Leishmaniasis: An Evolving Public Health Concern in Thailand

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ABSTRACT

Leishmaniasis is a parasitic disease caused by flagellated protozoa of the genus *Leishmania*. It is transmitted by infection from the bite of an infected sandfly. The 3 main clinical forms of the disease are cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL). Prior to 1996, all leishmaniasis cases were infected during the visit to the endemic areas. Thereafter, autochthonous leishmaniasis cases have been reported in Thailand. During 1996 to the present, at least 21 cases of autochthonous leishmaniasis have been confirmed in Thailand. *Leishmania siamensis*, a novel species of *Leishmania*, was suspected of being the causative pathogens in some of those cases, although the data supporting the existence of this new species is limited. Until recently, in-depth investigation using molecular characterization and isoenzyme analysis revealed that a suspected novel species consists of 2 different, but closely related strains: *L. siamensis and L. martiniquensis. L. martiniquensis*, a rare species firstly discovered on Martinique Island, is the cause of leishmaniasis in the majority of cases. Meanwhile, *L. siamensis*, a true novel species first and only reported from Thailand, was confirmed as the cause of leishmaniasis in two autochthonous cases. Two clinical forms (CL and VL) have been observed in both *L. martiniquensis and L. siamensis* infection. DNA of *L. martiniquensis* was found in black rats, suggesting their role as a natural reservoir. The presence of *L. martiniquensis* DNA in two sandfly species (*Sergentomyia gemmea* and *Sergentomyia barraudi*) that are commonly found in affected areas may also suggest their role as potential vectors. Here, we update the status of leishmaniasis in Thailand and its emergence as a potential public health concern.

Keywords: Thailand; Southeast Asia; *Leishmania*; autochthonous leishmaniasis; *Leishmania siamensis; Leishmania martiniquensis* (Siriraj Med J 2017;69: 398-411)

INTRODUCTION

Leishmaniasis is one of the world's neglected diseases caused by obligate intracellular flagellated protozoa in the genus *Leishmania*. It is transmitted by the bite of female Phlebotomine sandflies and over 90 species of this sandfly genus have been shown to transmit *Leishmania*. Approximately 20 species of *Leishmania* are capable of infecting humans.[1,2] This disease is primarily zoonotic which means that mammals such as rodents, hyrax and dogs are major natural reservoirs.[1,2] In some circumstances, this disease can also be anthroponotic which means that humans are a major reservior.[1,2] Clinical presentations of leishmaniasis vary depending on host immunity and the involved *Leishmania* species. The 3 main clinical presentations of the disease are cutaneous (CL), visceral (VL) and mucocutaneous (MCL) leishmaniasis.

Leishmaniases are prevalent in more than 98 countries in the tropics, the subtropics and Mediterranean regions.[1] The disease is often classified as 'Old World' and 'New World', according to the regions of the world where it primarily occurs and the vector presence at those sites.[1,2] Old World defines endemic regions in the Eastern Hemisphere including Asia, Africa and southern Europe.[1,2] New World leishmaniasis occurs mainly in the Western Hemisphere, extending from central to central to south Texas and from Central America and South

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America.[1,2] Prior to 1996, this disease was considered rare in Thailand and all reported cases were imported.[3,4] However, autochthonous leishmaniasis cases in patients with no history of travel outside Thailand have been reported since then.[5-25] Since 2005, a suspected novel species of *Leishmania*, proposed as *L. siamensis*, has been reported as a culprit pathogen in many autochthonous leishmaniasis cases in Thailand.[5,7,11-13,16-18] Those suspicious species were categorized into 2 lineages: TR and PG based on sequence polymorphisms of the ITS1, HSP70 and CYT B loci.[26] Recent data derived from thorough molecular genetic characterization and isoenzyme analysis confirmed that the 2 lineages are actually distinctive species. Specifically, lineage PG is *L. martiniquensis* and lineage TR is the true novel species *L. siamensis*. Both *L. martiniquensis* and *L. siamensis* have recently emerged in Thailand.[26,27] *L. martiniquensis*, a species endemic to Martinique (an island overseas region of France that is located in the Caribbean sea), was identified as the cause of the majority of autochthonous leishmaniasis cases in Thailand.[5,7,11-13,16,17,28,29] However, 2 known cases of autochthonous leishmaniasis cases were found to be caused by *L. siamensis*, a true novel *Leishmania* species first discovered in Thailand.[18,26,27]

The aim of this review was to update the status of leishmaniasis in Thailand and its emergence as a potential public health concern in Thailand. In this article, we focus on *L. martiniquensis* and *L. siamensis* relative to species characterization, clinical presentations and potential vectors as well as animal reservoirs. The retrospective data in this review was derived from published PubMed articles and from reports published by the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand from 1996 until the present.

Classification and biology of *Leishmania*

Leishmania is an obligate intracellular flagellate protozoa in the family Trypanosomatidae, order Kinetoplastida, Genus *Leishmania. Leishmania* has recently been classified into 2 major phylogenetic lineages i.e., *Euleishmania* and *Paraleishmania*, based on thorough molecular data and isoenzyme characterization.[1,30] The lineage Euleishmania is divided into 3 subgenera (*Leishmania, Viannia and Sauroleishmania*) and 1 complex (*L. enrietii* complex).[30-32] A few articles proposed *L. enrietii* complex as an additional *Leishmania* subgenus but this data remains inconclusive.[1,32-34] *Euleishmania* is a major pathogenic lineage that causes human leishmaniasis, in which subgenus *Leishmania* and *Viannia* are the most common groups that infect humans.[1] Subgenus *Leishmania* is the most common group that causes VL; while, subgenus *Viannia* can cause both VL and CL.[1,35] In contrast, *L. enrietti* complex mainly infects guinea pigs, and human infection is zoonotic and is considered rare.[1] Subgenus *Sauroleishmania* is a cause of leishmaniasis in lizards and no human infection has been reported.[1] The lineage *Paraleishmania* comprises 5 species but only *L. colombiensis* was found to infect humans.[1]

Transmission of *Leishmania* occurs when humans are bitten by female sandflies. In human stage, *Leishmania* motile promastigotes enter a human body at the bite site and are phagocytosed by resident macrophages, dendritic cells and neutrophils.[2] The promastigotes multiply intracellularly and then develop into aflagellate amastigotes. In case of CL and MCL, amastigotes are found in resident macrophages in the skin or mucocutaneous lesions. In cases of VL, amastigotes develop in phagocytes of the reticuloendothelial system including: liver, spleen and bone morrow causing hepatomegaly, splenomegaly and bone marrow suppression, respectively. The life cycle is then completed when sandflies receive parasitized cells containing amastigotes during blood feeding on infected humans. Amastigotes then transform into promastigotes in the gut and further migrate to the proboscis of the sandfly. The infection cycle is then repeated when an infected sandfly feeds on a new uninfected human.

Species characterization of emerging leishmania species: identification of *L. martiniquensis* and *L. siamensis*

Leishmania species are indistinguishable based on their morphological appearance; therefore, species discrimination requires molecular genetic identification and isoenzyme characterization.[1] A wide variety of genetic markers including small subunit ribosomal RNA (SSU-rRNA)[36], DNA polymerase a catalytic subunit (POLA)[37], RNA polymerase II largest subunit (RPOIILS)[37], heat shock protein 70 (HSP 70)[38], internal transcribed spacer 1 (ITS1)[36,39,40] and cytochrome b (CYT B)[41] have been used for species identification and phylogenetic diversity determination of *Leishmania*. Comparison of SSU-rRNA gene sequence has been used to effectively distinguish phylogenetic relationships among the genera of Kinetoplastida. However, and due to low interspecies variability within *Leishmania* spp., species discrimination based on SSU-rRNA gene sequence comparison is indecisive.[26] As such, other methods of genetic sequence analysis should be employed to facilitate decisive species taxonomic identification. Many studies have shown that multiple molecular sequence comparison produces reliable data that is capable of resolving questions relating to *Leishmania* taxonomic and phylogenetic diversity.

In 2005, a novel species of *Leishmania* was suspected

in an autochthonous leishmaniasis case that occurred in Phang Nga Province, Thailand, based on ITS1 and mini-exon rRNA sequence analysis.[17] Thereafter, many autochthonous leishmaniasis cases that were suspected of being caused by a new species of *Leishmania* were reported.[5,7,11,13,16,17] The baseline characteristics of autochthonous leishmaniasis cases reported from Thailand and Myanmar from 1996 onward are summarized in Table 1. The new suspected *Leishmania* species was proposed as *L. siamensis*, but at that time its taxonomy was still inconclusive. Lack of a conclusive determination regarding the given name and existence of this proposed new species was an ongoing source of confusion among scientists and clinicians. The ongoing confusion in the terminology of *L. siamensis* had occurred for a period of time. However, recent studies by *Leelayoova* et. al., shed light on taxonomic classification of this novel species.[26,27]

In those studies, species identification was performed by phylogenetic analysis of 4 genetic loci (SSU-rRNA, ITS1, CYT B and HSP70) as well as by isoenzyme analysis.[26,27] In phylogenetic analysis, all suspected novel *Leishmania* species, previously reported as *L. siamensis* were grouped together in the same clade as other *Leishmania* species in the division *Euleishmania*, subgenus *Leishmania* based on their SSU-rRNA sequence.[26,27] Phylogenetic tree analysis using Neighbor Joining method of SSU-rRNA sequences of all suspected novel *Leishmania* species were 100% identical as published previously.[26,27] Phylogenetic tree construction based on ITS1 region showed that most novel species reported were grouped into the same taxa as *L. martiniquensis*.[26,27] However, 2 strains previously reported as (JX195640) and *L. siamensis* (KX347438) cases after TR formed a separate branch from the others which indicated a specific strain. (Fig 1A)[26,27] HSP70 gene also defined *L. siamensis* strain to be distinct from the others as previously published.[26,27] The data showed that only 2 reported cases were truly *L. siamensis* (*L. siamensis* lineage TR [JX195640] and *L. siamensis* [KX347438]), but most of the other strains were actually *L. martiniquensis* based on genetic sequence analysis (Fig 1A&B). The novel *L. siamensis* (lineage TR) strain was found to be closely related to *L. enrietti* as shown in Fig 1B. The Genbank accession numbers and the names of both suspected and confirmed *Leishmania* species from reported studies are shown in Table 2. Isoenzyme analysis also confirmed the differences between those reported strains. A majority of those strains were in zymodeme MON-229, similar to *L. martiniquensis* reference strain.[26,27] In contrast, a true *L. siamensis* (previously *L. siamensis* lineage TR) was grouped in zymodeme MON-324 which is different from the isoenzyme profiles of *L. martiniquensis*.[26,27] In summary, the presence of a new *Leishmania* species, namely *L. siamensis* was confirmed in Thailand. However, most of the suspected novel Leishmaniasis cases were caused by *L. martiniquensis*.

Reported autochthonous leishmaniasis cases in Thailand

During 1960 to 1996, all reported leishmaniasis cases in Thailand were imported, with infections occurring during visits to endemic areas outside Thailand. The first autochthonous case of visceral leishmaniasis in Thailand, caused by an unknown *Leishmania* species, occurred in 1996, but was reported in the literature in 1999.[19] Since that time, sporadic cases of autochthonous leishmaniasis have been reported.[5-25] In 2005, a suspected new species of *Leishmania* was identified as a cause of autochthonous leishmaniasis in a man living in Phang Nga Province which is located in the south of Thailand.[17] Later, the causative species in that infection was confirmed to be *L. martiniquensis*.[26]

To date, at least 21 cases of autochthonous leishmaniasis have been reported from across Thailand.[5-20,23-25] All of those patients had no history of traveling abroad or visiting disease endemic areas. A majority of those patients lived in the southern region of Thailand at the time of their infection. Regions from which autochthonous leishmaniasis has been reported are shown in Fig 2. The mean age of these reported cases was 40.7±19.8 years (ranged: 3-81) and 13 (62%) and 8 (38%) cases were male and female, respectively. Twelve (57.1%), 5 (23.8%) and 4 (19%) cases lived in the southern, northern and central regions of Thailand, respectively. Ten (47.6%) patients had coinfection with HIV with a wide range of absolute CD4 cell count (range: <50 to 543 cells/mm^3. Sixteen out of 21 (76.2%) cases presented with visceral leishmaniasis, 4 (19%) cases had cutaneous leishmaniasis and 1 (4.8%) case was asymptomatic. Regarding the species that caused infection, 10 (47.6%) cases were infected with *L. martiniquensis*, 3 (14.3%) cases with *L. donovani*, 2 (9.5%) cases with *L. siamensis* and 1 (4.8%) case with *L. infantum* and 4 (23.8%) cases in which the species was unknown. Clinical presentations and baseline characteristics of reported autochthonous leishmaniasis cases are summarized in Table 1.

In addition, three leishmaniasis cases from Myanmar that were visiting Thailand to seek medical care or to accompany family members were diagnosed with *L. martiniquensis* infection. Those patients were moved to Thailand before symptoms developed and they had no history of traveling outside Thailand or Myanmar. The baseline characteristics of reported Burmese cases are also included in Table 1.

TABLE 1. Baseline characteristics and clinical manifestations of autochthonous leishmaniasis cases reported in Thailand and Myanmar from 1996 to present.

Case no.	Year	Location	Age (yr)	Sex	Occupation	HIV status (CD4 cells/mm^3)	Other underlying conditions	Clinical classification	Confirmed species	Laboratory investigation				References
										Microscopy (presence of amastigotes)	PCR positive in	Culture	DAT or IFA	
1	1996	Surat Thani	3	F	Student	Neg		VL	N/A	BM	BM	N/A	IFA 1:16,384	19, 20, 21
2	2005	Nan	40	M	Construction worker	Neg	Amphetamine, alcohol, and opium addict	VL	*L. donovani*	BM, skin nodule biopsy	BM	N/A	N/A	20, 21, 25
3	2005	Phang Nga	55	M	Rubber planter	Neg	-	VL	*L. martiniquensis**	BM	BM, blood	N/A	DAT 1:200¶	17, 20, 21
4	2006	Songkla	62	F	N/A	Neg	AIHA	VL	N/A	BM	N/A	N/A	N/A	20
5	2007	Nakhon Si Thammarat	44	M	Rubber planter	Neg	DM type 2 with retinopathy	VL	*L. donovani*	BM, LN	BM	N/A	N/A	20, 21
6	2007	Songkla	81	M	Shaman	Pos	-	VL	*L. donovani*	BM	BM	N/A	N/A	20, 21
7	2007	Bangkok	66	M	Retired officer	Neg	DM type 2, HT	VL	*L. infantum*	BM	BM	N/A	DAT 1:3,200	9, 20, 21
8	2008	Chaing Rai	45	M	Laborer	Pos (<50)	-	VL, DCL	*L. martiniquensis**	Skin biopsy	Skin biopsy, blood, saliva	Blood -, skin biopsy -, saliva -. and urine -	N/A	13
9	2008	Chantaburi	37	M	Fisherman	Pos (129)	TB, HCV, IVDU	VL	*L. martiniquensis**	BM, kidney biopsy	BM, kidney biopsy		DAT >1:1,600	16, 21
10	2008	Songkla	52	F	N/A	N/A	-	CL	N/A	Skin lesion	N/A	N/A	N/A	20
11	2009	Songkla	46	M	Rubber planter	Pos (175)	Evans syndrome	VL, CL	*L. martiniquensis**	BM, LN, blood smear	BM, LN, blood, buffy coat, saliva, urine	BM +, blood +, ulcer discharge -	N/A	7, 12, 13, 15, 20
12	2010	Satun	5	F	Student	Neg	β-thalassemia trait	VL	*L. martiniquensis**	BM, liver	BM, liver	N/A	DAT 1:3,200	11, 20
13	2010	Trang	32	F	Teacher	Pos (107)	-	VL, DCL	*L. siamensis*	BM, skin biopsy	Buffy coat, culture	BM +	N/A	5, 23

TABLE 1. Baseline characteristics and clinical manifestations of autochthonous leishmaniasis cases reported in Thailand and Myanmar from 1996 to present.

Case no.	Year	Location	Age (yr)	Sex	Occupation	HIV status (CD4 cells/mm^3)	Other underlying conditions	Clinical classification	Confirmed species	Laboratory investigation				
										Microscopy (presence of amastigotes)	PCR positive in	Culture	DAT or IFA	References
14	2011	Trang	30	M	Pet store owner	Pos (111)	-	VL, DCL	*L. martiniquensis**	BM, skin biopsy, blood smear	BM, blood, buffy coat, saliva, urine, skin biopsy	BM +	N/A	7, 13, 15
15	2012	Lamphun	52	M	Farmer	Neg	-	VL	*L. martiniquensis**	BM	BM	BM +	N/A	6, 14
16	2012	Chaing Mai	48	M	Industrial worker	Pos (121)	-	VL, DCL	*L. martiniquensis*	BM, skin nodule	BM, skin biopsy	Skin biopsy +, BM +	N/A	6
17	2012	Lamphun	38	M	Lumberjack	Pos (543)	-	VL, DCL	*L. martiniquensis*	BM, skin biopsy	BM, skin biopsy BM +	Skin biopsy +,	N/A	6
18	2012	Lopburi	3	F	Student	Neg	-	CL	N/A	Skin biopsy	N/A	N/A	N/A	8
19	2013	Songkla	28	F	N/A	Pos (617)	-	asymptomatic	*L. martiniquensis*	N/A	Saliva	N/A	N/A	15
20	2013	Nakhon Si Thammarat	45	F	Cook	N/A	-	CL	N/A	Skin biopsy	N/A	N/A	N/A	24
21	2015	Kanchanaburi	42	F	Housewife	Pos (89)	TB, PCP	DCL	*L. siamensis*	Skin biopsy	Skin biopsy	N/A	N/A	18
22	2011	Yangon, Myanmar	34	M	N/A	Pos	-	DCL	*L. martiniquensis**	Skin biopsy	Skin biopsy, blood, buffy coat, saliva	N/A	N/A	13
23	2011	Yangon, Myanmar	22	F	N/A	Neg	-	Asymptomatic	*L. martiniquensis**	N/A	Buffy coat, saliva	N/A	N/A	13
24	2013	Yangon, Myanmar	60	M	N/A	Neg	Prednisolone used for 2 months, DM	DCL	*L. martiniquensis**	Skin biopsy	Skin biopsy, blood, buffy coat, saliva, urine	N/A	N/A	10, 13

*Previously reported as *L. siamensis* or suspected new species, ¶Level post-treatment.

Abbreviations: HIV, human immunodeficiency virus; PCR, polymerase chain reaction; DAT, direct agglutination test; IFA, indirect immunofluorescence assay; N/A, not available; AIHA, autoimmune hemolytic anemia; DM type 2, diabetes mellitus type 2; HT, hypertension; TB, tuberculosis; HCV, hepatitis C virus; IVDU, intravenous drug use; PCP, pneumocystis carinii pneumonia; VL, visceral leishmaniasis; DCL, diffuse cutaneous leishmaniasis; CL, cutaneous leishmaniasis; BM, bone marrow; LN, lymph node

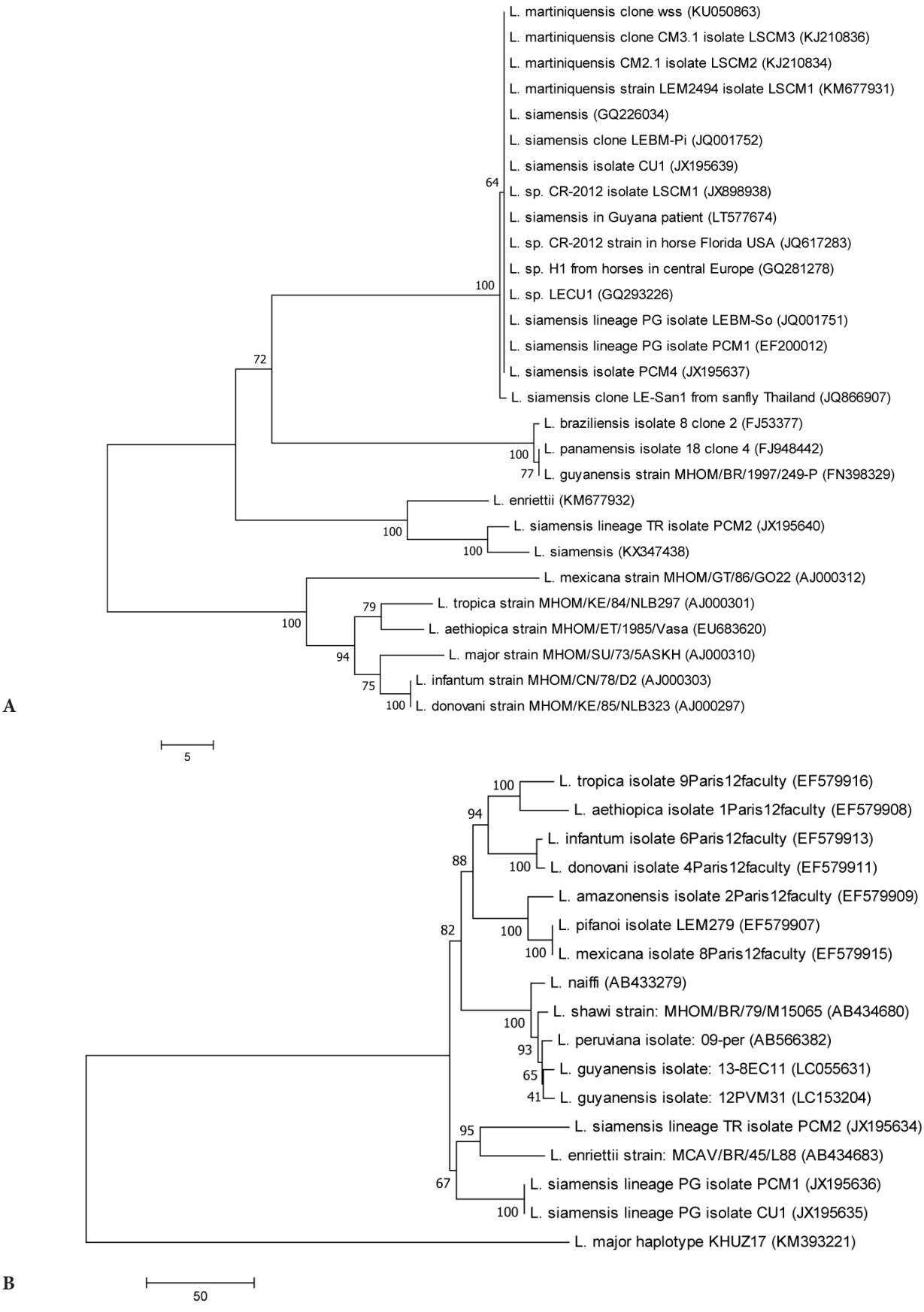

Fig 1. Unrooted phylogenetic trees were derived from 2 genetic loci [ITS1 (A) and CYT B (B)] using the Neighbor Joining Method.[66] The percentage of replicate trees in which the associated taxa clustered together during the bootstrap test (1,000 replicates) is shown next to the braches.[67] The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic trees. The evolutionary distances were computed using the number of differences method[68], and are in the units of the number of differences per sequence. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and/or missing data were eliminated. Evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 7.[69]

TABLE 2. Genbank accession numbers of suspected novel Leishmania species from autochthonous leishmaniasis cases reported from Thailand and Myanmar. The names of both suspected and confirmed species are shown.

Case no.	Year (yr)	Location	Age	SSU-rRNA	ITS1	HSP70	CYT B	RPOIILS	Suspected species	Confirmed species	References
3	2005	Phang Nga	55	JN885899	EF200012	N/A	JX195636	N/A	Suspected a novel species (*L. siamensis* lineage PG, isolate PCM1)	*L. martiniquensis**	17, 20, 21, 26
8	2008	Chiang Rai	45	N/A	N/A	N/A	N/A	N/A	Suspected *L. siamensis*	*L. martiniquensis**	13
9	2008	Chantaburi	37	N/A	GQ226034	N/A	N/A	N/A	Suspected a novel species (*L. siamensis*, isolate CU)	*L. martiniquensis**	16, 21
11	2009	Songkla	46	JX195633	JX195639 JQ001751 KU050860-KU050862 (saliva, buffy coat, and tissue)	KC202883	JX195635	N/A	Suspected a novel species (*L. siamensis*, lineage PG, isolate CU1/LEBM-So)	*L. martiniquensis**	7, 12, 13, 15, 20, 26
12	2010	Satun	5	JN087497	JX195637	KC202882	N/A	N/A	Suspected *L. siamensis* (*L. siamensis* lineage PG, isolate PCM4)	*L. martiniquensis**	11, 20
13	2010	Trang	32	JQ280883	JX195640	KC202880	JX195634	JQ586202	Suspected *L. siamensis* (*L. siamensis* lineage TR, isolate PCM2)	*L. siamensis*	5, 23
14	2011	Trang	30	N/A	JQ001752 KU050856-KU050859 (blood, buffy coat, and tissue) KF227887-KF227892 (bone marrow, blood, saliva, urine, and tissue biopsy)	N/A	N/A	N/A	Suspected a novel species (*L. siamensis*, lineage PG, isolate LEBM-Pi/PCM5)	*L. martiniquensis**	7, 13, 15, 26

15	2012	Lamphun	52	N/A	JX898938	KP244366	N/A	KM677933	*L. martiniquensis*	*L. martiniquensis* (LSCM1)	6, 14
16	2012	Chiang Mai	48	N/A	KJ210834 KJ210835	KP244367	N/A	KM820665	*L. martiniquensis*	*L. martiniquensis* (LSCM2)	6
15	2012	Lamphun	38	N/A	KJ210836 KJ210837	KP244368	N/A	KM820666	*L. martiniquensis*	*L. martiniquensis* (LSCM3)	6
19	2013	Songkla	28	N/A	KU050863 (saliva)	N/A	N/A	N/A	*L. martiniquensis*	*L. martiniquensis*	15
21	2015	Kanchanaburi	42	N/A	KX347438	N/A	N/A	KX347439	*L. siamensis*	*L. siamensis*	18
22	2011	Yangon, Myanmar	34	N/A	N/A	N/A	N/A	N/A	Suspected *L. siamensis*	*L. martiniquensis**	13
23	2011	Yangon, Myanmar	22	*Identical to GQ226033 (saliva and buffy coat)	N/A	N/A	N/A	N/A	Suspected *L. siamensis* (*L. siamensis*, isolate CU)	*L. martiniquensis**	13
24	2013	Yangon, Myanmar	60	N/A	*Identical to JQ001751	N/A	N/A	N/A	Suspected *L. siamensis* (*L. siamensis*, isolate LEBM-So)	*L. martiniquensis**	10, 13

*Sequence: 100% identical to the sequence previously reported (not submitted as a new accession number)

Abbreviations: SSU-rRNA, small subunit ribosomal ribonucleic acid; ITS1, internal transcribed spacer 1; HSP70, heat shock protein 70; CYT B, cytochrome B; RPOIILS, RNA polymerase II largest subunit; N/A, not available

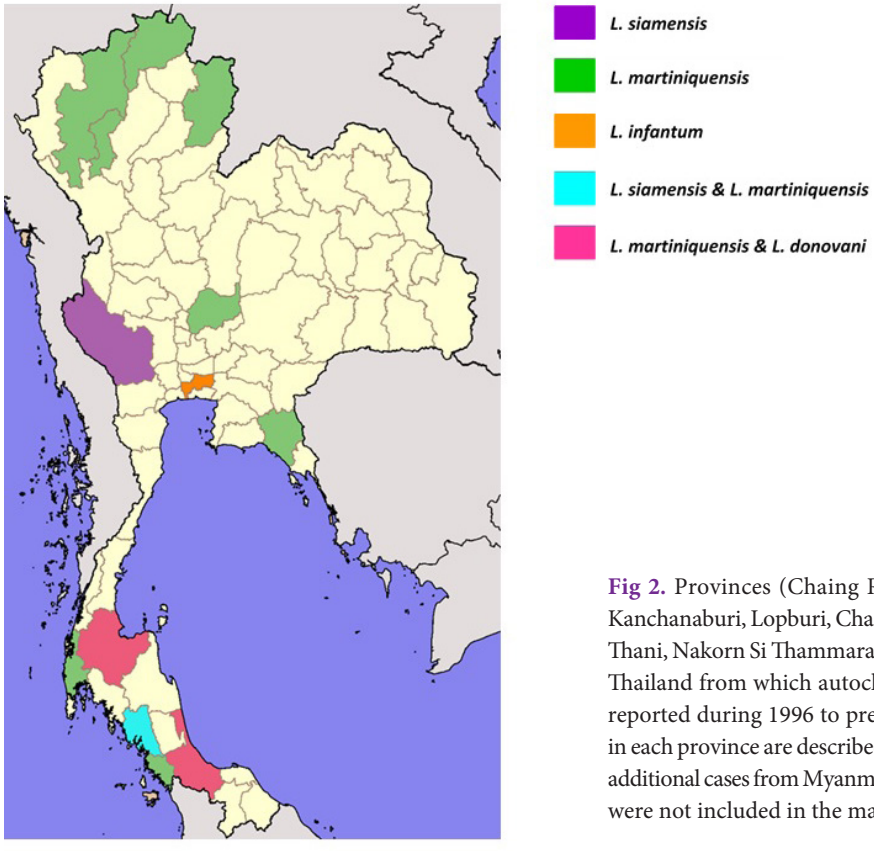

Fig 2. Provinces (Chaing Rai, Chaing Mai, Lumphun, Nan, Kanchanaburi, Lopburi, Chantaburi, Bangkok, Phang Nga, Surat Thani, Nakorn Si Thammarat, Trang, Satun and Songkla) within Thailand from which autochthonous leishmaniasis cases were reported during 1996 to present. Species of Leishmania found in each province are described and differentiated by color. (Three additional cases from Myanmar which were diagnosed in Thailand were not included in the map.)

L. martiniquensis is a major cause of autochthonous leishmaniasis in Thailand. *L. martiniquensis* is a rare *Leishmania* species that was originally discovered on Martinique Island.[28,29] Human cases of *L. martiniquensis* infection have been only sporadically reported from locations that include Martinique Island, Thailand and Myanmar.[26,28,29] In addition to being reported as a cause of human leishmaniasis, *L. martiniquensis* was found to cause CL in horses in Germany and Switzerland[42], in bovines in Switzerland[43] and a horse in the United States.[44] Moreover, *L. martiniquensis* is closely related to *L. enrietti* complex which mainly infects domestic guinea pigs, suggesting its zoonotic nature.[1,26,27]

L. siamensis is a novel *Leishmania* species that was first discovered in Thailand.[17,18] Only two cases of confirmed *L. siamensis* cases have been reported in the world and both were reported in Thailand.[17,18] Both cases had HIV co-infection with absolute CD4 count < 200 cells/mm^3 (Table 1). The first case developed VL[17] and the second case had DCL.[18] In their January 2017 review, *Leelayoova* et al., mentioned their preliminary unpublished finding that asymptomatic *L. siamensis* infection was observed in both immunocompetent and immunocompromised individuals.[27] No animal infection by *L. martiniquensis* has been reported. Overall, little is known about this novel *Leishmania* species.

L. donovani and *L. infantum* are species that commonly cause VL in India, Bangladesh, Nepal and Sudan. A few autochthonous cases caused by these 2 species have also been reported from Thailand (Table 1).[9,20,21,25] These two species are closely related members of the genus *Leishmania*. Given that both species are established, detailed information relating to their biology, taxonomy classification, epidemiology, and clinical manifestations are not mentioned in this review.

Diagnosis of leishmaniasis relies on clinical presentation and laboratory findings. Laboratory diagnosis of leishmaniasis is based on identification of *Leishmania* amastigotes in smear or tissues, by *in vitro* parasite cultivation, by molecular detection of parasite DNA and/or by serology testing of parasite antibody.[2,45,46] Definite diagnosis is confirmed when direct evidence of the presence of parasites, parasite DNA or positive culture is detected.[45,46] Serology testing such as direct agglutination test (DAT), recombinant K39 antigen based immunochromatographic test, immunofluorescence antibody assay (IFA) and enzyme-linked immunosorbent assays (ELISAs) can be used to support a diagnosis, but the sensitivity of the test depends on host immune status.[46-48] In immunocompromised patients such as patients with HIV, test sensitivity is diminished and false-negative results are often observed.[47,48] However, parasitic loads in immunocompromised patients are

usually high which increases the sensitivity of culture and molecular detection.[47,48] Accordingly, appropriate laboratory investigations should be employed and results should be interpreted taking all of the aforementioned factors into consideration before definite diagnosis is established.

Clinical manifestion of *L. martiniquensis* and *L. siamensis* infection

Various clinical presentations including asymptomatic, CL and VL, have been observed in individuals infected with *L. martiniquensis* or *L. siamensis* and these presentations are clinically similar to those observed in *L. donovani* and *L. infantum* infection. In disease endemic areas, the majority of *Leishmania* infected individuals are asymptomatic, especially in people with intact cell-mediated immunity.[48] Immunity defects such as children aged < 5 years, poor nutritional status, HIV co-infection and prolonged immunosuppressive drug use increases the susceptibility of an individual to develop symptomatic leishmaniasis.[48,49]

Of 13 reported cases of *L. martiniquensis* (10 Thai and 3 Burmese), two were symptomatic and 11 developed various clinical symptoms including CL and VL. Nine of 11 of symptomatic cases had VL. The main clinical symptoms found in VL cases included hepatomegaly (66.7%), splenomegaly (66.7%), prolonged fever (55.6%), and weight loss (33.3%). Bleeding tendency was also observed which was associated with thrombocytopenia. Abnormal laboratory findings in the VL group included anemia (100%), thrombocytopenia (88.9%), leukopenia (55.6%), pancytopenia (44.4%), elevated SGOT/SGPT or abnormal alkaline phosphatase (44.4%), hypoalbuminemia (44.4%) and hypergammaglobulinemia (44.4%).

Interestingly, 8 of 13 *L. martiniquensis* cases were co-infected with HIV. Most HIV infected individuals had absolute CD4 < 200 cells/mm^3. Of the 8 HIV infected patients, 6 (75%) cases developed VL. Remarkably, 5 of the 6 HIV infected patients who developed VL had at least one cutaneous lesion. In contrast, all non-HIV infected patients presented with or developed VL without skin lesions or disseminated CL without visceral organ involvement. These finding suggests that clinical presentations of *L. martiniquensis* infection are influenced by host immunity.

Cutaneous manifestations in reported *L. martiniquensis* and *L. siamensis* infection have ranged from single localized to diffuse cutaneous lesions. Skin manifestations vary from erythematous papules, nodules, plaques and ulcers. All lesions were chronic, discrete and painless, resembling lepromatous leprosy, but no neurological invasion was involved. Lesion distribution ranged from primary lesion at the exposed area to widely disseminated lesions throughout the body. Most reported CL cases had multiple diffuse cutaneous lesions, similar to what is typically observed in cases of New World leishmaniasis. However, a few Old World *Leishmania* species, (e.g., *L. aethiopica*) can also cause DCL. This condition is associated with impaired cell-medicated immunity which facilitates the dissemination of the parasite in subcutaneous tissues. Association between host immunity and cutaneous presentations was also observed in the reported cases because DCL was primarily found in HIV infected individuals. Skin lesions found in *L. martiniquensis* and *L. siamensis* reported cases are described in Table 3.

Potential vectors and reservoirs

The only confirmed vector of leishmaniasis is a female Phlebotomine sandfly. There are more than 800 known sandfly species, with 464 and 372 species discovered in the New World and Old World, respectively.[1] Not all sandfly species are thought to be capable of transmitting the disease, as only about 30 sandfly species are proven vectors of human leishmaniasis.[50,51] Old World sandflies include three genera (*Phlebotomus, Sergentomyia* and *Chinius*) and New World sandflies include three genera (*Lutzomyia, Warileya* and *Brumptomyia*).[1] Genus *Phlebotomus* and *Sergentomyia* are the major vectors of Old World; while, genus *Lutzomyia* is the primary New World vector.[1,50] Sandflies are mostly nocturnal, because they are not able to withstand dehydration.[50] In the New World, they are often found in caves and tree buttress roots while New World sandflies inhabit the contaminated soils of farm animal shelters, termite nests, rodent burrows and land under houses inhabited by humans.[50] They are short distance flyers, flying an average of 1.5 km per day.[1,50] Surveillance studies of sandfly species in Thailand were performed at various time points which showed that all 3 genera of Old World sandflies exist (*Phlebotomus, Sergentomyia* and *Chinius*; and some articles added *Idiophlebotomus* to the list as an additional genus, but currently *Idiophlebotomus* is a subgenus of genus *Phlebotomus*), with approximately 27 species being identified in the southern, central, northern and western regions of Thailand.[52-65] *S. gemmea* was the most common species found in the northern and southern regions[58,59,65]; whereas, *S. hodgsoni, S. anodontis* and *N. vietnamensis* were prominent species found in certain specific areas.[52,55,64] Although many sandfly species have been detected in Thailand, not all species have been proven to be vectors for leishmaniasis. Only one study reported the presence of *Leishmania* DNA in

TABLE 3. Clinical manifestations of skin lesions observed in patients infected with either *L. martiniquensis or L. siamensis*

Case no.	HIV status	Clinical classification	Characteristics of cutaneous lesions	Lesion distribution	References
8	Pos	VL, DCL	Lumpy skin lesions	ND	13
11	Pos	VL, CL	Single painless well-defined punched-out ulcer surrounded by erythematous plaque with serous oozing and granulation tissue on top (3 x 3 cm in size)	Left knee	7, 12, 13, 15
14	Pos	VL, DCL	Generalized painless discrete well-defined dusky red infiltrative papules and plaques with ulcers Punched-out ulcer surrounded by erythematous plaque with purulent discharge and granulation tissue on top (2 x 2 cm in size)	Torso, face, and extremities Right leg	7, 13, 15
16	Pos	VL, DCL	Hyperpigmented nodules	Face, both elbows, and later extended to both hips and legs	6
			Multiple discrete firm hypopigmented and brownish papules and nodules	Inner canthi, eyelids, nose, helices/antihelices of pinnae, and extensor surfaces of hands, forearms (prominence over knuckles, elbows, ulnar ridges, knees, tibial crests, malleoli, buttocks, palm, and soles)	
17	Pos	VL, DCL	Multiple discrete hypopigmented papules and nodules	Inner and outer canthi of eyes, helices/antihelices of both pinnae and extensor surfaces of hands, forearms, and legs (prominence on knuckles, elbows, ulnar ridges, knees, tibial crests, and malleoli)	6
			Multiple hypopigmented sclerotic plaques	Palms	
21	Pos	DCL	Multiple painless well-defined non-ulcerated erythematous to indurated hyperpigmented plaques and nodules varying in size; some atrophic hyperpigmented plaques; and, a few scattered erythematous macules and patches	Face and both legs Face Torso	18
22	Pos	DCL	Multiple umbilicated erythematous papules	Neck, arms, and chest wall	13
24	Neg	DCL	Multiple erythematous skinny infiltrative erythematous plaques and nodules with some developed ulcers	Face, torso, and all extremities	10, 13

Cases 8, 11, 14, 16, 17, 22, and 24 were infected with *L. martiniquensis* and case 21 was infected with *L. siamensis*.

Abbreviations: HIV, human immunodeficiency virus; ND, not described; VL, visceral leishmaniasis; DCL, diffuse cutaneous leishmaniasis

captured sandflies.[58] *Leishmania* ITS1 and HSP70 genes, similar to nucleotide sequences of *L. martiniquensis*, were detected in *S. gemmea* and *S. barraudi*, which suggest their role as potential vectors.[58]

A few studies were conducted to identify animal reservoirs in disease endemic areas of Thailand.[17,20,21,25] Some direct (presence of *Leishmania* in reservoirs) and indirect (serology) evidence exists strongly suggests that domestic animals may serve as natural reservoirs of the disease. [17,20,21,25] A few reported studies set forth to detect *Leishmania* antibody by direct agglutination test (DAT). Those studies found DAT test to be reactive in some domestic animals, such as dogs, cats, rats and cattle that were living or kept near the residence or housing of infected individuals.[17,20,21,25] However, only one study found direct evidence that suggested the presence of *Leishmania*.[7] *L. martiniquensis* DNA was detected by PCR of ITS1 and HSP70 genes in black rats (*Rattus rattus*) that were captured near the residence of infected patient's.[7] In addition, CL caused by *L. martiniquensis* was observed in Europe and America in farm animals.[42-44] Thus, the data suggests that *L. martiniquensis* transmission is likely zoonotic.

CONCLUSION

Leishmaniasis is a disease that was once considered rare in Thailand, given that all identified cases were thought to be imported. However, a number of autochthonous leishmaniasis cases have been reported since 1996 in patients who had no history of traveling abroad. The majority of these cases suffered from *L. martiniquensis* infection. However, a novel species of *Leishmania*, namely *L. siamensis*, which was first and only discovered in Thailand was found to be the causative pathogen in two confirmed cases of leishmaniasis. Black rats may serve as a natural reservoirs, suggesting it is zoonotic in nature. Two sandfly species, *S. gemmea* and *S. barraudi*, may be able to transmit the disease. The up to date knowledge on emerging leishmaniasis situation in Thailand emphasizes potential problematic public health concern especially in HIV-infected patients. Future research focusing on epidemiology of the disease, potential vectors and reservoirs are required for public health prevention and control. Early diagnosis of leishmaniasis in HIV-infected individuals should be performed in patients who have clinical manifestations suspicious of leishmaniasis. Moreover, in-depth study on biology of novel *Leishmania* species and host parasite immunity is crucial for drug development, since there are limited effective medical treatment options available for this disease.

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