# Evaluation of distinct inoculation routes in the course of experimental infection by *Leishmania infantum* in BALB/c mice

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Using experimental models in pre-clinical research is essential when investigating new therapeutic approaches, and vaccine development, among other studies. The most commonly used models are mice, hamsters, and dogs. Therefore, the present study aimed to compare distinct inoculation routes in the course of experimental infection by *Leishmania infantum* strain in BALB/c mice. We evaluated parasite load, the circulating leukocyte profile in the peripheral blood, and IgG total anti-*Leishmania* induced by the infection on the main disease-affected organs 45, 60, and 90 days post-infection. In total, 108 animals were divided into 4 experimental groups, i.e., 9 animals per group at each evaluation time (n=9/time/group). Animals were infected with $1 \times 10^7$ stationary-phase *Leishmania infantum* promastigotes (MCAN/BR/2008/OP46) via intraperitoneal (IP) or intravenously by tail vein (TV) and retro-orbital plexus (RO). As a control group, we used non-infected (NI) animals. After 45 days, we found increased parasite load on the splenic compartment in animals infected per intravenous course when comparing RO with IP. In addition, there was a significant increase in parasite load comparing IP and TV after both 60 and 90 days. On the liver, the parasite load was higher in animals infected through RO in comparison to IP and TV regardless of time. We also observed a consistent and progressive parasitism increase in RO compared to IP and TV, which indicates that RO is a potential route for providing a reliable and efficient infection in inducing the infection progression. Since it is an unconventional route, we highlight the need for training and monitoring to mitigate possible complications for the animals. The analysis of the circulating leukocyte in the peripheral blood 60 days post-infection showed an increase in monocytes in animals intravenously infected through TV and RO when compared to NI. Also, we found increased B lymphocyte percentual after infection by IP and TV routes. Moreover, the RO group showed a higher B lymphocyte population in comparison with TV. Lastly, we observed enhancement of T $CD4^+$ lymphocytes in TV compared to RO. Only after 90 days post-infection, there was an increase in IgG total when comparing infection via RO with both TV and IP. Based on those routes of investigation, we intended to contribute to a better understanding the experimental *Leishmania infantum* infection course as well as provide a basis for a more adequate experimental protocol, a solid comparison of effectiveness and, lastly, optimizing the animal experimentation by decreasing the number of animals per procedure.

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**Keywords:** *Leishmania infantum*; inoculation routes; BALB/c; parasite burden; humoral response; peripheral blood mononuclear cells.