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Cutaneous Leishmaniasis Caused by *Leishmania major*: A Potential Transmission Hotspot in Senegal: The Case of a 17-Year-Old Girl

Souleye Lelo[1,2*], Abdoulkarim Mhaji[1], Khadime Sylla[1,2], Aissatou Gaye[1,2], Ami Lam[1], Isaac Akhenaton Manga[1], Carole Pab Minlekib[1], Cheikh Binetou Fall[1], Magatte Ndiaye[1], Roger Tine[1], & Babacar Faye[1]

1. Department of Parasitology and Mycology, Cheikh Anta Diop University, Dakar, Senegal
2. Laboratory of Parasitology and Mycology, CHNU of Fann, Dakar, Senegal

Abstract: Introduction: This is a 13-year-old girl with negative immunological status who was referred to us by the dermatology department at Fann Hospital for a painless ulcerated lesion on her left forearm, which had been developing for three months. Methodology: A serous sample was taken from the lesion for smear and PCR testing. The slides were stained with a 10% Giemsa solution before being read under a microscope with a 100x objective lens. For PCR testing, DNA extraction was first performed using the Quick DNA miniprep Plus kit. Next, nested PCR was performed with the CSBIX-R and CSB2-F primers for the first amplification, and the 13Z and LIR primers for the second amplification. The positive controls used were*: Leishmania tropica, Leishmania infantum, and Leishmania major*, and water without Rase was used as a negative control. The amplification product was revealed after migration on a 1.5% agarose gel and reading under ultraviolet light. Result: Microscopy revealed amastigote forms of Leishmania, and PCR was used to conclude that the disease was caused by *Leishmania major*. Conclusion: Leishmaniasis is still present in Senegal with a potential residual transmission focus.

Keywords: *Leishmania major*, skin lesion, nested PCR, Senegal

INTRODUCTION

Leishmaniasis is a vector-borne parasitic disease caused by protozoa of the genus Leishmania and transmitted to humans through the bite of infected female sandflies. According to the World Health Organisation, the annual number of leishmaniasis cases is estimated to be between 700,000 and 1 million in 2023 [1]. This zoonosis encompasses several clinical forms, including cutaneous leishmaniasis, cutaneous-mucosal leishmaniasis and visceral leishmaniasis, the severity of which depends on both the species of parasite involved and the host's immune response [2]. Of these forms, cutaneous leishmaniasis is the most common and is generally characterised by chronic ulcerated lesions that can lead to permanent scarring and significant social stigma [1]. Every year, several hundred thousand new cases of cutaneous leishmaniasis are reported worldwide, with high levels of endemicity observed in certain regions of Africa, the Middle East, Asia and Latin America. In Senegal, several cases of leishmaniasis have been reported, particularly in the central and northern regions of the country [3-4]. *Leishmania major* is considered to be the predominant species responsible for the cutaneous forms observed. However, cases of leishmaniasis in dogs and rodents have also been reported [5-6-7]. However, epidemiological data remain limited, and the condition is likely to be underdiagnosed due to the wide variety of clinical presentations and the diagnostic challenges encountered in healthcare settings.

The diagnosis of leishmaniasis is traditionally based on direct parasitological methods such as microscopic examination or culture, the sensitivity of which can vary [8]. In recent years, molecular approaches, particularly PCR targeting kinetoplast DNA (kDNA), have significantly improved the detection of the parasite thanks to their high sensitivity and specificity [9-10-11]. Furthermore, molecular tools not only enable the diagnosis to be confirmed, but also allow the specific Leishmania species involved to be identified, which is essential for clinical management, epidemiological surveillance and understanding the dynamics of transmission.

In this study, we report a case of cutaneous leishmaniasis diagnosed in Senegal, confirmed by molecular methods that enabled the identification of the parasitic species involved.

PRESENTATION OF THE CASE

Patient Information

This is a 17-year-old girl from Malem Hoddar (central Senegal), a vocational student, who presented with a wound on her forearm that had been present for three months despite treatment with amoxicillin-clavulanic acid and paracetamol. A second consultation took place at a health centre, where a treatment regimen comprising amoxicillin-clavulanic acid, Fucidin cream and paracetamol was prescribed. As the symptoms persisted, she was referred to a dermatology department.

Clinical Results

The physical examination revealed a patient in good general health, fully alert, with no skin turgor loss and conjunctival mucosa showing little colouration. A large, infiltrated, rounded ulcerated patch, measuring 6 cm in its longest dimension, was located on the left forearm. There were no peripheral lymphadenopathies and the remainder of the physical examination was normal.

Diagnostic Approach

Biochemical tests showed the following blood count results: haemoglobin: 10 g/dl, leukocytosis: 12,500/mm^3, platelets: 340,000/mm^3. Other biochemical tests revealed the following: CRP: 12 mg/l, creatinine: 10 mg/l, urea: 0.4 g/l, blood glucose: 0.7 g/l, Na+: 132 mEq/l, K+: 4.4 mEq, Cl-: 98, Ca2+: 108 mg/l and normal liver function. At the parasitology laboratory at Fann Hospital Dermal fluid was collected by scarifying the periphery of the lesions. Two slides were used to create smears by applying them to the lesion. The smears were stained with May-Grünwald-Giemsa and then observed under a light microscope using a 100x objective and immersion oil **[12].** Microscopic examination of the smears revealed amastigote forms of Leishmania sp in the form of rounded, immobile elements within the macrophages (Figure 2).

Confetti was also made by applying Whatman® blotting paper to scarified lesions under sterile conditions for the search for Leishmania species deoxyribonucleic acid (DNA)

by Polymerase Chain Reaction (PCR). The nested PCR technique was carried out at the Parasitology Laboratory of the Cheikh Anta Diop University of Dakar (UCAD).

DNA extraction was performed using the Zymo Quick-DNA™ Miniprep plus Kit (USA) according to the manufacturer's instructions under sterile conditions. A nested PCR was carried out to amplify the variable region of the kinetoplast minicircle DNA (kDNA). The first round of amplification was performed using the primers CSB2XF (5′-ATTTTTCGCGATTTTCGCAGAAACG-3′) and CSB1XR (5′-CGAGTAGCAGAAACTCCCGTTCA-3′) **[13].** The PCR reaction mixture contained 1.0X TEMPase Hot Start Master Mix (including 3.0 mM MgCl2, 0.4 mM dNTPs, and 0.2 units/µL TEMPase Hot Start DNA Polymerase), 1 µM of each primer, and 5 µL of extracted DNA. Amplification was carried out in an Applied Biosystems™ ProFlex™ thermocycler with the following conditions: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 1 min, and extension at 72°C for 1 min 30 s; followed by a final extension at 72°C for 10 min. Positive controls consisting of *L. major* (MHOM/SU/73/5ASKH) and *L. tropica* (MHOM/SU/74/K27) **[14],** as well as a negative control (Nuclease-Free Water), were included in each PCR run.

The nested PCR reaction mixture was identical to that of the first round, except that primers LIR (5′-TCGCAGAACGCCCCT-3′) **[12]** and 13Z (5′-ACTGGGGGTTGGTGTAAAATAG-3′) **[15]** were used, with the first-round PCR product serving as the template. The amplified products were separated on a 1.5% agarose gel and sized using a 100 bp DNA ladder marker (New England Biolabs, Boston, MA). Electrophoresis was performed at 80 V for 1 h, and DNA bands were visualized under UV light using the Spectroline® transilluminator and a Bio-Rad Gel DocTM EZ System. The expected product sizes were ~680 bp for *L. infantum*, ~750 bp for *L. tropica*, and ~560 bp for *L. major* **[16].** Only *L. major* has been identified (Figure 3).

Therapeutic Intervention and Follow-up

The patient was treated with 1.5 g of meglumine antimoniate (1.5 g/5 ml in an injectable ampoule containing 0.3 g of meglumine antimoniate), administered by intralesional injection at a dose of 1 ml twice a week for four (04) weeks. Healing was achieved with the disappearance of the lesion, leaving a faint scar (Figure 1B). The patient was reviewed six months later

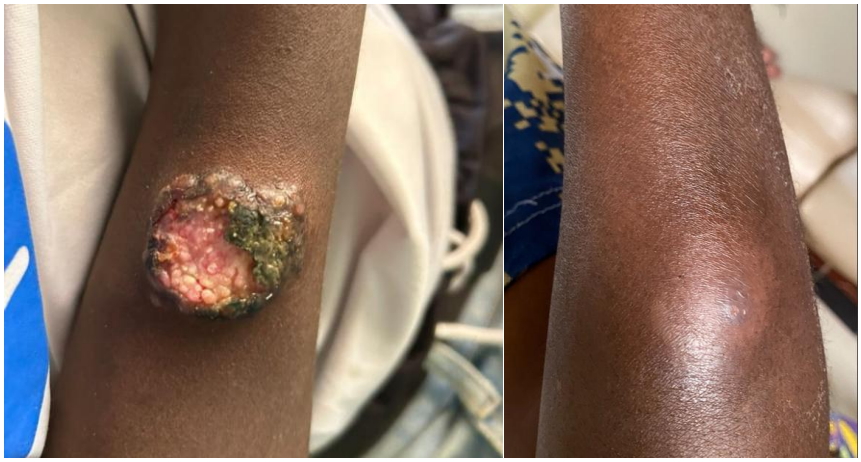

Figure 1 A, B: Aspect de la lésion avant et après traitement

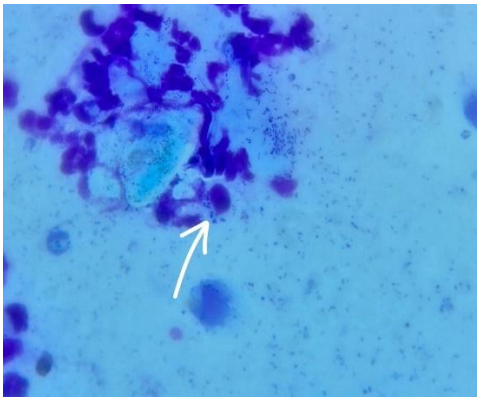

Figure 2: Amastigote form of Leishmania sp. under an optical microscope.

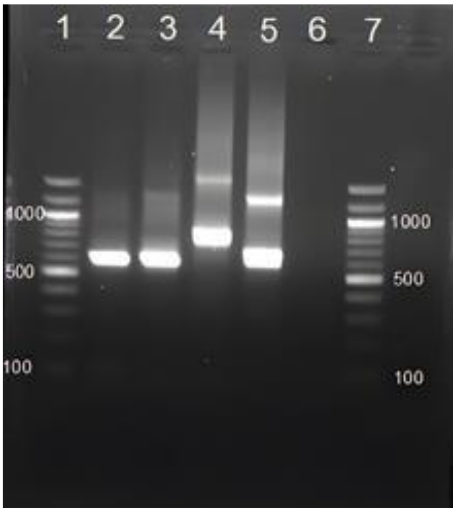

Figure 3: Agarose gel electrophoresis showing nested PCR products for the molecular diagnosis of *Leishmania* species: lane 1 and 7: Molecular weight (bp); lane 2 and 3: samples; lane 4: *L. tropica* (Positive control); lane 5: *L. major* (Positive control); lane 6: Negative control.

DISCUSSSION

Cutaneous leishmaniasis caused by *L. major* is frequently reported worldwide, but the majority of cases are not always reported to the authorities [17]. The gradual emergence of new transmission foci is a factor contributing to the spread of the infection and poses a significant risk of multiple Leishmania species coexisting [18]. Cutaneous leishmaniasis (CL) caused by *L. major* is endemic in Senegal. According to a case series by a Senegalese author, the disease affected young adults, with a predominance of males [19]. Ulcerative-crusted cutaneous leishmaniasis (CL) is the most common clinical form in Senegal, but pseudo-lepromatous and pseudo-tumourous forms have also been reported [19].

In our case presentation, our patient attended several appointments over a three-month period before receiving a diagnosis in July; this seasonal variation has been observed in several studies [20- 21]. The gender of our patient is also reflected in the literature. Both genders were affected, but with a strong predominance of males (accounting for 66.7% of

cases). This distribution is consistent with that observed in Morocco in a study conducted at a military training hospital [22].

Our patient presented with a lesion on the upper limbs; this pattern of lesions affecting the upper and lower limbs, which is also reported in the literature, can be explained by the fact that these parts of the body are usually exposed and therefore more accessible to sand fly bites [20, 22, 23]. Microscopy enabled the identification of the amastigote form as described in the literature. As described in the literature, PCR enabled species diagnosis. In the case of leishmaniasis, PCR is used to confirm the clinical diagnosis of both cutaneous and visceral leishmaniasis and to identify the Leishmania species responsible. It is currently the most widely used technique for diagnosing leishmaniasis because it is highly sensitive and highly specific. [24]

Our patient responded well to meglumine antimoniate, as described by the authors.

Other studies conducted in Tunisia and Morocco have also shown that meglumine antimoniate (Glucantime) remains the most commonly used drug for the treatment of cutaneous leishmaniasis. [24, 20, 23].

CONCLUSION

Leishmaniasis is still present in Senegal, with a potential transmission hotspot. There is a need to strengthen the epidemiological surveillance system for this disease (reservoir and vector). This work should encourage further research into species identification, on the one hand, and, on the other, investment in the genetic analysis of Leishmania isolates with a view to investigating the correlation between genotype and the clinical presentation of lesions.

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Ethics/Consent statement: This study was conducted according to the Declaration of Helsinki and existing national legal and regulatory requirements. The informed consent of the patient's father was obtained, and the child's assent was also sought. All information that could identify the patient has been removed to protect her privacy, and the father agreed to the use of this information, including photographs.

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Competing Interests: The authors declare that they have no competing interest

Authors' Contributions: SL conceived and designed the study. SL supervised the data collection. SL analyzed the data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Availability of Data and Material: The data used for this research article are available from the corresponding author upon request.

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