Molecular Analysis of the Immune Response and Parasite Control in Hamsters Immunized with Live-Attenuated *KHARON1*-Deficient *Leishmania infantum* as an Immunoprophylactic Strategy for Visceral Leishmaniasis

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Leishmanization, based on the inoculation of live parasites into the skin, was the only effective strategy against cutaneous leishmaniasis. With molecular advances, it has become possible to develop attenuated, non-pathogenic parasites capable of inducing protective immunity in a safe and long-lasting manner. The deletion of the *KHARON1* gene in *Leishmania infantum* resulted in an attenuated strain (LiKH) that can infect mice and trigger a robust immune response. In this study, we evaluated the immunogenicity and vaccine efficacy of LiKH in hamsters, a model more clinical representative of the disease. The animals were divided into groups that received one or two vaccine doses via subcutaneous or intradermal routes. They were analyzed one month after immunization and at one and eight months post-challenge with virulent *L. infantum*. Gene expression of immune cytokines (*TGF-β*, *TNF*, *IL-1*, *IFN-γ*, and *IL-10*) in the spleen was assessed by RT-PCR (Reverse Transcriptase Polymerase Chain Reaction), and parasite load in the spleen and liver was quantified by qPCR (Quantitative Polymerase Chain Reaction). Gene expression results indicate a strong immune stimulation in the subcutaneously vaccinated groups shortly after immunization. Eight months after the challenge, increased cytokine expression was observed in the intradermally vaccinated groups. Additionally, at both post-challenge time points (1 and 8 months), a significant reduction in hepatic parasite load was observed in animals that received two doses, regardless of the route. Those that received two doses via the subcutaneous route also exhibited a lower splenic parasite load compared to the control group. These findings suggest that immunization with LiKH is highly immunogenic, inducing a mixed Th1/Th2 response and promoting infection control, highlighting its potential as a promising immunoprophylactic strategy against visceral leishmaniasis.

Keywords: *Leishmania infantum*, leishmanization, KHARON1

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