

# CORRELATION OF TWO QUANTITATIVE TECHNIQUES: CIVTEST® CANIS LEISHMANIA AND IMMUNOFLUORESCENCE ANTIBODY TEST IN CANINE SERUM SAMPLES

Gascon\*<sup>1</sup>, S.; Coma<sup>1</sup>, E.; Rebordosa<sup>1</sup>, X.

<sup>1</sup>HIPRA, Amer (Girona), Spain.

\*Corresponding author (sandra.gascon@hipra.com)

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## INTRODUCTION

Canine Leishmaniasis is a vector-borne disease caused by *Leishmania infantum*. Leishmaniasis is a major global zoonosis that is potentially fatal to humans and dogs. Diagnosis is essential for the control of the disease. Canine Leishmaniasis is usually diagnosed through the detection of specific antibodies against *Leishmania spp.*, using quantitative serological techniques like immunofluorescence antibody test (IFAT), considered as the reference technique, and enzyme-linked immunosorbent assay (ELISA).

The aim of the present study is to correlate the values of the commercial ELISA CIVTEST® CANIS LEISHMANIA with the IFAT technique in order to obtain a quantitative interpretation of the values obtained with ELISA.

## MATERIALS AND METHODS

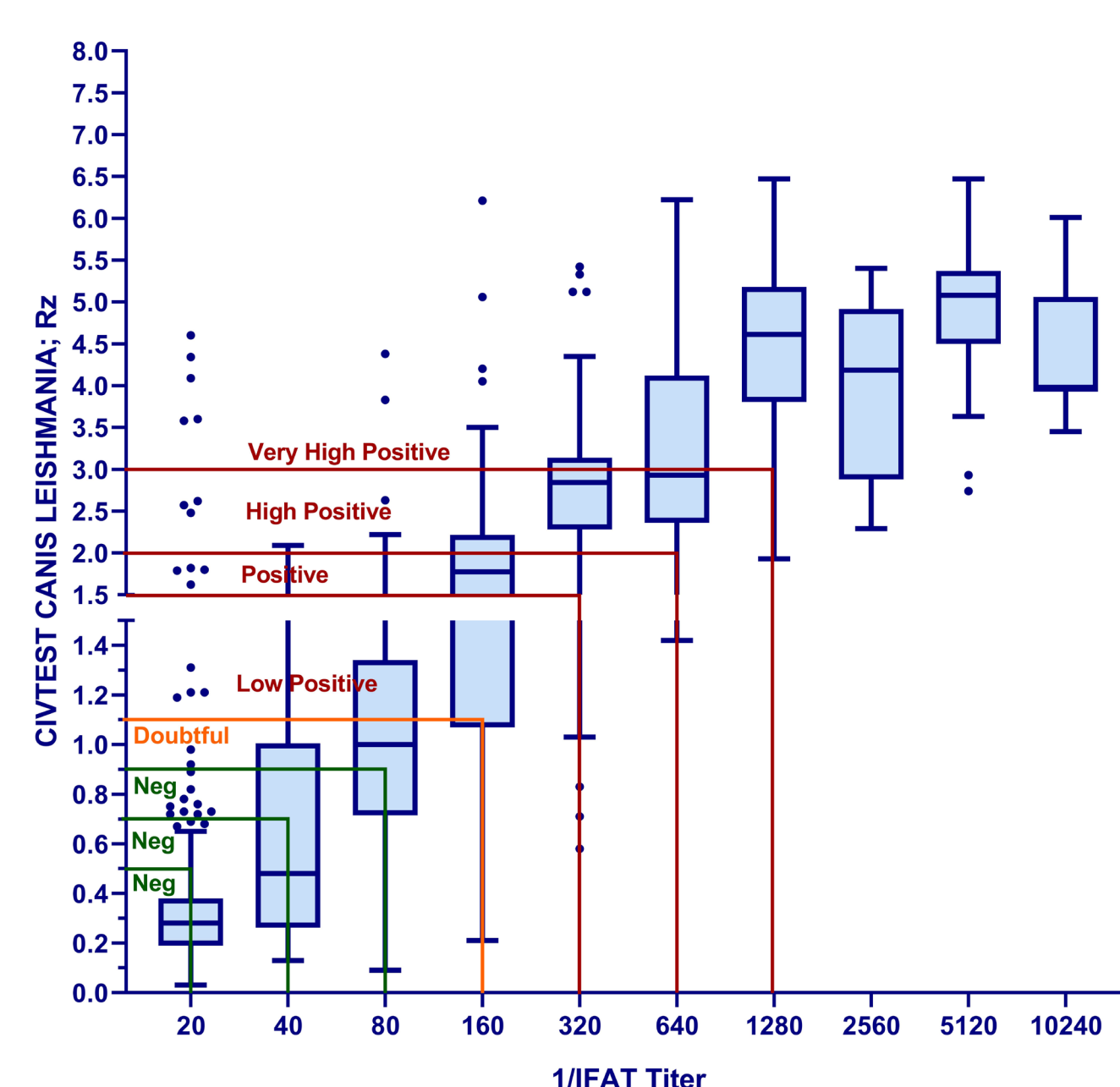
The study was conducted using 755 serum samples from Spain and Italy. 5 different laboratories were involved in the IFAT testing of the study. Each one analyzed a percentage of the samples using its own IFAT technique. The samples were classified in 10 different IFAT titre values (see Table 1). IFAT titre of 1/80 was considered the cut-off. All samples were also analyzed using CIVTEST® CANIS LEISHMANIA, according to manufacturer's instructions (the kit expresses results in Rz values, normalizing absorbance values against an internal control). Quantitative and qualitative correlation between both techniques were assessed.

IFAT	n
< 1:40	391
1:40	34
1:80	81
1:160	80
1:320	51
1:640	27
1:1280	53
1:2560	14
1:5120	17
1:10240	7
<b>Total</b>	<b>755</b>

**Table 1.** Number of samples (n) obtained within each group of IFAT values.

## RESULTS AND DISCUSSION

Good quantitative correlation was obtained between the median of Rz values and IFAT categories until IFAT titre of 1/1280 ( $R = 0.8$ ,  $p < 0.001$ ). At higher IFAT values correlation disappears ( $p = 0.33$ ). The ANOVA analysis of these groups shows that there are significant differences in the values of Rz as a function of the IFAT category ( $P = < 0.001$ ).



**Figure 1.** Correlation between the Rz values and the IFAT titre.

The qualitative analysis shows that CIVTEST® CANIS LEISHMANIA presented a sensitivity and specificity of 89.96% (85.60 – 93.11) and 89.92% (86.99 – 92.52), respectively (compared to IFAT technique).

The correspondence between eight ranges of Rz values and the categories of IFAT was defined in order to allow quantitative interpretation of the results obtained with the ELISA kit (see Table 2).

Rz (protocol 10 min)	Result	IFI Correspondence
$Rz < 0.5$	Negative	Negative
$0.5 < Rz < 0.7$	Negative	1/20 to 1/40
$0.7 < Rz < 0.9$	Negative	1/40 to 1/80
$0.9 < Rz < 1.1$	Doubtful	1/80
$1.1 < Rz < 1.5$	Low Positive	1/80 to 1/160
$1.5 < Rz < 2.0$	Positive	1/160 to 1/320
$2.0 < Rz < 3.0$	High Positive	1/320 to 1/640
$Rz > 3.0$	Very High Positive	$> 1/640$

**Table 2.** Correspondence of IFAT and Rz values.

However, given the existing variability and differences in the number of samples ( $n=38$ ) in the three higher IFAT titre categories (1/2560, 1/5120, 1/10240), it is not possible to distinguish with statistical significance these IFAT titres and define different Rz values for them.

Regarding the qualitative assessment, Se and Sp values obtained in this study were lower than the ones obtained in a previous study performed by Solan-Gallego et al [1] using samples from different epidemiological status (Se = 92.5% and Sp = 100%). IFAT is a laborious technique that can present differences of interpretation between laboratories. Having included results from five different laboratories in our study could explain the differences observed in the kit performance.

## REFERENCES

1. Laia Solano-Gallego *et al.* 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. *Parasites&Vectors*, 2014, 7:111.