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## Research note

Presence of parasite DNA in clinically unaffected nasal mucosa during cutaneous leishmaniasis caused by *Leishmania (Viannia) braziliensis*

A. Canário<sup>1,2,3</sup>, M. Queiroz<sup>3</sup>, G. Cunha<sup>3</sup>, T. Cavalcante<sup>3</sup>, V. Riesz<sup>1</sup>, R. Sharma<sup>1</sup>,  
 A. de Noronha<sup>1</sup>, T. Correia<sup>1</sup>, M. Barral-Netto<sup>1,2</sup>, A. Barral<sup>1,2</sup>, R. Khouri<sup>1,2</sup>,  
 V. Boaventura<sup>1,2,3,\*</sup>

<sup>1</sup> Instituto Gonçalo Moniz—Fundação Oswaldo Cruz, Bahia, Brazil

<sup>2</sup> Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal da Bahia, Bahia, Brazil

<sup>3</sup> Serviço de Otorrinolaringologia do Hospital Santa Izabel/Santa Casa de Misericórdia da Bahia, Bahia, Brazil

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## ABSTRACT

**Objectives:** We aimed to detect *Leishmania* DNA carriage in nasal mucosa of individuals with cutaneous leishmaniasis (CL) caused by *Leishmania (Viannia) braziliensis*.

**Methods:** A cross-sectional study was performed in all individuals with CL without nasal lesions ( $n = 153$ ) attended within 2 years in an endemic area of *L. (Viannia) braziliensis* in Bahia (Brazil). An otorhinolaryngologist assessed the clinical status of the nasal mucosa by anterior rhinoscopy and endoscopic examinations. Swab samples were collected for parasite DNA detection by PCR from all individuals before standard treatment for leishmaniasis. A second evaluation 3 months after treatment was performed to assess clinical outcomes.

**Results:** Parasite DNA was detected in 7.8% (12/153) of clinically healthy nasal mucosa of individuals with CL. Interestingly, DNA was more frequently identified in individuals with more skin lesions (median 1.5, interquartile range (IQR) 1–3.5 versus 1.0, IQR 1–1.5;  $p 0.044$ ), or larger injuries (median 2.7, IQR 2–3.8 versus 1.6, IQR 1–2.5;  $p 0.013$ ). Additionally, the disease of those individuals with positive PCR evolved more frequently to unusual forms of leishmaniasis (*recidiva cutis* and disseminated) (45.5% (5/11) versus 11.5% (14/122);  $p 0.009$ ), and required more cycles of treatment to reach clinical cure (median 2, IQR 1–4 versus 1, IQR 1–2;  $p 0.05$ ).

**Conclusion:** These findings suggest an early parasite tropism to nasal mucosa in *L. (Viannia) braziliensis* infection and a clinical phenotype of CL cases associated with parasite DNA in nasal mucosa. Future studies should evaluate whether PCR of nasal swab samples could serve as a prognostic tool for individuals at risk of mucocutaneous leishmaniasis. **A. Canário, Clin Microbiol Infect 2019;•:1**

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## Introduction

Tegumentary leishmaniasis (TL) represents a wide spectrum of disease caused by the protozoa *Leishmania*, comprising cutaneous (CL), mucocutaneous, disseminated and *recidiva cutis* leishmaniasis. Cutaneous leishmaniasis usually presents as a cutaneous ulcer that can later spread to the mucosa and cause destructive lesions in the

anterior portion of the nasal mucosa, leading to facial deformity [1–3].

As mucosal lesions occurs in about 4% of untreated CL cases within 3 years of disease onset [1,4], we aimed to evaluate the presence of parasite DNA in clinically healthy nasal mucosa of individuals with CL caused by *Leishmania (Viannia) braziliensis*, the only species that circulates in the endemic area of Bahia [5].

## Methods

We performed a cross-sectional study in a reference centre for TL in an area where *L. (Viannia) braziliensis* is endemic [5], at Vale do Jiquiriçá, Bahia, Brazil, from February 2015 to November 2017.

\* Corresponding author. V. Boaventura, Oswaldo Cruz Foundation—Bahia, R. Waldemar Falcão, 121, 40296-710, Salvador, Bahia, Brazil.

E-mail address: [vsboaventura@gmail.com](mailto:vsboaventura@gmail.com) (V. Boaventura).

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Clinical examination, leishmania skin test and biopsy were performed in all 160 individuals with CL. Diagnostic criteria were the presence of a typical skin lesion, with a painless ulcer, well circumscribed, with raised, hardened and erythematous borders [2,6], and a positive leishmania skin test (detected in 84.8%) or parasite detected in histopathological examination (43.1%). Seven cases (4.4%) of concomitant mucosal and cutaneous lesion were excluded from the analysis. The remaining 153 individuals with CL without nasal lesion detected by otorhinolaryngological examination underwent nasal swab collection (Catch-All™ Sample Collection Swab; Epicentre, Illumina, Madison, WI, USA). The swab was rotated two to three times in each nasal fossa at the anterior septum and inferior turbinate head, without the introduction of a nasal speculum into the nasal cavity. Swabs were immediately stored in dry ice in an isolated sterile container until storage at  $-80^{\circ}\text{C}$  in the Laboratory of Emerging Infections Transmitted by Vectors at the Instituto Gonçalo Moniz of the Oswaldo Cruz—BA Foundation for subsequent processing.

After treatment of CL with intravenous meglumine antimoniate (15–20 mg  $\text{Sb}^{+5}$ /kg/day, for 20 days) as previously described [2], the clinical status of cutaneous disease and nasal mucosa was assessed in a second clinical evaluation. No additional swab sample was obtained. Disseminated leishmaniasis was defined as the presence of more than ten lesions localized in at least two body segments. *Recidiva cutis* leishmaniasis was defined as the presence of a papular and vesicular lesion around the scar of a CL lesion.

DNA extraction from the nasal swab samples was performed using the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The presence of *Leishmania* DNA in swab samples was evaluated by PCR assays using sense primer 150 (3'-GGGKAGGGCGTTCTSCGAA-5') and anti-sense primer 152 (3'-SSSWCTATWTTACACCAACCC-5') directed to the conserved region of *Leishmania* genus minicircle kDNA and following the protocol adapted from Oliveira et al. [7]. Negative and positive controls were included in all PCR assays. The PCR tests were performed only after all samples were collected and follow up was completed. Results were described as median and interquartile range (IQR). Fisher exact test (for categorical variables) and Mann–Whitney *U*-test (for continuous variables) were performed according to the Kolmogorov–Smirnov test for normality, using SPSS software (version 20.0; IBM, Chicago, IL, USA). These

were all two-tailed with a significance level of 5%. The study was approved by the IGM-Fiocruz Review Board.

## Results

Parasite DNA was detected in 12 out of 153 (7.8%) nasal swab samples of patients with CL and clinically healthy mucosa (Table 1). PCR-positive individuals presented with more lesions (1.5 versus 1.0;  $p$  0.044) and larger lesion size (2.75 versus 1.6;  $p$  0.013) when compared with individuals with negative nasal PCR.

During clinical follow up (median 3 months) an increased frequency of unusual forms of TL was detected in PCR-positive individuals (45.5% (5/11) versus 11.5% (14/122),  $p$  0.009). Among 11 *Leishmania*-positive individuals with complete follow up, 4 (36.4%) evolved to *recidiva cutis* leishmaniasis and one (9.1%) to disseminated leishmaniasis. Individuals with positive PCR test also required more cycles of treatment to reach clinical cure (2 versus 1;  $p$  0.031). None of these 11 patients developed nasal complaints or mucosal lesions.

## Discussion

In this study, *Leishmania* DNA carriage in nasal mucosa was detected in 7.8% of CL cases. Additionally, clinical outcome and therapeutic response were different in PCR-positive cases compared with PCR-negative. Positive cases presented with more skin lesions or larger injuries, evolved more frequently to unusual forms (*recidiva cutis* leishmaniasis and disseminated leishmaniasis) of TL and required more cycles of treatment to reach clinical cure.

Interestingly, recent reports from Colombia demonstrated a higher occurrence of *Leishmania* DNA in mucosal swab samples (45%–61.5%) from individuals with acute CL with apparently unaffected mucosa [8,9]. We speculate that this difference may be due to the mucosal tropism of the *Leishmania* (*V.*) subspecies circulating in Colombia since *Leishmania panamensis* causes concomitant cutaneous and mucosal lesions more frequently than other subspecies [10]. Additionally, an RNA virus infecting some species of *Leishmania* detected in Colombia has been associated with an increased risk of subsequent mucosal lesion [11]. In the endemic area of Bahia, only *L. (V.) braziliensis* circulates [5] and this virus has not been detected (data not shown; Aldina Barral, 2001), possibly

**Table 1**  
Characteristics of cutaneous leishmaniasis patients with positive or negative PCR tests

	PCR test		p <sup>a</sup>
	Positive	Negative	
	12 (7.8%)	141 (92.3%)	
Gender, male, n (%)	9 (75%)	79 (56%)	0.239
Age (years), median (IQR)	47 (22, 54)	36 (25.5, 49)	0.724
Cutaneous lesion			
Number, median (IQR)	1.5 (1, 3.5)	1 (1, 1.5)	0.044
Size (cm), median (IQR)	2.75 (2, 3.8)	1.6 (1, 2.5)	0.013
Location above belt-line, n (%)	6 (50%)	51 (36.2%)	0.365
Duration of symptoms (months), median (IQR)	2 (1, 2.75)	2 (1, 3)	0.853
LST (mm), median (IQR)	11.5 (4, 18)	15 (10, 20)	0.277
Treatment with MA (number of cycles), median (IQR)	2 (1, 4)	1 (1, 2)	0.031
Follow up until clinical cure (1.5–7 months), n (%)	11 (91.7%)	122 (86.5%)	0.516
Outcome			
Recidiva cutis, n (%)	4 (36.4%)	11 (9%)	0.022
Disseminated leishmaniasis, n (%)	1 (9.1%)	3 (2.5%)	0.295
Clinical cure/complete healing, n (%)	6 (54.5%)	82 (67.2%)	0.295

Abbreviations: CL, cutaneous leishmaniasis; IQR, interquartile range; LST, leishmania skin test; MA, meglumine antimoniate.

<sup>a</sup> Fisher (categorical variables) or Mann–Whitney *U*-test (numerical variables).

contributing to a reduced frequency of mucosal lesions among individuals with CL.

Parasite DNA detected in nasal mucosa might not necessarily represent metastasis of a cutaneous lesion and ongoing parasite replication at this site, although it is unlikely that it represents direct infection by a sand fly bite. Of note, several studies have demonstrated *Leishmania* amastigotes in mucosal lesions by histopathology, suggesting that parasites can both establish and replicate at the nasal site [4].

In our series, some clinical variables previously recognized as risk factors for mucosal involvement [1,6,12,13], including multiple and larger cutaneous ulcers, were present in the patients with parasite DNA detected in the nasal mucosa. Furthermore, individuals with *Leishmania* DNA carriage in the nasal mucosa evolved more frequently to atypical forms of TL (45.5% versus 11.5%). The prevalence of mucosal involvement in individuals with disseminated leishmaniasis, an atypical form of TL, is higher than in patients with CL [14]. Given these findings, parasite DNA carriage in nasal mucosa may be linked to subsequent development of mucosal lesions.

We cannot address the impact of therapy on eliminating the parasite from mucosa because no nasal swab sample was obtained after treatment. Considering the finding that early treatment of mucosal lesions results in successful outcomes [15], a different management strategy, with routine PCR analysis of nasal swabs, could be evaluated as a screening tool in CL. Additionally, given the study design, we cannot predict the risk of developing a mucosal lesion. Of concern, mucosal lesions can occur many years after CL and individuals with positive PCR should be monitored for a long period of time to evaluate the occurrence of mucocutaneous leishmaniasis. A further limitation is the absence of a PCR test for all cutaneous lesions. Even considering the fact that only *L. braziliensis* has been characterized in this endemic area [5], we cannot exclude the existence of other species circulating in the same region.

In conclusion, the carriage of parasite DNA in the nasal mucosa of individuals with CL was associated with a more severe clinical presentation and disease outcome. Whether the presence of parasite DNA in the nasal mucosa of patients with CL due to *L. (Viannia) braziliensis* might be a risk factor for subsequent development of mucocutaneous leishmaniasis still needs to be studied in prospective cohort studies of adequate size.

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### Transparency declaration

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