PROBIOTIC AS A VACCINATION PLATFORM AGAINST VISCERAL LEISHMANIASIS: CONSTRUCTION, COMPARISON WITH THE RECOMBINANT APPROACH AND IMMUNE RESPONSE ANALYSIS IN A MURINE MODEL

José Bryan da Rocha Rihs[1*]; Isabela de Brito Duval[1]; Jordânia Costa Pinto[1]; Tatyane Martins Cirilo[1]; Chiara Cássia Oliveira Amorim[1]; Marcelo Eduardo Cardozo[1]; Gabriela Gomes Monteiro Lemos[1]; Ana Rafaela Antunes Porto[1]; Luisa Vitor Braga do Amaral[1]; Izabela da Silva Oliveira[1]; Getúlio Motta Silva Junior[1]; Ana Clara Santana de Sousa[1]; Jorge Lucas Nascimento Souza[1]; Ana Laura Grossi de Oliveira[1]; Fernanda Alvarenga Lima Barroso[1]; Lilian Lacerda Bueno[1]; Vasco Ariston Azevedo[1]; Janete Soares Coelho dos Santos[2]; Ricardo Toshio Fujiwara[1].

[1]Federal University of Minas Gerais, Minas Gerais, Brazil; [2]Ezequiel Dias Foundation, Minas Gerais, Brazil

Visceral leishmaniasis (VL) is a severe zoonosis, potentially fatal if untreated. Despite efforts to develop VL vaccines, improving antigen presentation remains crucial. Probiotics like *Lactococcus lactis* are promising as delivery systems. This study aimed to develop and evaluate a probiotic-based vaccine against *Leishmania*, comparing its immunogenicity to a recombinant approach. Recombinant vectors pEXU-LALU:TryPan and pET-28a-TEV:TryPan were synthesized and transformed into *L. lactis* FnBPA+ and *Escherichia coli* Shuffle, respectively. Clone identity was confirmed via PCR, and the chimera was expressed and purified for cromatography. To determine the appropriate vaccine dose, growth curves of vaccine and control strains were performed, establishing the correlation between OD, CFU/mL, and cultivation time. A transfection assay in CHO cells with pEXU-LALU:TryPan assessed vector functionality. BALB/c mice were orally immunized with the probiotic, TryPan protein + MPLA, or both approaches combined. Antibody responses were analyzed via ELISA. PCR confirmed the vaccine probiotic and *E. coli* constructs, with 220 bp and 2000 bp fragments, respectively. The chimera was successfully expressed, purified, and identified immunologically. Growth curves showed cultures reached ~1×10^9 CFU/mL in 2–2.5 hours. Transfection success was validated by ELISA, indicating constitutive chimera production in CHO cells. ELISA from immunized mice showed recombinant immunization induced a strong systemic antibody response, unlike the oral probiotic. However, combining both strategies with a protein booster significantly enhanced specific antibody production. Future steps involve in vivo testing and assessing the cellular immune response to VL.

Supported by: FAPEMIG; CNPQ; CAPES.

Keywords: oral vaccine; probiotics; visceral leishmaniasis.