Short Communications

Survey of Wild Mammal Hosts of Cutaneous Leishmaniasis Parasites in Panamá and Costa Rica

Kadir González[1], José E. Calzada[1], Azael Saldaña[1], Chystrie A. Rigg[1], Gilbert Alvarado[2], Bernal Rodríguez-Herrera[2], Uriel D. Kitron[3], Gregory H. Adler[4], Nicole L. Gottdenker[5], Luis Fernando Chaves[6,7] and Mario Baldi[7]*

Abstract: The eco-epidemiology of American cutaneous leishmaniasis (ACL) is driven by animal reservoir species that are a source of infection for sand flies that serve as vectors infecting humans with *Leishmania* spp parasites. The emergence and re-emergence of this disease across Latin America calls for further studies to identify reservoir species associated with enzootic transmission. Here, we present results from a survey of 52 individuals from 13 wild mammal species at endemic sites in Costa Rica and Panama where ACL mammal hosts have not been previously studied. For *Leishmania* spp. diagnostics we employed a novel PCR technique using blood samples collected on filter paper. We only found *Leishmania* spp parasites in one host, the two-toed sloth, *Choloepus hoffmanni*. Our findings add further support to the role of two-toed sloths as an important ACL reservoir in Central America.

Key words: zoonotic disease, reservoir, *Leishmania* spp.

Zoonotic vector-borne diseases are a major cause of morbidity and mortality in Latin America and the Caribbean. American cutaneous leishmaniasis (ACL) is a common but neglected tropical disease in both Panamá and Costa Rica where annual incidence is estimated to be 2,188 and 1,249 cases per year, respectively, with reported cases suspected to account for only 1/3 of the total infections [1]. Mammalian reservoirs of *Leishmania* spp. play a major role in epidemiology and transmission, given their role as sources of infections for phlebotomine sandflies (Diptera: Psychodidae) vectors and humans [2–4]. Several wild mammal species, including sloths and rodents, were identified as reservoirs of cutaneous leishmaniasis parasites by pioneering studies on the eco-epidemiology of the disease in Panamá [2, 5–8] and Costa Rica [9, 10]. Nevertheless, the emergence and re-emergence of ACL in Latin America calls for further studies to identify hosts associated with enzootic transmission [11, 12]. Here, we present results from wild mammal hosts of *Leishmania* spp. parasites in endemic areas of Panamá and Costa Rica not previously studied, employing novel molecular PCR tests on samples collected on filter paper and by parasite isolation from blood to detect *Leishmania* parasites.

In Panamá, our samples were collected from April to June 2010 in Trinidad de Las Minas (8°46'32"N, 79°59'45"W), where ACL is enzootic and endemic [13]. We collected samples from wild mammals prior to the implementation of an insecticide thermal fogging control trial for ACL vectors [14, 15]. In Costa Rica, our samples were collected from August 2010 to June 2012 in Savegre Abajo (9°30'50"N, 83°49'27"W), where ACL is endemic [16]. The sampling sites in Costa Rica (Fig. 1A) and Panamá (Fig. 1B) are rural areas where secondary forest patches and agricultural land are mixed within the landscape matrix.

In Panamá we set a total of 15 Sherman® (for small

[1] Departamento de Parasitología, Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES), Apartado Postal No. 0816-02593, Ciudad de Panamá, República de Panamá
[2] Escuela de Biología, Universidad de Costa Rica, Apartado Postal 11501-2060, San Pedro, Costa Rica
[3] Department of Environmental Studies, Emory University, Atlanta, GA, 30322 USA
[4] University of Wisconsin-Oshkosh, Oshkosh, WI, 54901 USA
[5] Department of Pathology, School of Veterinary Medicine, University of Georgia, Athens, GA, 30602 USA
[6] Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
[7] Programa de Investigación en Enfermedades Tropicales (PIET), Escuela de Medicina Veterinaria, Universidad Nacional, Apartado Postal 304-3000, Heredia, Costa Rica
*Corresponding author:
Tel: +506-22380761
Fax: +506-22381298
E-mail: mario.baldi.salas@una.cr

Fig. 1.   Study Settings, Traps and Wild Mammals. (A) Savegre Abajo, Costa Rica (B) Trinidad de las Minas, Panamá (C) Sherman
Trap (D) Havahart Trap with a grey four eyed opossum, *Philander opossum* (E) Spiny rat, *Proechimys semispinosus* (F)
Two toed sloth, *Choloepus hoffmanni*

mammals, Fig. 1C) and 15 Tomahawk® (for medium-size mammals) traps, baited with bananas, over 7 nights to collect wild mammals. Traps were set in peridomestic environments. Sloths were captured by visually searching for them in trees followed by manual capture by an experienced individual. Most wild mammals were manually restrained. However, sloths were anesthetized with ketamin [10 mg/kg] and xylacin [1 mg/kg] following the method of Vogel *et al.* [17]. Blood was collected from wild mammals by venipuncture using standard laboratory animal procedures [18]. In addition, skin biopsies were taken from the nose in sloths using a 3.5 mm sterile biopsy punch (Acuderm, Inc., Ft. Lauderdale, FL, USA) and from the tail with a tail clip and/or ear with a sterile surgical blade in wild rodents. In Costa Rica, we collected samples from 12 wildlife species. We deployed 54 Sherman® (for small mammals) and 5 Havahart® (for medium-size mammals, Fig. 1D) traps, baited with a 1:1 oatmeal and peanut butter mix, over 71 nights evenly distributed across the dry and rainy seasons. Traps were set in grids that included peridomiciliary environments, agricultural land and secondary forest. In Costa Rica, all animals were manually restrained

and blood was collected using the same procedure as in Panamá. In Costa Rica no skin biopsies were taken given the absence of two-toed sloths in the study area.

Our study protocol was approved by the National Review Board, Comité Nacional de Bioética de la Investigación, Instituto Conmemorativo Gorgas de Estudios de la Salud, ICGES (561/CNBI/ICGES/06) and by the ICGES Institutional Animal Care and Use Committee (IACUC, 2006/02) in Panamá. In Costa Rica our study protocol was approved by the Animal Welfare Committee at Universidad Nacional de Costa Rica (FCSA-EMV-CBA-Oll-2010).

To identify mammal hosts infected with *Leishmania* spp. parasites, DNA was extracted from Costa Rican and Panamanian samples from whole blood and from skin scrapings on filter paper using the DNeasy extraction kit (Qiagen, Valencia, CA, USA). Primers targeting the heat-shock protein 70 of New World *Leishmania* spp. [19] and the entire 750-bp minicircle *of Leishmania Viannia* sp. [20] were used for PCR diagnosis and species characterization. Amplification reactions were performed in a final volume of 50 μl as described by Miranda *et al.* [21].

In Panamá, for parasite isolation purposes, the blood

from our samples was inoculated into biphasic Senekjie's medium with M199 (Sigma, St Louis, MO, USA). Hemoculture tubes were incubated at 26°C and were checked weekly for 1 month to verify the presence of *Leishmania* spp. promastigotes.

Differences in the sampling protocols were mainly due to the limited funding for wild mammal sampling in Panamá. In Panamá, wild mammals were sampled as part of a trial evaluating the impacts of insecticide thermal fogging on sand fly control [13–15].

In Panama, all sampled *Proechimys semispinosus* (N = 4, Fig. 1E) were negative by PCR tests in blood or biopsies (both primer sets). Three of the *Choloepus hoffmanni* (N = 4, Fig. 1F) were *Leishmania* positive by PCR (both primer sets) from the blood, but all were negative for PCR (both primer sets) from skin biopsies, and we isolated parasites from 2 sloths by hemoculture. The isolated parasites belonged to *L. panamensis*, the ACL parasite species circulating among humans in the area [13] and the main parasite causing ACL in Panamá [22–23], an important indication that two-toed sloths are a likely reservoir [11] in the studied site in Panamá. Our results confirm previous observations for Panamá [2, 6–8], but the use of a more sensitive diagnostic method, i.e., PCR of blood collected on filter paper [21], suggests that previous studies based on parasite isolation [6, 8] underestimated the prevalence of *L. panamensis* in two-toed sloths, given the ability to find circulating DNA in a sloth from which we were unable to isolate parasites from blood. In Costa Rica, where we did not trap any two-toed sloths, all samples were negative when PCR (both primer sets) was performed on blood from the following wild animals: one opossum *Didelphis marsupialis*, one Goldman's water mouse *Rheomys raptor*, one fulvous pygmy rice rat *Oligoryzomys fulvescens*, one Coues rice rat *Oryzomys couesi*, one forest spiny pocket mouse *Heteromys desmarestianus*, one Talamancan harvest mouse *Reithrodontomys creper*, two cotton rats *Sigmodon hispidus*, two gray four-eyed opossums *Philander opossum* (Fig. 1D), three harvest mice *Reithrodontomys* spp., four spiny rats *Proechimys semispinosus* (Fig. 1E), ten rice rats *Melanomys caliginosus* and 16 kinkajous *Potos flavus*.

In general, our results add further support to previous observations from Panamá [2] and Costa Rica [10] implicating two-toed sloths as a major reservoir for ACL transmission, as originally hypothesized by Garnham [24] when describing the natural history of ACL transmission in Belize. Surprisingly, we found no infections in rodents commonly infected with *Leishmania* spp. parasites in South America, such as *Proechimys* spp. spiny rats [25], but we suspect this was due to our relatively small sample size. Similarly, our samples were negative for other species

incriminated as ACL parasite reservoirs, such as opossums [26] and kinkajous [27]. Nevertheless, further studies that consider landscape structure and include long-term sampling will allow for a better understanding of the community ecology of ACL hosts in Panamá and Costa Rica.

CONFLICTS OF INTEREST

All authors declare that no competing interests exist.

REFERENCES

1. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis Worldwide and Global Estimates of Its Incidence. PLoS ONE 2012; 7: e35671.
2. Christensen HA, Fairchild GB, Herrer A, et al. The ecology of cutaneous leishmaniasis in the republic of Panama. J Med Entomol 1983; 20: 463–484.
3. Reithinger R, Dujardin J-C, Louzir H, et al. Cutaneous leishmaniasis. Lancet Infect Dis 2007; 7: 581–596.
4. Chaves LF, Hernandez M-J. Mathematical modelling of American Cutaneous Leishmaniasis: incidental hosts and threshold conditions for infection persistence. Acta Tropica 2004; 92: 245–252.
5. Telford SR Jr, Herrer A, Christensen HA. Enzootic cutaneous leishmaniasis in eastern Panama. 3. Ecological factors relating to the mammalian hosts. Ann Trop Med Parasitol 1972; 66: 173–179.
6. Herrer A, Telford SR Jr, Christensen HA. Enzootic cutaneous leishmaniasis in Eastern Panama. I. Investigation of

the infection among forest mammals. Ann Trop Med Parasitol 1971; 65: 349–358.

7. Herrer A, Telford SR Jr. *Leishmania braziliensis* Isolated from Sloths in Panama. Science 1969; 164: 1419–1420.

8. Herrer A, Christensen HA. *Leishmania braziliensis* in the Panamanian Two-Toed Sloth, *Choloepus hoffmanni*. Am J Trop Med Hyg 1980; 29: 1196–1200.

9. Zeledón R, McPherson B, Ponce C. Isolation of *Leishmania braziliensis* from a Wild Rodent in Costa Rica. Am J Trop Med Hyg 1977; 26: 1044–1045.

10. Zeledón R, Ponce C, De Ponce E. The Isolation of *Leishmania braziliensis* from Sloths in Costa Rica. Am J Trop Med Hyg 1975; 24: 706–707.

11. Chaves LF, Hernandez M-J, Dobson AP, et al. Sources and sinks: revisiting the criteria for identifying reservoirs for American cutaneous leishmaniasis. Trends Parasitol 2007; 23: 311–316.

12. Chaves LF, Hernandez M-J, Ramos S. Simulación de modelos matemáticos como herramienta para el estudio de los reservorios de la Leishmaniasis Cutánea Americana. Divulgaciones matemáticas 2008; 16: 125–154.

13. Saldaña A, Chaves LF, Rigg CA, et al. Clinical Cutaneous Leishmaniasis Rates Are Associated with Household *Lutzomyia gomezi*, *Lu. panamensis*, and *Lu. trapidoi* Abundance in Trinidad de Las Minas, Western Panama. Am J Trop Med Hyg 2013; 88: 572–574.

14. Chaves LF, Calzada JE, Rigg C, et al. Leishmaniasis sand fly vector density reduction is less marked in destitute housing after insecticide thermal fogging. Parasit Vectors 2013; 6: 164.

15. Calzada JE, Saldaña A, Rigg C, et al. Changes in phlebotomine sand fly species composition following insecticide thermal fogging in a rural setting of western Panamá. PLoS One 2013; 8: e53289.

16. Chaves LF, Cohen JM, Pascual M, et al. Social Exclusion Modifies Climate and Deforestation Impacts on a Vector-Borne Disease. PLoS Negl Trop Dis 2008; 2: e176.

17. Vogel I, de Thoisy B, Vié J-C. Comparison of injectable anesthesic combinations in free-ranging Two-Toed Sloths in French Guiana. J Wild Dis 1998; 34: 555–566.

18. Hoff J. Methods of blood collection in the mouse. Lab Anim 2000; 29: 47–53.

19. Garcia L, Kindt A, Bermudez H, et al. Culture-Independent Species Typing of Neotropical *Leishmania* for Clinical Validation of a PCR-Based Assay Targeting Heat Shock Protein 70 Genes. J Clin Microbiol 2004; 42: 2294–2297.

20. Vergel C, Walker J, Saravia NG. Amplification of human DNA by primers targeted to *Leishmania* kinestoplast DNA and Post-Genome considerations in the detection of parasites by a polymerase chain reaction. Am J Trop Med Hyg 2005; 72: 423–429.

21. Miranda A, Saldaña A, González K, et al. Evaluation of PCR for cutaneous leishmaniasis diagnosis and species identification using filter paper samples in Panama, Central America. Trans R Soc Trop Med Hyg 2012; 106: 544–548.

22. Miranda A, Carrasco R, Paz H, et al. Molecular Epidemiology of American Tegumentary Leishmaniasis in Panama. Am J Trop Med Hyg 2009; 81: 565–571.

23. Chaves LF, Calzada JE, Valderama A, et al. Cutaneous Leishmaniasis and Sand Fly fluctuations are associated with El Niño in Panamá. PLoS Negl Trop Dis 2014; 8: e3210.

24. Garnham PCC. The Leishmanias, with Special Reference to the Role of Animal Reservoirs. Am Zool 1965; 5: 141–151.

25. Alexander B, Lozano C, Barker DC, et al. Detection of *Leishmania (Viannia) braziliensis* complex in wild mammals from Colombian coffee plantations by PCR and DNA hybridization. Acta Tropica 1998; 69: 41–50.

26. Scorza JV, Rezzano S, Márquez JC. *Didelphis marsupialis*, reservorio primario de *Leishmania* spp. en la ciudad de Trujillo, Venezuela. Boletín de Malariología y Saneamiento Ambiental 1986; 26: 1–5.

27. Thatcher VE, Eisenmann C, Hertig M. A natural infection of *Leishmania* in the kinkajou, *Potos flavus*, in Panama. J Parasitol 1965; 51: 1022–1023.