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Histological Evidence of the Migration of *Leishmania mexicana* from the Plantar Pad to the Heart in a Murine Model

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ABSTRACT

Leishmaniasis is a global tropical disease that is far from being eradicated. There are three types: cutaneous, visceral, and mucocutaneous. We reproduced cutaneous leishmaniasis in a murine model and are interested in parasite migration because we believe that studying this process will allow us to halt the parasite's spread to the organs of infected patients. Therefore, in this study, we aimed to demonstrate that the parasite does indeed migrate, in this case from the mouse's foot pad to its heart. Using histological techniques, we found the inoculated parasite not only in the mice's foot pads but also in their hearts.

Keywords

Cutaneous leishmaniasis, *Leishmania spp*, Parasite migration, Parasite dissemination, Murine model, Histopathology, Hematogenous dissemination.

Introduction

Leishmania spp. is an intracellular protozoan that affects humans and animals and is transmitted by the bite of blood-sucking sandflies. Each year, 1.5 million new cases occur in humans [1-4]. There are three forms of the disease: cutaneous, mucosal, and visceral. Cutaneous leishmaniasis causes chronic skin lesions ranging from nodules to large ulcers that can persist for months or years, although they may eventually heal. Mucocutaneous leishmaniasis affects the nasopharyngeal tissues and can cause visible mutilation of the nose and palate. Visceral leishmaniasis causes irregular fever, hepatosplenomegaly, hypergammaglobulinemia and is associated with a high mortality rate [2]. Diagnosis is based on demonstrating the parasites in smears or cultures, and increasingly on PCR-based assays (Polymerase Chain Reaction). Serological tests can be useful in the diagnosis of visceral leishmaniasis, and oral miltefosine is used to treat all three forms of the disease [3]. Due to the scarcity of *in situ* morphological information on *Leishmania mexicana*, and especially regarding parasite migration, we set out to demonstrate the parasite's invasion of the heart. Using a murine model, we found the hearts of inoculated mice invaded by parasites shortly after infection [5].

Objective

To demonstrate the migration of *Leishmania mexicana* from the plantar pad to the heart of BALB/c mice, using histological techniques.

Methods

Inoculation: Five BALB/c strain mice were inoculated with *Leishmania mexicana* in the plantar pad of the right paw ($10x10^6$ million) and after 6 weeks the mice were sacrificed, the infected paw was dissected and the heart was removed.

Fixation and Infiltration in Paraffin: The tissues were fixed overnight in 10% formalin buffered with PBS 1x pH neutral.

Dehydration was performed in graded alcohols and three changes in xylene, after that were incubated parafin at 60°C with three 1-hour changes, before being embedded in paraffin blocks.

Hematoxylin and Eosin (H&E) Staining: 2 micron sections were made; deparaffinized for 30 min at 60° in a histological oven; 2 steps of 10 min in xylene; graded alcohols, running water, Hematoxylin (3 min.); wash in running water; acid alcohol (quick bath); running water; ammonia water (bath); running water; 96% alcohol (bath); Eosin, (3 min); dehydration, graded alcohols (baths); xylene (2 baths); mount with Entellan resin.

Results

The five mice inoculated with *Leishmania mexicana* showed the same morphological results: in the footpad (Figures 1 and 2), the parasites were seen inhabiting abundant brown adipose tissue. In the heart (Figures 3 and 4), the parasites were seen in the extracellular space of the cardiac muscle fibers. In Figure 3, a fat droplet (FD) appears to be present, and in Figure 4, numerous nuclei, seemingly from macrophages, were also observed. It is worth noting that hematoxylin and eosin staining provided significant results, leaving no doubt about the morphology of the parasites in both the footpad and the heart.

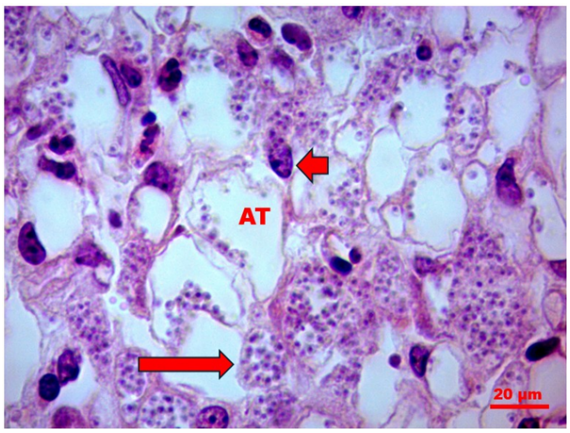


Figure 1: Plantar pad infected, 100x magnification, H&E stain, 2-micron section. The long arrow points to the parasites, and the short arrow points to the nucleus of the adipose tissue (AT).

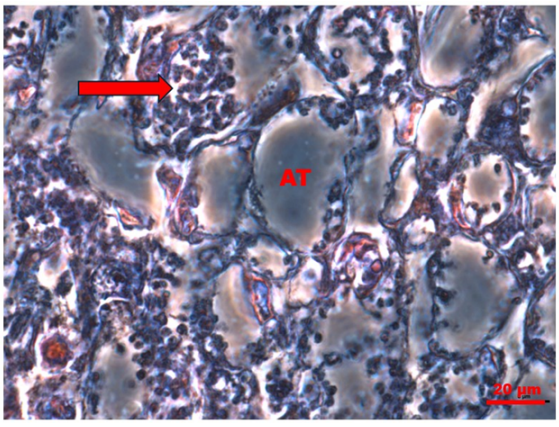


Figure 2: Plantar pad infected, 100x magnification, H&E stain, 2-micron section, blue filter. The long arrow points to the parasites populating the adipose tissue (AT).

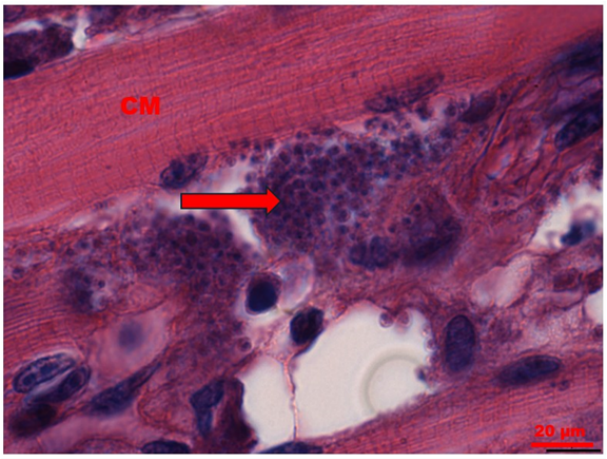


Figure 3: Infected heart, 100x magnification, H&E-stained 4-micron section. The arrow points to parasites populating the extracellular space of the cardiac muscle (CM).

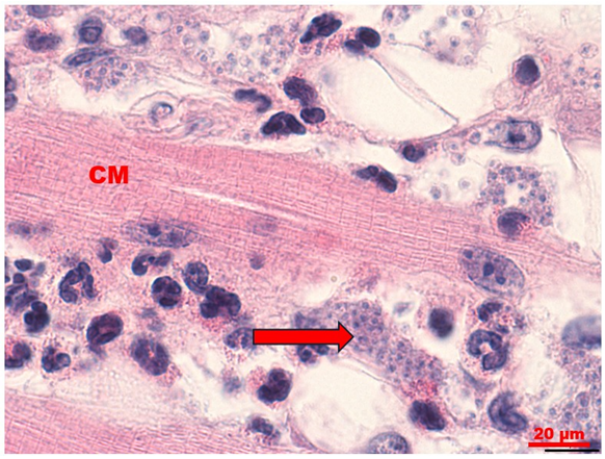


Figure 4: Infected heart, 100x magnification, H&E stain, 2-micron section. The arrow points to parasites populating the extracellular space of the cardiac muscle (CM).

Discussion

There are very few studies regarding the migration of *Leishmania spp.* to various organs, whether in animal models or in patients suffering from leishmaniasis. The observations in this study indicates that following the bite of the sandfly—which infects not only humans but mammals in general—nodules grow in the organs and ulcers develop on the skin after a certain period. These manifestations of the disease warrant further study to help prevent the parasite's migration and, consequently, its invasion of the infected host's body.

Conclusion

The results obtained in this study, conducted using a murine model, demonstrate the migration of *Leishmania mexicana* from the footpad to the heart; this migration appears to occur shortly after infection, given the abundant number of parasites found in

the heart. It is worth noting that significant results can be obtained even when using simple techniques, such as Hematoxylin and Eosin staining.

Acknowledgement

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