

Detection of *Leishmania* RNA Virus 1 in *Leishmania (Viannia) panamensis* Isolates, Panama

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We detected *Leishmania* RNA virus 1 (LRV1) in 11 isolates of *Leishmania (Viannia) panamensis* collected during 2014–2019 from patients from different geographic areas in Panama. The distribution suggested a spread of LRV1 in *L. (V.) panamensis* parasites. We found no association between LRV1 and an increase in clinical pathology.

Leishmania RNA virus 1 (LRV1) belongs to the *Totiviridae* family, *Leishmaniavirus* genus, and infects different *Leishmania* lineages. This virus is not enveloped and is composed of a viral capsid ≈ 40 nm in diameter and a double-stranded RNA (dsRNA) of 5,280 nt (1,2). The genome has 3 open reading frames (ORF), 2 of which are coding. The *orf2* codes for the capsid protein and the *orf3* codes for an RNA-dependent RNA polymerase (RdRp). *orf1* has been described in other members of the family, but its function is unknown (1,3). This virus has been categorized in LRV1 and LRV2, according to the subgeneruses of *Leishmania* in which they have been identified (4,5). The presence of LRV1 has been reported more frequently in specific regions of South America associated with cases of cutaneous leishmaniasis (CL) and mucocutaneous

leishmaniasis (MCL) (6,7). *L. (Viannia) panamensis* is the predominant species and is responsible for most cases of CL in Panama (8,9) and the presence of LRV1 has been reported in 2 isolates of *L. (V.) panamensis* from Ecuador and Costa Rica (7,10).

The Study

We analyzed *Leishmania* spp. parasite isolates from clinical samples from 2014–2018 that were cryopreserved at Gorgas Memorial Institute's parasitology research department (Panama City, Panama). The Bioethics Committee of the Gorgas Memorial Institute for Health Studies approved this study (protocol no. 056/CBI/ICGES/19). We extracted clinical and epidemiologic data such as sex, age, clinical classification (location, severity, and number of lesions), and province of origin from the database. The disease was classified as nonsevere or severe according to Infectious Disease Society of America guidelines (11). We activated the isolates at 26°C by using Schneider's medium enriched with 25% fetal bovine serum until reaching exponential growth ($2\text{--}3 \times 10^7$ parasites/mL) (9). We centrifuged this concentration of parasites for 10 minutes at 3,500 rpm and divided it into 2 pellets; we used 1 pellet to extract DNA from *Leishmania* spp. for characterization and confirmation and the other to extract RNA and detect LRV1. We characterized the isolates as *L. (V.) panamensis* by the RFLP/PCR-Hsp70 methodology (12). For the detection of LRV1, we amplified 245 nucleotides corresponding to the *orf1* gene region using the primers described by Ito et al. (6,13) and sequenced the product by the Sanger method.

We recovered parasite isolates from 56 patients. Of those isolates, 11 (20%) were positive for LRV1, 63.3% from female patients and 36.4% from male

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patients. Patient age range was 8–59 years; mean (\pm SD) age was 34 (\pm 5.4) years (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/29/6/22-0012-App1.pdf>). All the patients came from leishmaniasis-endemic areas in Panama: 36.4% from Panama Oeste, 18.2% from Panama, 18.2% from Colón, 18.2% from Darién, and 9.0% from Coclé (Figure 1). Most of the patients had single lesions (7/11 [63.6%]); mean (\pm SD) was 1 (\pm 0.2) and range 1–3 lesions per patient. Mean (\pm SD) time of evolution of the lesion was 50 (\pm 9.6) days and range was 21–120 days. Most (6/11 [54.5%]) patients had an evolution time of 30 days. All the lesions were CL and were classified as nonsevere; lesions consisted of a crusty, moist ulcer with raised margins and a clean base (Table) (11). The lesions were distributed mainly on the arms (9/11: 81.8%); only 2 were visible elsewhere, on the leg (1/11: 9.1%) and face (1/11: 9.1%).

We performed data analysis using GraphPad Prism 5.0 software (GraphPad, <https://www.graphpad.com>). We performed the Kolmogorov-Smirnov test to assess the normality of the samples. To analyze the differences between groups, we performed a *t* test for Gaussian distribution data. We considered differences statistically significant when *p* was <0.05 . We found no significant difference to suggest that those with LRV1-positive parasites developed more severe diseases (data not shown). From 10 sequences downloaded from obtained in the study (GenBank accession nos. OL389058–67), we selected 6 sequences based on phylogenetic analysis quality

(Appendix Table 2); those sequences clustered within the phylogenetic group of LRV1 sequences detected in the species of the subgenus *Viannia*, close to those found in isolates of *L. (V.) guyanensis* (Figure 2).

Conclusions

We detected LRV1 in 11/56 (20%) of *L. (V.) panamensis*-evaluated isolates, all of them in patients with CL, consistent with the preliminary description of the presence of LRV1 in 2 isolates of *L. (V.) panamensis* from clinical samples from Ecuador and Costa Rica, countries geographically close to Panama (7). The prevalence of LRV1 has been reported as higher in *Leishmania* spp. isolates from the New World (39.1%) than in those from the Old World (8.4%); prevalence also is higher in isolates from patients with severe skin forms of leishmaniasis, such as disseminated leishmaniasis and MCL, than from patients with CL (14).

The use of *Leishmania* spp. isolates could be a limitation for the analysis because we were able to analyze only the parasites that grew in medium. To avoid this bias, future studies analyzing the presence of the virus directly from clinical samples are needed. In South American countries, prevalence of $\approx 25\%$ of LRV1 has been described in isolates of *L. (V.) braziliensis* and *L. (V.) guyanensis* from Peru (7), Bolivia (14), and Brazil (15). The presence of LRV1 in *L. (V.) panamensis* in this study (20%) indicates circulation of this virus in Panama, suggesting LRV1 is likely widespread across the Americas and in different *Leishmania (V.)* species. Future analysis using a higher

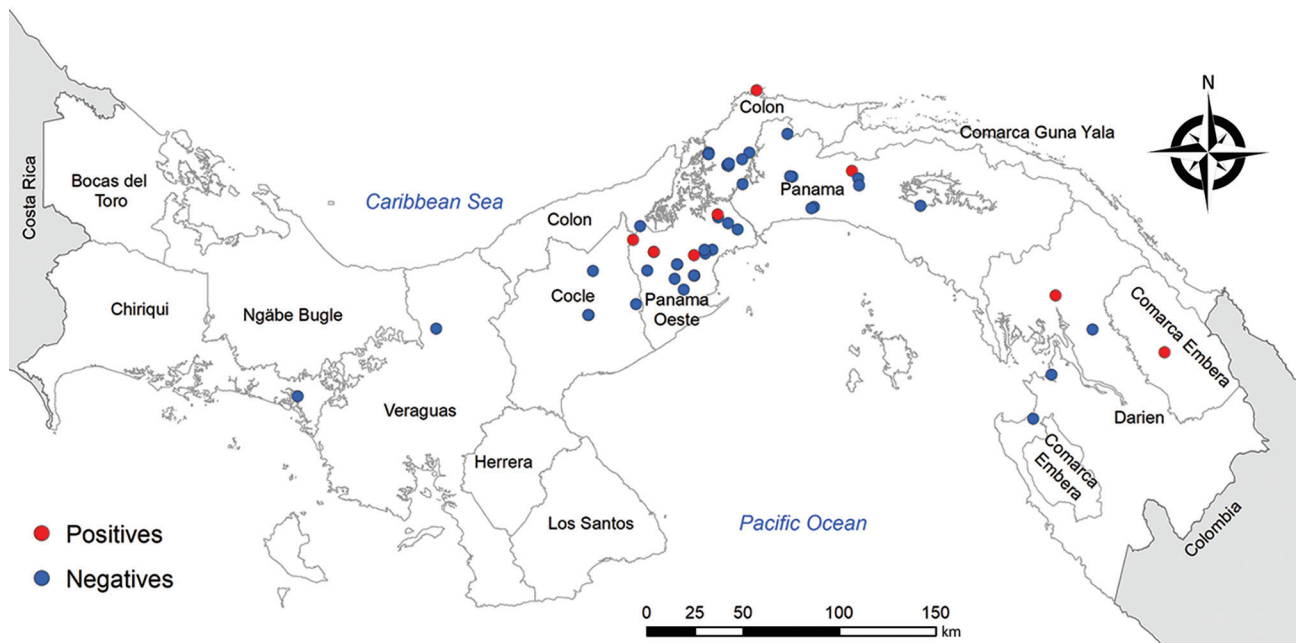


Figure 1. Distribution map of *Leishmania* RNA virus 1 positive and negative isolates analyzed in Panama, 2014–2018.

Table. Epidemiologic description of *Leishmania (Viannia) panamensis* isolates analyzed for LRV1, Panama, 2014–2018*

LRV1 status	No. isolates, N = 56	Mean age, y (SD)	Age range, y	Sex, no.	Duration of disease, d		No. lesions	
					Mean (SD)	Range	Mean (SD)	Range
Positive	11	34 (5.4)	8–59	4 M, 7 F	50 (9.6)	21–120	1 (0.2)	1–3
Negative	45	30 (3.1)	3–72	26 M, 19 F	67 (11)	15–365	1 (0.2)	1–6

*LRV1, *Leishmania* RNA virus 1.

number of samples is necessary to estimate LRV1 prevalence in Panama.

In this study, we found no evidence that correlates the presence of LRV1 with severe clinical forms of leishmaniasis caused by *L. (V.) panamensis*, which was consistent with previous findings of no predisposition of the Th2 response induced by LRV1 for the favorable survival of the parasite for *L. (V.) panamensis* (7). In addition, previous studies described a general decrease in the expression of virulence factor transcription in *L. (V.) panamensis* (7) compared with an earlier study of *L. (V.) braziliensis* (10). It is possi-

ble that *L. (V.) panamensis* strains infected with LRV1 have low expression of virulence factor, which would be reflected in the presence of uncomplicated symptoms of CL cases in the analyzed samples.

The role of LRV1 and its subtypes modulating the immune response in infection caused by *L. (V.) panamensis* is unclear. It is important to carry out studies of the virus subtypes that are circulating in the country and analyze whether the differences in the modulation of the immune response reflected in the clinical manifestations are because of intrinsic factors of the virus, the *Leishmania* species that it infects, or both.

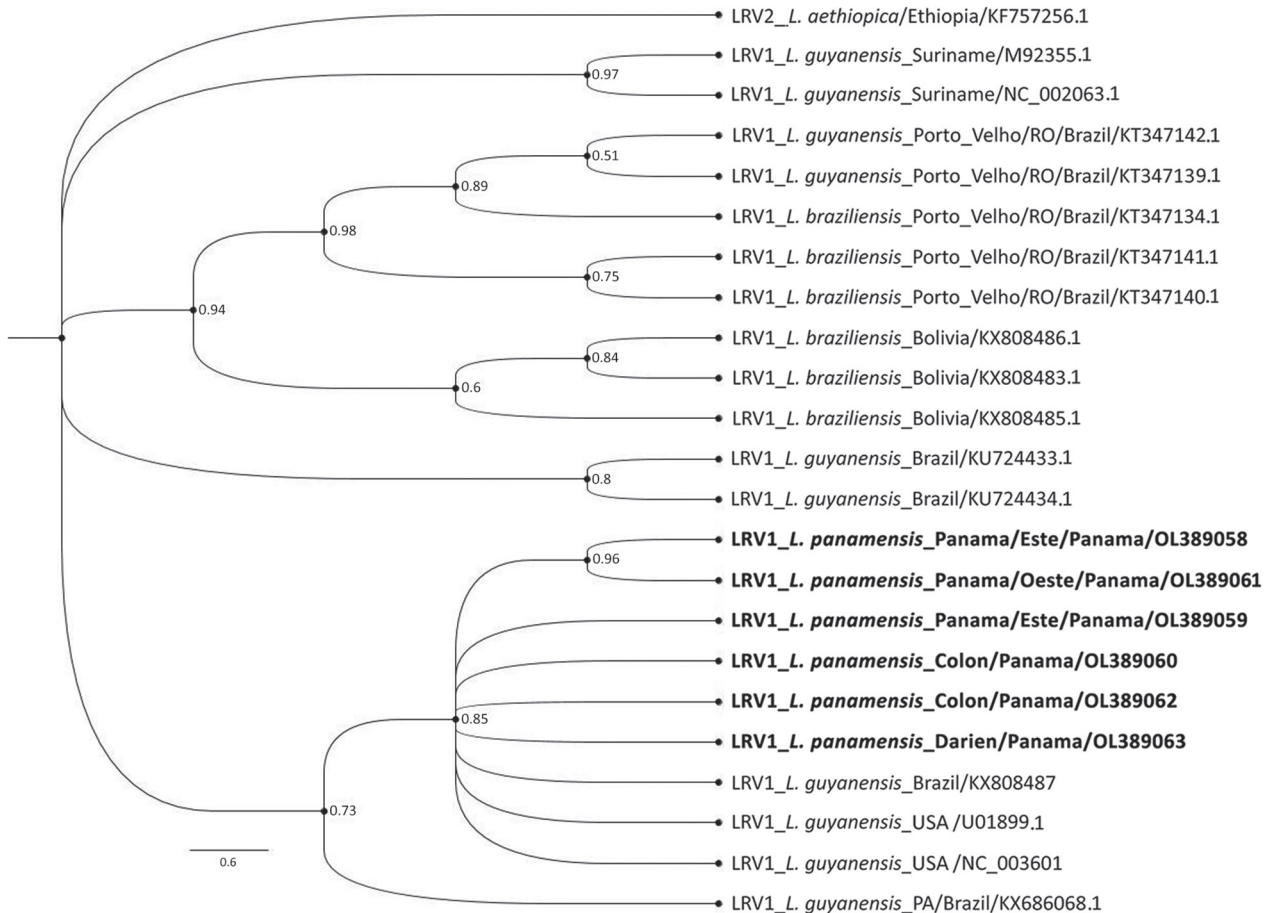


Figure 2. Phylogenetic analysis of *Leishmania* RNA virus 1 isolates analyzed in Panama, 2014–2018, and reference isolates. A phylogenetic tree reconstruction was implemented, applying Bayesian inference with the general time reversible plus gamma 4 plus invariable sites model using MrBayes version 3.2.6 phylogenetic software (<https://nbisweden.github.io/MrBayes>). Boldface indicates sequences obtained in this study, which are in the same clade with reference sequences from *Leishmania (Viannia) panamensis* isolates, mostly from Brazil. Numbers at each node represent clade credibility values. GenBank accession numbers are provided. Scale bar indicates substitutions per site.

In conclusion, the data we obtained show the presence of LRV1 in isolates of *L. (V.) panamensis* from Panama from different years and locations, suggesting wide spread of the virus in this species. In addition, the recent documented circulation of *L. (V.) guyanensis* and *L. (V.) braziliensis* in Panama (9) and the proposed association of LRV1 presence in these species with severity of disease highlight the necessity of future studies on the presence of LRV1 in non-*L. (V.) panamensis* species in Panama. The role of *Leishmania* in disease severity may depend on the species infected and the role of viral, parasite, and human host factors in pathogenesis.

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Dr. González is a medical technologist and senior health researcher at Gorgas Memorial Institute, Panama City, Panama. Primary research interests are immunopathology of cutaneous leishmaniasis and molecular characterization of *Leishmania* spp., and in vitro studies of *Leishmania (V.) panamensis* infection.

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Appendix

Appendix Table 1. Clinical and epidemiologic description of the 56 *Leishmania (V.) panamensis* isolates analyzed in this study

ID	Province of origin	Age, y	Sex	Duration of disease,		LRV1 status	GenBank accession no.
				d	No. lesions		
1	Panama	8	F	60	1	Positive	OL389058
2	Panama	46	F	21	1	Positive	OL389059
3	Colon	41	M	90	1	Positive	OL389060
4	Panama Oeste	17	M	60	1	Positive	ND
5	Panama Oeste	59	F	21	3	Positive	OL389064
6	Darien	57	F	30	2	Positive	OL389065
7	Panama Oeste	20	F	60	1	Positive	OL389066
8	Panama Oeste	51	F	30	1	Positive	OL389061
9	Cocle	14	F	120	2	Positive	OL389067
10	Colon	39	M	42	1	Positive	OL389062
11	Darien	31	M	21	2	Positive	OL389063
12	Panama Oeste	44	M	30	1	Negative	ND
13	Chiriqui	9	M	90	1	Negative	ND
14	Panama Oeste	25	M	30	4	Negative	ND
15	Veraguas	17	F	60	2	Negative	ND
16	Colon	18	M	90	1	Negative	ND
17	Panama	30	M	120	1	Negative	ND
18	Panama	23	F	90	1	Negative	ND
19	Darien	40	F	30	1	Negative	ND
20	Cocle	12	M	21	1	Negative	ND
21	Colon	8	M	20	1	Negative	ND
22	Darien	17	F	120	2	Negative	ND
23	Panama	48	M	42	5	Negative	ND
24	Panama	35	F	ND	4	Negative	ND
25	Darien	38	M	360	1	Negative	ND
26	Panama	9	F	30	1	Negative	ND
27	Panama	ND	F	ND	1	Negative	ND
28	Panama Oeste	52	F	30	1	Negative	ND
29	Darien	64	M	15	6	Negative	ND
30	Panama	63	M	365	1	Negative	ND
31	Panama	65	M	21	1	Negative	ND
32	Colon	54	F	30	2	Negative	ND
33	Panama	4	M	30	1	Negative	ND
34	Panama Oeste	20	F	42	1	Negative	ND
35	Cocle	30	M	60	2	Negative	ND
36	Colon	18	M	30	1	Negative	ND
37	Panama Oeste	15	F	60	2	Negative	ND
38	Panama Oeste	14	M	30	2	Negative	ND
39	Cocle	7	M	30	1	Negative	ND
40	Panama Oeste	20	M	21	1	Negative	ND
41	Colon	3	F	60	1	Negative	ND
42	Panama Oeste	28	F	60	1	Negative	ND
43	Panama Oeste	19	M	30	1	Negative	ND
44	Panama Oeste	15	M	60	1	Negative	ND
45	Panama Oeste	9	F	45	2	Negative	ND
46	Panama Oeste	69	F	60	2	Negative	ND

ID	Province of origin	Age, y	Sex	Duration of disease, d	No. lesions	LRV1 status	GenBank accession no.
47	Panama Oeste	14	M	90	1	Negative	ND
48	Panama Oeste	41	M	23	3	Negative	ND
49	Panama Oeste	36	F	60	2	Negative	ND
50	Cocle	48	F	90	2	Negative	ND
51	Colon	20	F	60	1	Negative	ND
52	Panama Oeste	16	M	60	3	Negative	ND
53	Cocle	54	F	90	2	Negative	ND
54	Colon	53	M	45	1	Negative	ND
55	Panama	72	M	60	1	Negative	ND
56	Colon	12	M	90	2	Negative	ND

*LRV1, Leishmania RNA virus 1; ND: data not available

Appendix Table 2. Sequence quality and access codes for the GenBank of the *Leishmania*. (*Viannia*) *panamensis* isolates positive to LRV1

Strain ID	Parasite	Leishmania International code	Sequence length, bp	GenBank accession no.
C-16-34	<i>L. (V.) panamensis</i>	MHOM/PA/2016/C1634	214	OL389058*
C-16-72	<i>L. (V.) panamensis</i>	MHOM/PA/2016/C1672	211	OL389059*
C-16-78	<i>L. (V.) panamensis</i>	MHOM/PA/2016/C1678	212	OL389060*
C-18-151	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18151	158 (Forward)	OL389064
C-18-163	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18163	143 (Forward)	OL389065
C-18-167	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18167	147 (Reverse)	OL389066
C-18-176	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18176	214	OL389061*
C-18-185	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18185	146 (Forward)	OL389067
C-18-186	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18186	215	OL389062*
C-18-196	<i>L. (V.) panamensis</i>	MHOM/PA/2018/C18196	212	OL389063*

*Sequences used in the phylogenetic analysis