P-29

The significance of very low titres in IFAT for the detection of *Leishmania* infection: comparative result in 484 imported Spanish Galgos and 150 dogs from a non-endemic area in France

Patrick Bourdeau[1], Nicolas Soetart[1], Cecile Roux[1], Fanny Alexandre[1], Floriane Hubert[1], Caroline Dumont[1], Mansala Mukoko[1], Laetitia Jaillardon[1].

1. Lab. DPM/Laboniris. National College of Veterinary Medicine, Food Science and Engineering, Nantes, France.
*pjbourdeau44@gmail.com

Background

The laboratory diagnosis and follow-up of canine leishmaniosis (*L. infantum*) (CanL) is mainly based on the detection of specific anti-leishmanial antibodies by quantitative serological techniques. IFAT (indirect fluorescent antibody test) and ELISA (enzyme-linked immunosorbent assay) are the most common diagnostic techniques required by veterinary practitioners in many countries and used in clinical or research studies [1]. IFAT has been initially, and is still, usually performed with a cutoff titre (T) of 1/80 (1/100 in some laboratories), used to qualify dogs as seropositive or seronegative for the diagnosis of CanL [2]. However little information is available on the use of IFAT to detect infected but clinically healthy dogs. The aim of this study was to assess the significance of very low titers (LT) in dogs from a laboratory perspective (independently of clinical status), by comparing potentially infected imported Spanish Galgos with indigenous dogs living in a non-endemic area.

Material and methods

Sera from two groups of dogs were compared: A) originating/living in a non-endemic area of France (negative control) and, B) sera of Galgos sent to the laboratory for confirmation of clinical suspicion or detection of *Leishmania* infection or CanL. These dogs were tested at varying intervals after their importation to France. IFAT was performed (strain DPM IV74) at serial dilutions ranging from 1/20 up to 1/2560 (or higher if necessary). The results were grouped as follows: Negative N (T<1/20); Low titers: LT1 (T=1/20), LT2 (T=1/40) or “classically” Positive T≥1/80; with titers classified as moderate: T1(1/80-1/160), high: T2: (1/320-1/640) and very high: T3(≥1/1280).

Results

Group A included 150 dogs (healthy or sick regardless of the cause); Group B compiled 484 imported Galgos, with 92,5% of them living in non-endemic parts of France. Among these, 75 underwent follow-up testing once to up to 23 times, with at least 50% of them being tested twice or more over long periods. In Group A, as expected, no dog tested positive for Leishmania antibodies. Among these, 92,7% tested negative, while low LT were detected in only 7.3% of dogs (Table 1, Figure 1).

In Group B, 33.7% of sera were positive (T≥1/80) (n= 163), while 41.5 % (n= 201) were negative. LT were detected in 24.8% (n= 120) (Table 1), which sometimes persisted for extended periods (Table 2).

During the follow-up period, 3 out of the 13 dogs initially displaying LT1 changed to LT2, while 4 others shifted to positive titers, and among the 6 dogs initially presenting LT2, 3 subsequently tested positive. Among positive dogs undergoing treatment and follow-up, 3 dogs progressed to LT2, while one transitioned to LT1 before eventually returning to positive values (relapse), demonstrating the association between low titers and persistent infection (Table 2).

Conclusion

IFAT is suited for assessing low titers, representing 25% of IFAT results in our laboratory. In non-infected dogs, titers of 1/20 and 1/40 are very rare, if not entirely absent (see comments*, ** in Figure 1), unlike probably infected dogs. Among the non-seronegative dogs in Group B, 57.6% exhibited classical seropositive titers (≥1/80), while 42.4% displayed infra-diagnostic titers (1/20, 1/40), sometimes persisting for years (Figure 1).

These findings suggest that: a) a titre of 1/40 is virtually synonymous with *Leishmania* spp. infection (unless cross-reacting with *Trypanosoma* sp. is present); b) a titre of 1/20 is "compatible" with infection, necessitating further follow-up; c) only titers below 1/20 can be classified as (potentially) "seronegative".
These results may have implications for: 1) providing more accurate and appropriate laboratory interpretations of IFAT results (both in serosurveys and individual cases); 2) enhancing the precision of methodologies in seroprevalence studies, potentially warranting a revision of cutoff values to increase sensitivity for detecting clinically healthy infected dogs; and 3) shaping future considerations for vaccination protocols and travel guidelines for dogs.

Table 1: Distribution of IFAT values in control dogs and Galgos.

	Control		**Galgos**	
	n =	**%**	**n=**	**%**
Neg (<1/20)	139	92.7	201	41.6
LT1 (1/20)	9*	6	77	15.9
LT2 (1/40)	2**	1.3	13	8.9
T1 (1/80-1/160)	0		61	12.6
T2 (1/320-1/640)	0		74	15.3
T3 (≥1/1280)	0		28	5.8
Total	150	100	484	100

*,** see corresponding dogs in Figure 1.

Table 2: IFAT values and follow-up in Galgos.

				Follow-up					
Titre	**Initial**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th-10th**	**11th-15th**	**16th-23rd**
Neg (<1/20)	201	24	0	10	4	4	5	3	0
LT 1/20	77	17	11	3	3	1	6	2	0
LT 1/40	43	3	8	5	3	0	2	5	2
T1 (1/80-1/160)	61	17	11	6	5	5	14	4	6
T2 (1/320-1/640)	74	8	5	6	0	1	5	4	6
T3 (≥ 1/1280)	28	6	4	3	1	1	3	0	0
Nb. of tested dogs	484	75	39	33	16	12	7	5 to 2	2

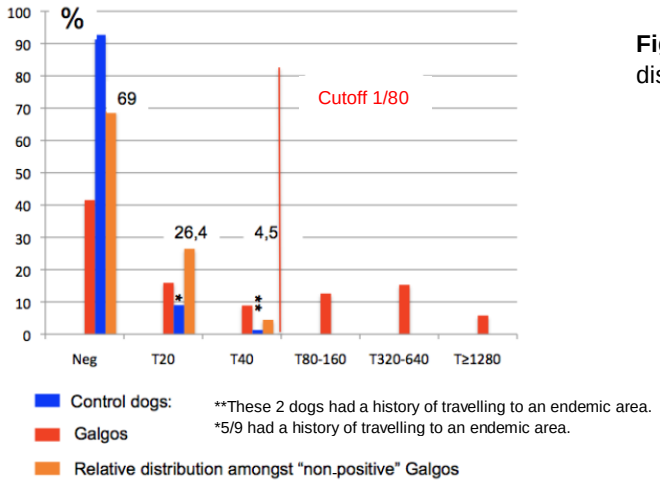

Figure 1: Comparative histograms of the distribution of IFAT in control dogs and Galgos.

Funding: self-funded.

Conflict of interest: none declared.

References

1. Bourdeau P, Saridomichelakis M.N, Oliveira A, Oliva G, Kotnik T, Galvez R, Foglia Manzillo V, Koutinas A.F, Pereira da Fonseca I, Miro G: Management of canine leishmaniosis in endemic SW European regions: a questionnaire-based multinational Survey Parasites & Vectors 2014, 7:1 10
2. Solano-Gallego L, Koutinas A, Miro G, Cardoso L, Pennisi MG, Ferrer L, Bourdeau P, Oliva G, Baneth G: Directions for the diagnosis, clinical staging, treatment, and prevention of canine leishmaniosis. Vet Parasitol. 2009, 165 (1–2): 1-18