## VAC.11 - Developing an intranasal vaccine against canine visceral leishmaniasis: a study of efficacy in mice

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**Introduction:** Visceral leishmaniasis (VL) is caused in America and in Europe by *L. infantum* and is lethal if not treated. Although dogs are the main domestic reservoirs, available canine vaccines are administered only after 4 months of age, leaving younger puppies unprotected. Our group has developed a tolerogenic strategy based on mucosa vaccination to prevent the early Th2 counter-protective response elicited by the infecting parasite. Indeed, intranasal (i.n.) vaccination with whole *Leishmania amazonensis* antigens (LaAg) has proven to protect mice and hamsters against both cutaneous and VL, showing a broad spectrum of action. Moreover, retinoic acid (a vitamin A metabolite) encapsulated in nanoparticles (RA-NP) acts as an adjuvant for i.n. LaAg, increasing protection in BALB/c mice against *L. amazonensis* and in hamsters against *L. braziliensis* infection by enhancing $CD4^+$ $Foxp3^+$ $T^{reg}$ population in nasal mucosa draining lymph nodes. Besides being needle-free, an i.n. vaccine could be administered in dogs from 3 weeks of age, conferring earlier protection.

**Objective:** Based on these findings, we proposed to evaluate the LaAg/RA-NP i.n. vaccine efficacy against *L. infantum* infection in mice, aiming at the development of an innovative i.n. vaccine against canine VL.

**Methodology**: For comparative evaluation, BALB/c young (8 weeks-old) or newborn (10 days-old) mice were immunized with 2 i.n. doses of LaAg/RA-NP with a 7 day-interval between them. Controls were not vaccinated or received 3 s.c. doses of the marketed Leish-Tec® (10 μg protein + 50 μg saponin/dose) with a 7-day-interval. Seven days after immunization, Leishmania-specific antibodies were quantified in serum and animals were intravenously challenged with *L. infantum* promastigotes ($2 \times 10^7$). Seven days after infection, transcription factor and cytokine expression in spleen were evaluated by quantitative real-time PCR and 35 days after infection parasite loads were evaluated in liver and spleen by limiting dilution assay.

**Results:** As Leish-Tec®, our vaccine does not induce antibodies that interfere with the detection of active infection, using serum from infected animals as positive control. During early infection, LaAg/RA-NP modulates expression of transcription factors and cytokines in spleen, increasing, mainly, IL-10 expression, suggesting a peripheral suppressive response. We found that LaAg/RA-NP is more effective than Leish-Tec®, reducing 94% of the parasite load in the spleen and 91% in the liver compared to the non-immunized group against 54% and 82% reduction promoted by the latter. In addition, LaAg/RA-NP proved to be effective in newborn mice, reducing the parasite load in the spleen (75%) and liver (81%) compared to non-immunized animals.

**Conclusion**: Therefore, our results demonstrate that LaAg/RA-NP is a promising vaccine to be tested against canine VL particularly aimed at newborn animals.

**Keywords:** Canine visceral leishmaniasis; intranasal vaccine; mucosa