our knowledge, this is the first report of autochthonous visceral leishmaniasis in an HIV-infected patient. The patient readily responded to conventional treatment. With an increasing number of patients with autochthonous leishmaniasis in association with the presence of potential vector, it remains to be determined whether this vector-borne disease will become an emerging infectious disease in Thailand.

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Authors' addresses: Chusana Suankratay and Gompol Suwanpimolkul, Division of Infectious Diseases, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand, E-mail: chusana.s@chula.ac.th. Henry Wilde, Department of Medicine and Division of Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Padet Siriyasatien, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

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