Imported Cases of Cutaneous Leishmaniasis in Cuba, 2017: Role of Human Movement.

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Research Article

Keywords: Cutaneous leishmaniasis, Epidemiology, Imported cases

Posted Date: October 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-968613/v1

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Abstract

Background: Leishmaniasis is a vector-borne disease caused by several species from genus *Leishmania*. An increase in the number of cases related to human movement has been informed in the last years. Due to the increase of suspicious leishmaniasis cases arriving in Cuba during 2017, an general analysis is presented herein.

Methods: Clinical samples were collected from 5 patients suspicious of leishmaniasis, received from January to December 2017 at the Institute of Tropical Medicine Pedro Kourí, Cuba. Skin lesion samples were analyzed using different diagnostic assays: direct smear, histological examination, and molecular analysis for species identification. Epidemiological and demographic data were requested from each case and analyzed. Treatment and follow up of patient was also performed.

Results: Five cases were confirmed as *Leishmania* infection according to microscopic observation and molecular methods results. PCR-18S, PCR-N/RFLP and PCR-F/RFLP identified the following species: *L. panamensis* (2 cases), *L. braziliensis* (1 case), *L. panamensis/L. guyanensis* (1 case), *L. mexicana* complex (1 case). In treated patients, drugs were well tolerated, cure were documented and no relapse have been currently reported (3 years later).

Conclusions: Clinical characteristics, demographic data, and epidemiological features of infection for each case evidence the potential risk related with travel to endemic areas of leishmaniasis.

Background

Leishmaniasis is a vector-borne diseases caused by around 20 species of the genus *Leishmania* which are transmitted through the bite of female sandflies to mammalian hosts. Different clinical presentations, including cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL), are present in 98 countries worldwide, whit 2 million new cases reported per year [1]. The epidemiology of leishmaniasis is dynamic and the circumstances of transmission are continually changing concerning the environment, demography, human behavior, socioeconomic status, and other factors [2]. In line with this, an increase in cases due to migration, traveling, and military conflicts have been notified around the world in the recent past [3–5].

In particular, the American continent represents a special scenario for the disease due to: (i) a high disease burden, (ii) competent vectors for transmission, (iii) circulation of 20 different species in the geographical area, (iv) up to ten different species within the same territory/country (per example 15 species have been reported in Brazil and 11 in Peru), and (v) at least 26 animal reservoir, human included [6]. The disease is present in 19 countries, with important transmission incidence from Mexico to Argentina, which 66, 941 cases [1]. Argentina, Brazil, Colombia, Ecuador, Venezuela, Paraguay and Perú report stable transmission [6–8]. However, information about imported cases in non-endemic countries from Latin America is extremely scarce.
In Cuba, leishmaniasis is not endemic [9]; although few imported cases with cutaneous leishmaniasis were diagnosed and treated in the ’70s and ’80s of the last decade, which were not reported (Statistical Department of Institute of Tropical Medicine Pedro Kouri). Recently, five out of 16 suspicious imported cases investigated in our laboratory in ten years (2006-2016) were confirmed and documented [9]. They all shared a common epidemiologic feature since the patients in this study visited different settings where contact with vectors was possible. In general, several factors such as human activities and tourism could increase the risk in the number of Leishmania cases. In this sense, a continuous surveillance and international sanitary control is a permanent task nowadays. However, health professionals must be alert and when interrogating the patients take into consideration the possibilities of conditions favoring the spread of this disease.

In particular, during 2017, an increase of suspicious leishmaniasis imported cases arriving in Cuba was observed, which motivate to perform an individual and general analysis. In the present report, the epidemiological contexts of infections, their clinical presentation, the diagnostic methods used, treatment and follow up are described. This will serve not only as an update on the disease in the country context but also as a contribution to the discussion about the influence of human movement in the epidemiology of this parasitic disease.

**Methods**

**Study area**

The present study took place at the Institute of Tropical Medicine Pedro Kouri (IPK), Havana, Cuba, where five cases of suspicious of leishmaniasis arrived during 2017. The regulations of the Institutional Ethical Committee at IPK (CEI-IPK-8918) concerning the use of human clinical samples for research purposes were respected. All the patients voluntarily participated, signed the agreement through the informed consent for the use of their information, clinical samples and photographs for diagnostic, research, and academic purposes.

**Study subjects and clinical evaluation**

Patients (with code: 17-01, 17-02, 17-03, 17-04, and 17-05, in order of arrival date) were received in the IPK out patient clinic as suspicious cases of leishmaniasis from January to December 2017. They all had cutaneous lesions in different locations and variable time of evolution. Personal and general data about each one was obtained during interview or from Hospital's Clinical Records. All the information concerning the epidemiological conditions pertaining to the probable infection was also collected.

Clinical and dermatological evaluation of each patient and their lesion(s) were made at the Hospital from the IPK by physician experts in parasitology and dermatology, who indicated laboratory analysis. Re-evaluations were routinely performed during their admittance in the hospital, treatment administration and follow-up period.

**Sample collection and diagnostic**
Skin biopsies were taken from more than one lesion/patient whenever possible. Scrapings (sterile lancets) and biopsies (disposable punches) were taken from the edge of the lesions according to their location and time of evolution, preferring active lesions. The algorithm followed for diagnosis comprised the use of parasitological and molecular tools to analyze the samples. Firstly, smears were prepared from the lesion's material, fixed with methanol and stained with Giemsa for detecting intracellular parasites (amastigotes) under light microscopy at 1000x. Five microns thick tissue sections were obtained from paraffin-embedded skin tissues, stained with hematoxilin-eosin and analyzed under light microscopy with at 1000x, except for case 17-05.

**Species identification**

A portion of fresh skin tissue from each patient was used for DNA extraction with High Pure PCR Template Preparation Kit (Roche, Germany) following the manufacturer's instructions. Then, PCR targeting the 18S rRNA (namely PCR-18S) was performed, using the primers and conditions described by Deborggraeve et al. [10] for *Leishmania* genus detection. Afterwards, PCR-F and PCR-N assays, which amplify different fragments of *hsp70* gene and their corresponding stepwise RFLP's algorithm were previously described by Montalvo et al. [11] and Fraga et al. [12], to determine the infecting species.

**Treatment**

All the patients considered as positive for leishmaniasis that remained in Cuba after diagnosis were treated with conventional drugs. According international guidelines [13], the following formulations were selected: Amphotericin B® (deoxycholate 50 mg/ampule; Empresa Laboratorios AICA, La Habana, Cuba), Ampholip® (liposomal Amphotericin B 50 mg/vial; ALFARMA S.A., Panamá, República de Panamá) and Fluconazol® (150 mg/capsule; Empresa Laboratorios MEDSOL, La Habana, Cuba). The cure was defined if complete reepithelization of the cutaneous tissue was clinically observed without surging of new lesions. On the other hand, therapeutic failure was defined as the presence of active lesions at day 30 / 60 / 90 or 180 after last treatment (time of re-evaluation). Patients were followed up for at least one year after the end of the last treatment regimen, including clinical and laboratory evaluations.

**Results**

*Leishmania* infection was confirmed in all five cases suspicious of having the disease. Figure 1 showed the lesion of each patient at arrival time and clinical description, which was consistent with *Leishmania*. No systemic signs of infection nor other symptoms were appreciated and according to the laboratory test performed, all patients were immunocompetent and common laboratory tests within normal ranges.

Epidemiological data of the five patients were summarized in Table 1, which 4/5 (80%) were men and 1/5 (20%) female. Four were Cuban citizens (80%) and the other one (20%) to a British residing in Mexico. In all cases, the epidemiological background supported the possible natural infection, through the bite of *Leishmania* vectors when their natural environment was invaded.
All the studied cases corresponded to travelers that under uncontrolled conditions passed/crossed through several Latin American countries where parasites and vectors are endemic (Table 1). Three of them (cases 17-01, 17-02, and 17-04) followed irregular trails, including long journey jungle walking, no safe ground nor river transportation. On the other hand, the case 17-03 agreed to be hired for working on mines without actual information about living conditions, exposing himself to the forest, and sleeping outdoors barely protected. Finally, the patient represented as 17-05, traveled straight from Mexico to Cuba by airplane. However, he got probably infected during previous stays in rural areas visited for professional purposes, several weeks before the lesion appearance. According to the data provided, geographical mapping of the route followed by patients was prepared in Figure 2. Although it was impossible to determine the country or place of infection, two possible “hot spots” for transmission were suggested: Turbo (Colombia) and Darien Jungle (Panama), but other ones could be considered as well.

Related to laboratory diagnostic methods, Table 2 showed that direct microscopical observation of amastigotes was possible in cases 17-01, 17-02, and 17-05; while the histological study was positive for *Leishmania* parasite and kinetoplast in all analyzed biopsies (except for case 17-05 that was not studied). PCR-18S and PCR-N were positive in all five of them; whereas PCR-F was only negative in case 17-05, and the amplicons obtained using PCR-F were weak for 17-03. Then, species or species complex were identified in all cases, which is described in Table 3. According to the combined result of PCR-F/RFLP and PCR-N/RFLP, in three cases a unique species could be identified: *L. braziliensis* for case 17-01 and *L. panamensis* for cases 17-02 and 17-04. However, in case 17-03, although PCR-F product was restricted with *Bcd*, expecting to discriminate between parasites belonging to *L. panamensis*/*L. guyanensis*, the obtained pattern was not conclusive. In this sense, bands corresponding to *L. panamensis* (787 bp, 429 bp) and *L. guyanensis* (544 bp) were obtained. Finally, case 17-05 was found to be infected by a species from *L. mexicana* complex, which cannot be discriminated by either of the approaches used.

Patients were treated according to protocols, except case 17-05 who returned to his home country after diagnostic confirmation, so the treatment and skin lesion resolution are unknown. The most employed treatment was the liposomal amphotericin B: Ampholip® in monotherapy in three patients, which case 17-01 and 17-02 was used to started the treatment with it as the first option. Cases 17-01 required 3 cycles of Ampholip® at a total dose between 850-3300 mg and 9-21 days by intravenous route, interchanging with 2 cycles with Fluconazol® capsules at 150 mg/day during 30 days by oral route. In contrast, case 17-02 only needed a cycle with Ampholip® (total dose of 450 mg and 9 days), followed by Fluconazol® (300 mg/days during 15 days). Case 17-04 started with Amphotericin B® administered at increased doses until a total of 230 mg during 7 days by intravenous route; following a cycle with Fluconazol® (300 mg/days during 30 days). In this case, 9 months later, an additional cycle of Ampholip® (total dose of 1800 mg and 15 days) and Fluconazol® (300 mg/days during 45 days) were applied. The case 17-03 was treated with a cycle of Amphotericin B® (increased doses until a total of 130 mg during 7 days) and two cycles of Fluconazol® (150 mg/days during 15 days) at interval of 3 months. In all cases, drug-associated side effects did not limit patient compliance and treatment was well tolerated. All treated patients were cured and no relapse has been currently reported (3 years later).
However, complete remission of the lesions was observed after different treatment periods: case 17-01, 16 months; case 17-02, 2 months; case 17-03, 4 months; and case 17-04, 12 months. As it happened with these cases, *L. braziliensis* required prolonged treatment regimens.

**Discussion**

CL is recognized as one of the most frequent skin diseases occurring after traveling in endemic areas [14]. The diagnosis may be also a challenge because unusual presentations can occur [15] and parasitological detection, which is often the most available method, usually relies on technical expertise. Taking all this into consideration, travel clinics and referral centers must be prepared to offer not only a prompt but an accurate diagnosis.

All the cases presented here were positive to *Leishmania*. As a laboratory in a non-endemic area, we continue promoting the use of different methods, eluding subjectivism or inexperience, so parasitological and molecular tools are currently utilized during the diagnostic process. It is known that a combination of laboratory methods increased the sensitivity for diagnosis and also provides the possibility to identify the infecting species [4].

Our results indicated that each case had at least one positive parasitological result whereas molecular detection of DNA was possible in all of them, using more than one target (*hsp70* and *rDNA* genes) which makes the final diagnostic robust. It is not surprising that weak DNA product had been obtained after PCR-F in case 17-03, due to the amplicon’s size. While PCR-N is 593 bp length, PCR-F is 1286 bp, a feature that can affect diagnostic sensitivity, as it has been previously reported by our group [16].

In regards to typification, the species identified corresponded with those reported in the countries where the infected persons stayed, and the results were concordant according to the RFLP scheme used. The results of PCR-F/RFLP-*BccI* in case 17-03 are remarkable. According to the *hsp70* and *hsp20* sequences analysis, *L. panamensis* and *L. guyanensis* were previously considered as a monophyletic group [17]. Nevertheless, both of them could be distinguished using *BccI* as a restriction enzyme for PCR-F product [18], which was validated in the differentiation of isolates and clinical samples from some endemic countries [19, 20]. However, the pattern obtained after PCR-F/RFLP was not unequivocal, as it showed bands expected for both entities: *L. panamensis* and *L. guyanensis*. Considering that *L. panamensis* have not been reported in the territories visited by patient 17-03, where *L. guyanensis*, *L. braziliensis* and *L. amazonensis* are main species involved in CL [21], one possible explanation could be that this patient suffering of a mixed infection of *L. guyanensis* and other species. Another possibility is that the pattern observed corresponds to a different *L. guyanensis* population, agreeing with the significant genetic diversity associated with this species reported, for example, infecting miners in that country [22, 23]. As the detection of polymorphism within each species varies according to the genetic markers used, it is possible that further studies, using multilocus sequencing, could shed light on this matter.

Concerning treatment, different protocols were used, since the selected drug, dosage and duration of therapy was personalized for each case; taking into account drug availability, the clinical aspect of the
lesions, the infecting *Leishmania* species, and the response of each patient. We acknowledge that international guidelines exist; however, specific treatment regimens are mainly guided by practical considerations and the personal experience of the treating physician [24]. Nevertheless, is evident that for CL a systemic treatment and drugs with different effectiveness against *Leishmania* is considered mandatory. In this regard, in the analyzed cases, drugs targeting ergosterol were used, including amphotericin B that binds to membrane sterols, forming complexes that arrange into ion channels and increase membrane permeability [25] or fluconazole that interfere with ergosterol biosynthesis by inhibiting the C-14 demethylation of sterols in *Leishmania* [26]. In addition, the use of lipid complex of amphotericin B (Ampholip®) represents an advantage, such as: (i) deliver the drug on-site, (ii) minimizing the dosage by many folds, and (iii) reducing the side effects related to drug toxicity, which is preferable over using conventional amphotericin B [27].

Although cure was achieved for all treated patients, response was very different. However, we can not determined the real causes of this due to the influence of different factors; among them: (i) time elapse between infection and treatment start, (ii) severity of disease when treatment started, (iii) clinical characteristics related with single/multiple or nodule/ulcerated lesion, and (iv) immunological status of patients. Nevertheless, longer and complex treatment was administered to *L. braziliensis* (case 17-01). It is known that *L. braziliensis* is the main causal agent of CL and MCL in the Americas [28] with the greatest relative abundance in Colombia and frequently results in therapeutic failure [29]. Recently, a study to determine drug susceptibility profiles of amphotericin B and fluconazole in cultured isolates of Old World and New World *Leishmania* spp, showed reduced susceptibility to drugs against New World species compared with Old World strains. In particular, some clinical isolates of *L. braziliensis* and *L. panamensis* displayed lower susceptibility compared with reference strain [30].

According to World Health Organization (WHO), leishmaniasis remains as a group of diseases without current control measures. Prophylactic vaccines do not exist nor vector control is effective in most of the settings where these parasitoses are endemic [31, 32], which makes harder the task. Besides, it has been recently recognized that “in an interconnected world, change is occurring across social, environmental and climatic scales affecting human, animal and natural systems” [33] and leishmaniasis is not an exception. Among the multiple epidemiological features surrounding the possible occurrence of leishmaniasis, the human movement between low- and high-risk areas is also important, mainly when uncontrolled displacements take place. In this study, 4 out of 5 cases departed from a country where *Leishmania* is not present, to enter a whole region where the disease is highly distributed [1, 6, 34]. Even more, some of the countries with the highest number of CL cases reported in Latin America, such as Brazil, Peru, Colombia, and Panama [6], were intruded on by most of these persons on their route, increasing, in particular, their risk. As it was described for all the cases (except 17-05), the persons infected traveled rural areas unsafely and slept outdoors without protection. All five cases spent partial or complete journeys in the forest or the jungle; without protecting from insect bites capable of transmitting leishmaniasis or another vector-borne disease. Remarkably, the interviews corroborated that none of the travelers knew about the disease nor other probable infections transmitted by vectors, except for dengue. Therefore, unknowingly they disregarded that possibility and displaced under inappropriate protection.
measures and totally vulnerable to a serie of transmissible diseases. These cases can serve as an example of the serious risks assumed by persons that decide to travel, in particular, by irregular routes, whatever be the reason.

In Cuba, there is currently no evidence about the presence of recognized *Lutzomya* species that could transmit *Leishmania* parasite [35]. However, considering the ecology of sandflies, it is unlikely that phlebotomus could enter the country with travelers, adapt to new ecology conditions and expand their habitat. Although the possibility of the disease spreading certainly is low, notification of imported cases is necessary in the age of globalization.

A recent retrospective analysis between 2006-2016 showed that in 10 years only 5 patients with positive *Leishmania* infection were confirmed out from 16 suspicious cases. Thus, it may cautiously be assumed to our best knowledge that this series of 5 cases constitute the great majority of imported cases/year diagnosed and treated in Cuba. In several non-endemic countries, the number of cases has increased in the past decade. In particular, some reports from Europe, like Belgium [36], Poland [37], and Sweden [4], are showing an increase in cases imported from America. In parallel, the human movement towards of the island, not only causes an increment in leishmaniasis (as described herein), same challenge has been also evidenced in other non-endemic parasitic diseases such as trypanosomiasis [38] and malaria [39].

**Conclusion**

The results presented herein suggest that new efforts should be addressed in terms of educating the general population from non-endemic diseases in relation to the risk of travelling in unsafely conditions into dangerous geographic zones. In addition, it is necessary to strengthen the continuous education to refresh our physicians in the differential diagnosis of skin lessions from traveleres returning from endemic regions of leishmaniasis in the primary healthcare centre. But for international travelers control centers updating of cutaneous and mucocutaneous leishmaniasis should be mandatory.

**Abbreviations**

CEI-IPK: Institutional Ethical Committee at Institute of Tropical Medicine Pedro Kouri; CL: cutaneous leishmaniasis; IPK: Institute of Tropical Medicine Pedro Kouri; MCL: mucocutaneous leishmaniasis; VL: visceral leishmaniasis; WHO: World Health Organization

**Declarations**

**Acknowledgments**

The authors would like to thank to Lic. Dialys González for her assistance related with pharmaceutical information and to all patients that voluntarily participated.

**Funding**
This research did not receive any specific grant from funding agencies.

**Availability of data and materials**

Data related with cases and laboratory tests are available from the corresponding author on reasonable request. All relevant data are within the manuscript.

**Authors’ contributions**

AMM conceived the study. AMM, JF and VC performed the parasitological analysis; while DG, OB and AH performed the clinical monitoring of patients. AMM, DG, AH and LM were involved in data collection and analysis. The first draft of the manuscript was written by AMM and LM, and all authors commented on previous versions of the manuscript.

**Ethics approval and consent to participate**

Authorization of the patient to voluntarily participate was obtained throughout the application of informed consent and expected benefits were explained to each patient. Applied protocols were approved by Institutional Ethical Committee at IPK (CEI-IPK-8918). The handling of all the data of each patient, the clinical-epidemiological record and the results, were carried out under the strictest standards of confidentiality.

**Consent for publication**

Consent for the scientific/academic use of samples and data was obtained from the patients.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671.

2. Reveiz L, Maia-Elkhoury ANS, Nicholls RS, Sierra Romero GA, Yadon ZE. Interventions for American Cutaneous and Mucocutaneous Leishmaniasis: A Systematic Review Update. PLoS One.
3. Matlin SA, Depoux , Schütte S, Flahault A, Saso L. Migrants’and refugees’ health: towards an agenda of solutions. Public Health Rev. 2018;39(27):2-55.

4. Söbirk SK, Inghammar M, Collin M, Davidsson L. Imported leishmaniasis in Sweden 1993–2016. Epidemiol Infect. 2018;146(10):1267–74.

5. Beiter KJ, Wentlent ZJ, Hamouda AR, Thomas BN. Nonconventional opponents: a review of malaria and leishmaniasis among United States Armed Forces. PeerJ. 2019;7:e6313.

6. Herrera G, Barragán N, Luna N, Martínez D, De Martino F, Medina J, Niño S, Páez L, Ramírez A, Vega L, Velandia V, Vera M, Zúñiga MF, Bottin MJ, Ramírez JD. An interactive database of Leishmania species distribution in the Americas. Scientific Data. 2020;7(1):110.

7. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018;392(10151):951–70.

8. Pan American Health Organization. Leishmaniasis. Epidemiological Report of the Americas. Report No. 8 (Pan American Health Organization/World Health Organization, 2019).

9. Montalvo A, Fraga J, Blanco O, González D, Monzote L, Soong L, Capó V. Imported leishmaniasis cases in Cuba (2006-2016). What have we learned? Trop Dis, Travel Med Vaccines. 2018;4:7.

10. Deborggraeve S, Laurent T, Espinosa D, Van der Auwera G, Mbuchi M, Wasunna M, El-Safi S, Al-Basheer AA, Arévalo J, Miranda-Verástegui C, Leclipteux T, Mertensa P, Dujardin JC, Herdewijn P, Büscher P. A simplified and standardized polymerase chain reaction format for the diagnosis of leishmaniasis. J Infect Dis. 2008;198(10):1565–72.

11. Montalvo AM, Fraga J, Maes I, Dujardin JC, Van Der Auwera G. Three new sensitive and specific heat-shock protein 70 PCRs for global Leishmania species identification. Eur J Clin Microbiol Infect Dis. 2012;31(7):1453–61.

12. Fraga J, Montalvo AM, Maes I, Dujardin JC, Van der Auwera G. HindII and SduI digests of heat-shock protein 70 PCR for Leishmania typing. Diag Microbiol Infect Dis. 2013;77(3):245–7.

13. Gradoni L, López-Vélez R, Mokni M. Manual on Case Management and Surveillance of the Leishmaniases in the WHO European Region; World Health Organization: Copenhagen, Denmark, 2017.

14. Lavergne RA, Iriart X, Martin-Blondel G, Chauvin P, Menard S, Fillaux J, Cassaing S, Roques-Malecaxe C, Arnaud S, Valentin A, Magnaval JF, Marchou B, Berry A. Contribution of molecular diagnosis to the management of cutaneous leishmaniasis in travellers. Clin Microbiol Infect. 2014;20(8):O528-30.

15. Harrison N, Walochnik J, Rambasebner R, Veletky L, Lagler H, Ramharter M. Case report: Progressive perforation fo nasal septum due to Leishmania major. A case of mucosal leishmaniasis in a traveler. Am J Trop Med Hyg. 2017;96(3):653-5.

16. Fraga J, Veland N, Montalvo AM, Praet N, Boggild AK, Valencia BM, Arévalo J, Llanos-Cuentas A, Dujardin JC, Van der Auwera G. Accurate and rapid species typing from cutaneous and mucocutaneous leishmaniasis lesions of the New World. Diag Microbiol Infect Dis. 2012;74(2):142–50.
17. Fraga J, Montalvo AM, Van der Auwera G, Maes I, Dujardin JC, Requena JM. Evolution and species discrimination according to the *Leishmania* heat-shock protein 20 gene. Infect Gen Evol. 2013;18:229–37.

18. Montalvo AM, Fraga J, Montano I, Monzote L, Marin M, Van Der Auwera G, Dujardin JC, Velez ID, Muskus C. Differentiation of *Leishmania* (*Viannia*) *panamensis* and *Leishmania* (*V.*) *guyanensis* using Bccl for hsp70 PCR-RFLP. Trans Roy Soc Trop Med Hyg. 2010;104(5):364–7.

19. Montalvo AM, Fraga J, Montano I, Monzote L, Van der Auwera G, Marín M, Muskus C. Identificación molecular con base en el gen hsp70 de aislamientos clínicos de *Leishmania* spp. en Colombia. Biomédica. 2016;36(Supl.1):37-44.

20. Montalvo AM, Fraga J, Tirado D, Blandón G, Alba A, Van der Auwera G, Vélez ID, Muskus C. Detection and identification of *Leishmania* spp.: application of two hsp70-based PCR-RFLP protocols to clinical samples from the New World. Parasitol Res. 2017;116(7):1843–8.

21. Simon S, Nacher M, Carme B, Basurko C, Roger A, Adenis A, Ginouves M, Demar M, Couppie P. Cutaneous leishmaniasis in French Guiana: revising epidemiology with PCR-RFLP. Trop Med Health. 2017;45(1):5.

22. Rotureau B, Joubert M, Clyti E, Djossou F, Carme B. Leishmaniasis among Gold Miners, French Guiana. Emerg Infect Dis. 2006;12(7):1169-70.

23. Rotureau B, Ravel C, Nacher M, Couppie P, Curtet I, Dedet JP, Carme B. Molecular Epidemiology of *Leishmania* (*Viannia*) *guyanensis* in French Guiana. J Clin Microbiol. 2006b;44(2):468–73.

24. Blum J, Buffet P, Visser L, Harms G, Bailey MS, Caumes E, Clerinx J, van Thiel PPAM, Morizot G, Hatz C, Dorlo TPC, Lockwood DNJ. LeishMan recommendations for treatment of cutaneous and mucosal leishmaniasis in travellers, 2014. J Travel Med. 2014;21(2):116–29.

25. Gray KC, Palacios DS, Dailey I, Endo MM, Uno BE, Wilcock BC, Burle MD. Amphotericin primarily kills yeast by simply binding ergosterol. Proc Natl Acad Sci USA. 2012;109(7):2234–9.

26. Keighobadi M, Emami S, Fakhar M, Shokri A, Mirzaei H, Teshnizi SH. Repurposingazole antifungals into antileishmanials: Novel 3-triazolylflavanones with promising in vitro antileishmanial activity against *Leishmania major*. Parasitol Int. 2019;69:103–9.

27. Saleem K, Khursheed Z, Hano C, Anjum I, Anjum S. Applications of Nanomaterials in Leishmaniasis: A Focus on Recent Advances and Challenges. Nanomaterials. 2019;9(12):1749.

28. Cincurá C, de Lima CMF, Machado PRL, Oliveira-Filho J, Glesby MJ, Lessa MM, et al. Mucosal leishmaniasis: A Retrospective Study of 327 Cases from an Endemic Area of *Leishmania* (*Viannia*) *braziliensis*. Am J Trop Med Hyg. 2017;97(3):761–6.

29. Correa-Cárdenas CA, Pérez J, Patino LH, Ramírez JD, Duque MC, Romero Y, Cantillo-Barraza O, Rodríguez O, Alvarado MT, Cruz C, Méndez C. Distribution, treatment outcome and genetic diversity of *Leishmania* species in military personnel from Colombia with cutaneous leishmaniasis. BMC Infect Dis. 2020;20(1):938.

30. Kariyawasam R, Challa P, Lau R, Boggild AK. Susceptibility testing of *Leishmania* spp. against amphotericin B and fluconazole using the Sensititre™ YeastOne™ YO9 platform. BMC Infect Dis.
31. Srivastava S, Shankar P, Mishra J, Singh S. Possibilities and challenges for developing a successful vaccine for leishmaniasis. Parasites Vectors. 2016;9(1):277.

32. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, Lindsay SW. The importance of vector control for the control and elimination of vector-borne diseases. PLoS Negl Trop Dis. 2020;14(1):e0007831.

33. Bardosh KL, Ryan SJ, Ebi K, Welburn S, de Burton C. Addressing vulnerability, building resilience: community-based adaptation to vector-borne diseases in the context of global change. Infect Dis Poverty. 2017;6(1):166.

34. Maia-Elkhoury ANS, Valadas SYOB, Puppim-Buzanovsky L, Rocha F, Sánchez-Vázquez MJ. SisLeish: A multi-country standardized information system to monitor the status of leishmaniasis in the Americas. Plos Negl Trop Dis. 2017;11(9):e0005868.

35. Montalvo AM, Monzote L. Leishmania y leishmaniasis. 20 años de estudio en el IPK, aportes y perspectivas. Rev Cubana Med Trop. 2017;69(3):1-19.

36. Vandeputte M, van Henten S, van Griensven J, Huits R, Van Esbroeck M, Van der Auwera G, Cnops L, Bottieau E. Epidemiology, clinical pattern and impact of species-specific molecular diagnosis on management of leishmaniasis in Belgium, 2010–2018: A retrospective study. Travel Med Infect Dis. 2020;38:101885.

37. Kuna A, Gajewski M, Bykowska M, Pietkiewicz H, Olszański R, Myjak P. Imported cutaneous leishmaniasis: a 13-year experience of a Polish tertiary center. Adv Dermatol Allergol. 2019;XXXVI(1):104–11.

38. Delgado JP. Introducción de la técnica de la RCP ADNk (S35-S36) para el diagnóstico de la Enfermedad de Chagas en el Laboratorio Nacional de Referencia de Parasitología del IPK [Master thesis in Parasitology]. Institute of Tropical Medicine, Havana, Cuba 2018, pp: 97.

39. López JL. Paludismo importado: caracterización y riesgos asociados a la gravedad, Camagüey, 1986-2018. [Master thesis in Parasitology]. Institute of Tropical Medicine, Havana, Cuba 2019, pp: 97.

Tables

Table 1 General epidemiological data of patients with cutaneous leishmaniasis that arrived to IPK, Havana, Cuba, 2017
| Case    | Sex / Age | Depart | Route                                                                 |
|---------|-----------|--------|----------------------------------------------------------------------|
| 17-01   | Female / 49 | Havana | Guyana, Brazil, Peru, Ecuador, Colombia, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Mexico |
| 17-02   | Male / 55  | Havana | Guyana, Venezuela, Colombia, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Mexico |
| 17-03   | Male / 39  | Havana | Guyana, Suriname, Guyana                                             |
| 17-04   | Male / 27  | Havana | Brazil, Peru, Ecuador, Colombia, Panama                              |
| 17-05   | Male / 43  | Mexico |                                                                      |

The countries comprising all the routes followed by each patient before their arrival in Cuba are mentioned.

**Table 2** Samples and diagnostic results of patients with cutaneous leishmaniasis. IPK, Havana, Cuba, 2017

| Case    | Samples                              | Microscopy | Histology | PCR 18S | PCR F | PCR N |
|---------|--------------------------------------|------------|-----------|---------|-------|-------|
| 17-01   | Lancet scrapings and punch biopsy     | +          | +         | +       | +     | +     |
| 17-02   | Lancet scrapings and punch biopsy     | +          | +         | +       | +     | +     |
| 17-03   | Lancet scrapings and punch biopsy     | -          | +         | +       | Positive (w) | +     |
| 17-04   | Lancet scrapings                     | -          | +         | +       | +     | +     |
| 17-05   | Lancet scraping                      | +          | ND        | +       | -     | +     |

+: Positive result to diagnostic of *Leishmania* spp.

-: Negative result to diagnostic of *Leishmania* spp.

ND: Not done.

Positive (w): A weak amplicon was obtained.

**Table 3** Identified species or species complex in skin lesion from patients with cutaneous leishmaniasis. IPK, Havana, Cuba, 2017
| Case  | PCR-F / RFLP | PCR-N / RFLP | Species identified          |
|-------|--------------|--------------|----------------------------|
| 17-01 | L.bra        | L.bra        | L. braziliensis             |
| 17-02 | L. pan       | L. pan/L. guy| L. panamensis              |
| 17-03 | L. pan/L. guy* | L. pan/L. guy | L.pan/L.guy               |
| 17-04 | L. pan       | L. pan/L.guy | L. panamensis              |
| 17-05 | ND           | L. mexicana complex | L. mexicana complex |

*: The pattern obtained corresponded to bands characterizing *L. panamensis* (787 bp, 429 bp) and *L. guyanensis* (544 bp)

**Figures**
| Case  | Lesion | Clinical description |
|-------|--------|----------------------|
| 17-01 | ![Image](image1.png) | A lesion in the external upper part of the nose, boarding with the palpebral area (1 ½ cm diameter). Another one in the left arm (3 ½ cm diameter). Both active, well defined and ulcerated. |
| 17-02 | ![Image](image2.png) | An active lesion in the external side of the left leg (4 cm diameter). Border defined, ulcer and crust, over-infected and purulent. |
| 17-03 | ![Image](image3.png) | Two lesions ulcerated, crusty, on both sides of the back. Other numerous ones smaller, all around the area and near the shoulder. |
| 17-04 | ![Image](image4.png) | Several lesions in the border of the right ear. Lesions were crusty and over-infected. |
| 17-05 | ![Image](image5.png) | One lesion in the right leg, ulcerated and not crusty. |

**Figure 1**

Lesions and clinic description of patients with cutaneous leishmaniasis. IPK, Havana, Cuba, 2017 (Patients appear in chronological order to arrive at IPK)
Figure 2

Map route followed by the travellers arriving with cutaneous leishmaniasis that arrived to IPK, Havana, Cuba, during 2017. Arrows indicate the routes followed and countries visited by these five patients although it was impossible to identify the exact places/cities they passed by/stayed in.