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Comparative results between IFAT and ELISA for serological diagnosis of Canine Leishmaniosis and evaluation on possible use of intermediate IFAT titration

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Background

The laboratory diagnosis of infection, prognosis, and follow-up of Canine Leishmaniosis (*L. infantum*) remain mainly based on quantitative serology. The aim of the study was to compare Immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) techniques on a series of serum samples with a focus on very low titres and the possibility and reliability to propose intermediate titres for a more precise titration in IFAT.

Material and methods

A series of 234 sera were tested by: 1) IFAT (in-house strain DPM.IV74) at titres from 1/20 to 1/20480, with 1/80 defined as a classical positive diagnostic cutoff; 2) ELISA: (MEGA ELISA Leish Can. MEGACOR) (cutoff 10 MU = optical density OD 0.45). These techniques were previously validated separately through a ring test study presented at ALIVE 1 Conference [1].

Intermediate IFAT titres were calculated by comparison of results of two blind lectures of IFAT (positive: +, negative: - or doubtful:?) done by highly experimented technicians with potentially 3 divergences (- ?, ??, ?+) resulting in corresponding proposed intermediate titres (i.e.: 1/80, 1/100, 1/120, 1/140, 1/160). When observed, these titres were then compared to corresponding ELISA variations. Statistical analysis was performed using the Chi-squared test, with a significance level set at $p < 0.05$.

Results

The distribution of sera, including intermediate IFAT, is illustrated in Figure 1. A remarkably high concordance of 90.2% was observed between IFAT and ELISA regarding positive/negative classifications, as confirmed by the Chi-square test ($p < 7.34$ E-35). Relative sensitivities and specificities of IFAT according to ELISA, and vice versa, are indicated in Table 1.

Table 1: Relative specificity and sensitivity of IFAT (1/80) and ELISA (OD 0.45).

	ELISA +	**ELISA-**	**Total**	**IFAT according to ELISA**
IFAT +	133 (TP)	21 (FN)	154	Sensitivity 0,864
IFAT -	2 (FP)	78 (TN)	80	Specificity 0,975
Total	135	99	234	
ELISA according to IFAT	Sensitivity 0,985	Specificity 0,788		

TP: true positive; TN: true negative; FP: false positive; FN: false negative. The apparent lower sensitivity of IFAT is influenced by the high number of false negatives in ELISA for low positive titres (see Figure 1), and similarly for the specificity of ELISA.

However, the concordance varied based on the levels of anti-*Leishmania* antibodies present. For high IFAT titers (≥ 1/320), the concordance for "positive/negative" classifications was nearly complete at 99.1%. For sera classified as "negative" (< 1/80), the concordance for "positive/negative" classifications was 97.5%. In the case of positive sera falling within the interval 1/80-1/160, the concordance was notably lower at 54.3%.

For 44,8% of the sera, discrepancies in IFAT readings resulted in intermediate titres, each with equivalent intermediate variations in ELISA as shown in Figure 1. The calculated slopes of the straight lines (0,06 +/-

0,005) show a similar trend between real titres and intermediate titres, with dispersion around the line depicted in Figure 4 (R^2=0.88) (Figure 3). Moreover, the average values of ELISA OD obtained corroborate the reliability of IFAT for low titres (< 80) (Figure 2).

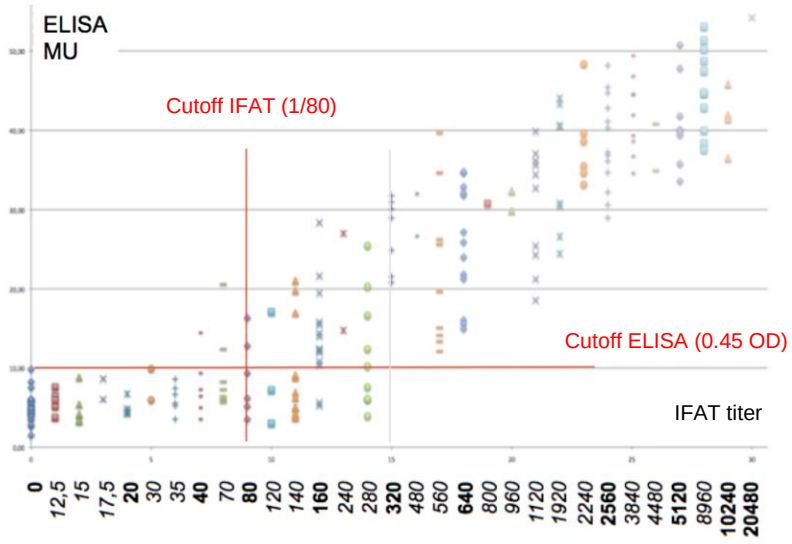

Figure 1: Distribution of ELISA/IFAT values. Similar variations with intermediate titres. Good correlation observed for "positive/positive" and "negative/negative" results. Discrepancies noted for sera with titers ranging from 1/80 to 1/280. Sera were obtained from dogs from non-endemic areas and tested "undiluted" to confirm negative IFAT titers (titer 0).

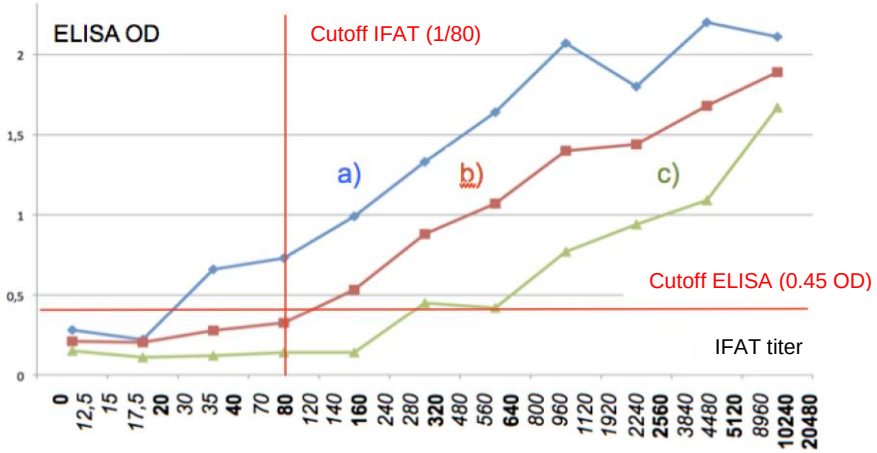

Figure 2: Comparison of ELISA and IFAT curves. **a)** and **c)** represent maximal and minimal OD at different IFAT titers, respectively; **b)** illustrates average OD values at different IFAT titers, demonstrating a progressive increase starting at low titers (≤1/80) in IFAT.

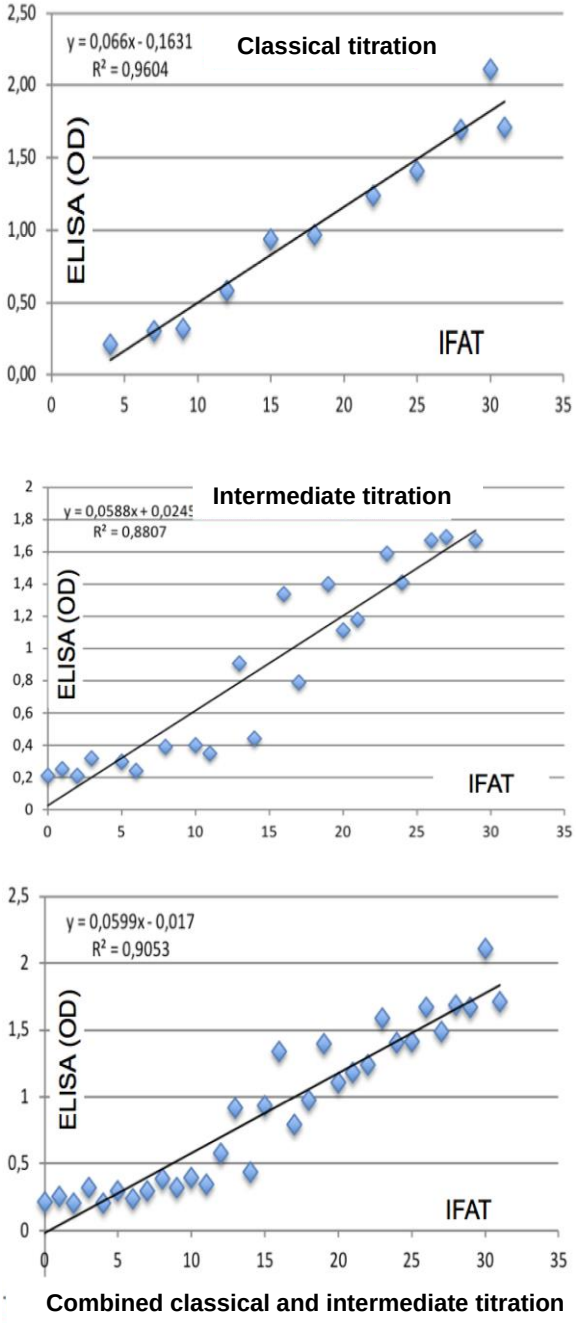

Figure 3: calculated slopes of lines: classical, intermediate, or combined IFAT titres.

Conclusion

Although an excellent overall concordance for "positive/negative" results was found at cutoff values, a variation in ELISA was present for low IFAT titers (1/80-1/320) in individual sera, despite the average OD still correlating well with IFAT. Furthermore, IFAT appears to be reliable for measuring very low titers (<1/80), whereas ELISA shows high variation. These limitations are partly attributed to the structural sigmoid variation in optical density (OD) observed in any analysis, which consequently reduces precision at the extremities of the curve regardless of the quality (specificity) of antigens used. Intermediate values in IFAT do not interfere with the calculation and could therefore be considered as part of the serological interpretation, with potential importance in the follow-up of treated dogs for a more accurate evaluation of titers decrease. These intermediate values are confirmed by corresponding variations in ELISA.

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References

1. Mahachi K, Miro G, Bourdeau P, Solano-Gallego L, Cardoso L, Oliva G, Ferrer L, Baneth G, Pennisi MG, Kontowicz E, Ozanne M, Toepp A, Petersen CA. Comparison of diagnostic accuracy of ELISA and IFAT within pan-Mediterranean veterinary diagnostic laboratories for canine *Leishmania infantum* infection via latent class analysis Proceed. ALIVE 1, MALAGA 2022; S15 pp 113-115.