Vaccination With LetiFend®, A Novel Canine Leishmaniosis Vaccine, Does Not Interfere With Serological Diagnostic Tests

Virginia Iniesta[a], Javier Fernández-Cotrina[a], Laia Solano-Gallego[b], Isabel Monroy[a], Adela Gómez-Luque[a], Rubén Muñoz-Madrid[a], José Luís Barneto[a], Luis Carlos Gómez-Nieto[a], Mercedes Fabra[c], Dolors Balsa[c], Pilar Brazis[c].

[(a)] LeishmanCeres Group, Unidad de Parasitología, Facultad de Veterinaria, Universidad de Extremadura, Cáceres (Spain); [(b)] Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Autónoma de Barcelona, Barcelona (Spain); [(c)] Animal Health Unit, Laboratorios LETI S.L.U. Barcelona (Spain)

Background and Objectives

The administration of a vaccine may elicit antibody production that could be detected by standard diagnostic techniques. This fact may not allow Differentiating Infected from Vaccinated Animals (DIVA) (1). A DIVA vaccine makes possible the mass vaccination of a susceptible animal population without compromising the serological identification of convalescent individuals.

In canine leishmaniosis, antibody response to vaccines may be detected by standard diagnostic techniques, preventing the discrimination of vaccinated from naturally infected dogs. Moreover, anti-*Leishmania* antibody levels in vaccinated dogs may be detectable for months (2, 3).

Recently, the European Commission has granted the marketing authorization to LetiFend®. The active ingredient of this vaccine is Protein Q, a recombinant protein formed by the genetic fusion of five antigenic fragments from *L. infantum* intracellular proteins (3). LetiFend® is indicated for active immunization of non-infected dogs from 6 months of age to reduce the risk of developing an active infection and/or clinical disease after exposure to *L. infantum*. The vaccination course of LetiFend® is based in one injection, followed by annual booster injections.

The aim of the present study was to evaluate the potential interference of vaccination with LetiFend® with a wide range of *L. infantum* serological diagnostic tests.

Materials and Methods

Vaccine: Each dose of 0.5 ml vaccine contained recombinant Protein Q from *L. infantum* MON-1 lyophilisate (≥ 36.7 ELISA Units).

Dogs

- Vaccine group (ID1-ID10): Ten healthy Beagle dogs (4 male, 6 female) of 6 months of age were selected based on negative results in Immunofluorescence Antibody Test (IFAT) (titre <1/80), ELISA Soluble *Leishmania* Antigen (SLA) (O.D. < 0.437), smear test (4) and qPCR (6) in lymph node (LN) and bone marrow (BM). Dogs were subcutaneously vaccinated with one dose of LetiFend® at day 0. Sera samples were obtained on days 0, 7, 14, 21 and 28 post-vaccination (dpv).
- Negative Control (ID11-ID20): Ten healthy Beagle dogs (4 male, 6 female) of 6 months of age and not infected with *L. infantum* were selected based on negative results in IFAT (titre <1/80), ELISA SLA (O.D. < 0.437), smear test (4) and qPCR (5) in LN and BM.
- Positive Control (ID21-ID30): Ten adult dogs of different breeds (3 male, 7 female) naturally infected with *L.infantum* were selected based on positive results in IFAT (titre ≥1/80), ELISA SLA (O.D ≥ 0.437) and qPCR in BM and/or LN (5).

Analytical methods

Serological response to vaccination

The IgG2 antibody levels against Protein Q (the antigenic component of LetiFend®) were evaluated at days 0, 7, 14, 21 and 28 after vaccination by ELISA PQ (4).

Leishmaniosis serological diagnostic tests

Detection of *L.infantum* antibodies was performed by ten different diagnostic techniques, covering the main range of tests available throughout the EC.

- Indirect immunofluorescence Test (IFAT) was performed according to (4).
- ELISA
  - *in house* ELISA SLA were performed according to (4).
  - *In house Leishmania* ELISA was performed according to (6).
  - Leiscan®, and INgezim® *Leishmania* were performed according to the manufacturer's instructions.
- Rapid diagnostic tests: Kalazar Detect™ (InBios), Snap© *Leishmania* (IDEXX), Speedleish K™ (Virbac), WITNESS® *Leishmania* (Zoetis) and Uranotest Leishmania (Urano®Vet) were performed according to the manufacturer's instructions.

Parasitological analysis:

LN and BM Smear Tests and qPCR (4) were performed at day 0 and 28 dpv for parasite burden.

Statistical analysis

Pearson chi square test (with or without the Yates correction) or exact Fisher test was used for categorical data. Normal distribution was evaluated by Kolmogorov-Smirnov test for continuous variables. Student's t-test and the Mann-Whitney U test were used to analyze the effect of Group. The Student's test and the Wilcoxon signed rank sum test were used to investigate variations with time. The hypotheses were tested at the 5% significance level (alpha = 0.05) with 80% statistical power (1-beta = 0.8), using two-tailed tests.

Table 1: Parasitological characterization of selected dogs

	Vaccine group (Days post-vaccination)					Negative control group	Positive control group
	0	7	14	21	28		
Smear tests (BM, LN)	0	ND	ND	ND	0	0	ND
qPCR (BM and/or LN)	0	ND	ND	ND	0	0	100

Results are expressed as percentage of positive dogs. BM: Bone Marrow; LN: Lymph Node; ND: Not Determine

Tabla 2: Positive dogs (%) analyzed by different serological *Leishmania* tests

Serological methods		Vaccine group (Days post-vaccination)					Negative control group	Positive control group
		0	7	14	21	28		
IFAT		0	0	0	0	0	0	100
ELISA	ELISA SLA	0	0	0	0	0	0	100
	Leiscan®	0	0	0	0	0	0	100
	Ingezim® Leishmania	0	0	0	0	0	0	100
	In house Leishmania ELISA	0	0	0	0	0	0	100
Rapid Diagnostic Tests	Kalazar Detect™	0	0	0	0	0	0	60
	Snap© Leishmania	0	0	0	0	0	0	100
	Speedleish K™	0	0	0	0	0	0	70
	WITNESS® Leishmania	0	0	0	0	0	0	80
	Uranotest Leishmania	0	0	0	0	0	0	90

Data is expressed as percentage (%) of positive dogs in each group

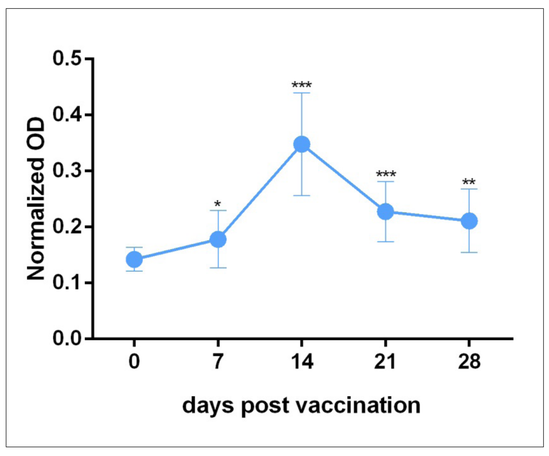

Figura 1: Kinetics of specific protein Q IgG2 response after vaccination in dogs
Data are expressed as the mean of normalized O.D. (± SD) of sera from ten vaccinated dogs analysed by ELISA at different days after vaccine administration. * p<0.05; **p<0.01; ***p<0.001 (Student's t-test)

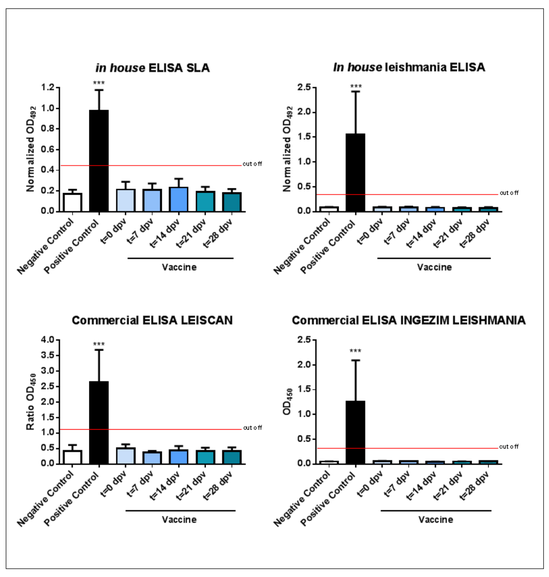

Figura 2: Serological diagnosis of leishmaniosis by different ELISA methods
Data are expressed as the mean of O.D. (± SD) of sera from dogs (n=10 in each group) analysed by the different ELISAs. ***p<0.001 vs Negative Control (Student's t-test); dpv: days post vaccination

Conclusions

The results of this study allow to conclude that:

- Vaccination with LetiFend® induces a transient increase in anti-Protein Q IgG2 antibodies in dogs that peaked at 14 days after vaccination and declined thereafter.
- Vaccination with LetiFend® does not interfere with the most widely used *L.infantum* serological diagnostic tests, allowing thus the discrimination between vaccinated animals and naturally infected dogs.

Results

Serological response to vaccination

The titers of antibodies (IgG2) against Protein Q, the antigenic component of LetiFend®, were evaluated at different time points after vaccination. The antibody levels transiently peaked at day 14 after vaccine administration and declined to baseline levels thereafter, being this increase significant at 7 (p=0.022), 14 (p<0.001), 21 (p<0.001) and 28 (p=0.001) days post administration (**Figure 1**).

Parasitological results

Vaccinated animals were negative to the presence of *L. infantum* in LN and BM at day 0 (pre-vaccination) and 28 post-vaccination (**Table 1**).

Detection of *L.infantum* antibodies

The proportion (%) of positive dogs analyzed by different serological and parasitological *Leishmania* tests is listed in **Table 2**. The results of the different ELISAs are showed in **Figure 2**. **Figure 3** shows the results of Snap Leishmania and Uranotest Leishmania as an example of rapid test.

The results obtained show that the anti-parasite antibodies were only detected in animals of the Positive Control group (infected).

All dogs of the Vaccine group (not infected) were shown to be negative at all the different time points after vaccination.

As expected, antibody levels were also negative in all dogs of the Negative Control group (non-vaccinated, non-infected).

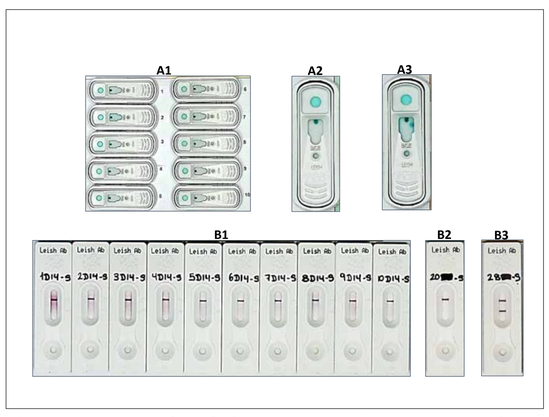

Figura 3: Example of the negative (A1, A2, B1 and B2) and positive (A3 and B3) results obtained with some of the *Leishmania* rapid diagnostic tests.
(A) Snap Leishmania and (B) Uranotest Leishmania were used to analyse sera from (1) vaccinated animals (at day 14 after vaccine administration), (2) Negative Control and (3) Positive Control dogs.

Bibliography

1. Van Oirschot JT: Diva vaccines that reduce virus transmission. J Biotechnol 1999; 73(2-3): 195-205.
2. Marcondes M, de Lima VM, de Araújo M de F, et al: Longitudinal analysis of serological tests officially adopted by the Brazilian Ministry of Health for the diagnosis of canine visceral leishmaniasis in dogs vaccinated with Leishmune®. Vet Parasitol 2013; 197(3-4): 649-52.
3. Ribeiro RA, Teixeira-Neto RG, Belo VS, Ferreira EC, Schallig HD, Silva ES: Ability of immunodiagnostic tests to differentiate between dogs naturally infected with Leishmania infantum and Leishmune(®)-vaccinated dogs. Vet Res Commun 2015; 39(2): 87-95.
4. Carcelén J, Iniesta V, Fernández-Cotrina J, et al. The chimerical multi-component Q protein from Leishmania in the absence of adjuvant protects dogs against an experimental Leishmania infantum infection. Vaccine 2009; 27(43): 5964-73.
5. Belinchón-Lorenzo S, Iniesta V, Parejo JC, et al. Detection of Leishmania infantum kinetoplast minicircle DNA by Real Time PCR in hair of dogs with leishmaniosis. Vet Parasitol 2013; 192 (1-3): 43-50.
6. Solano-Gallego L, Villanueva-Saz S, Carbonell M, Trotta M, Furlanello T, Natale A. Serological diagnosis of canine leishmaniosis: comparison of three commercial ELISA tests (Leiscan, ID Screen and Leishmania 96), a rapid test (Speed Leish K) and an in-house IFAT. Parasit Vectors 2014; 7:1-10.

SEVC X SOUTHERN EUROPEAN VETERINARY CONFERENCE GRANADA 2016 20-22 October - Spain