SENSITIVE ANTIBODY DETECTION SYSTEMS FOR LEISHMANIA INFECTION IN DOGS

Diane Dogcio[1], Luis Cardoso[2], John Jardine[3], G-Halli Rajasekariah[1], Anthony M. Smithyman[1].

[1]Cellabs Pty Ltd., 7/27 Dale St. Brookvale, NSW 2100, Australia; [2]University of Tras-Montes Alto Douro, Vila Real, Portugal;
[3] VETPATH Laboratory Services, WA, Australia. E mail: diane@cellabs.com.au

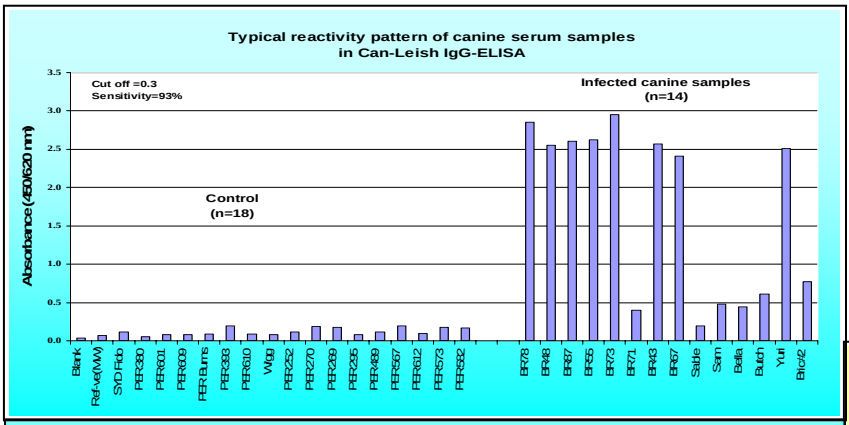


Fig 1 The Cellabs ELISA showing the reaction of canine serum samples from Australia where Leishmania is non endemic and canine serum samples from the endemic regions of South America and Europe. A cut- off value of 0.3 based on the mean +3SD of control sera determines the sensitivity of the assay at 93%.

Background Leishmania infection in dogs is increasingly becoming a problem in endemic and non-endemic countries. The significance of canine species as reservoir for human Leishmania infections makes correct diagnosis of the disease in canine very important. Cellabs Pty Ltd has constructed two assay formats to detect circulating Leishmania antibodies in canine serum samples, the **Canine Leishmania IgG ELISA** and a highly specific **Canine Leishmania IFAT**. The ELISA is based on a cocktail of exo-antigens from *L.infantum* and *L.donovani* promastigotes harvested in serum and protein free medium. The IFAT was developed using *L.infantum* surface antigens fixed on diagnostic slides to detect specific antibodies against Leishmania in canine serum samples.

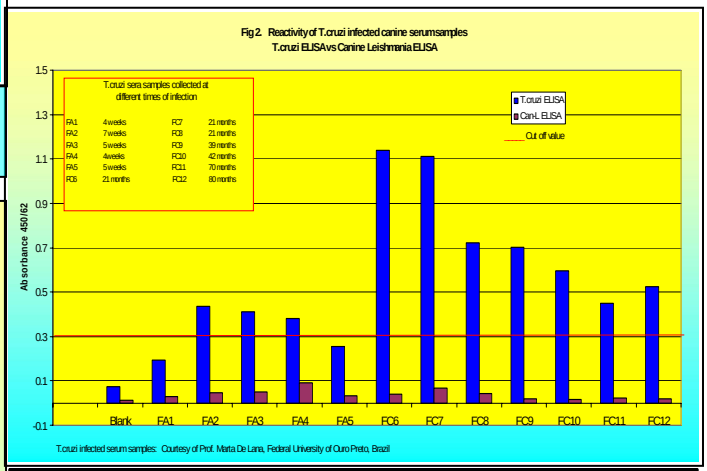


Table 1 Comparison of Cellabs ELISA and Traditional Serology Tests

*Sample	*DAT	*IFAT	ELISA	*Parasitology
Blank	-	-	0.073	-
PT47	1:20	1:20	0.126	-
PT38	1:20	1:20	0.128	-
PT74	1:20	1:20	0.241	-
PT61	1:320	1:80	0.315	+
PT94	1:10240	1:80	0.528	ND
PT72	1:1280	1:160	0.840	+
PT30	1:20480	1:80	1.006	ND
PT49	1:20480	1:80	1.096	ND
PT68	1:20480	1:320	1.121	+
PT19	1:640	1:80	1.175	+
PT86	1:10240	1:80	1.127	+
PT95	1:20480	1:320	2.085	+
PT12	1:20480	1:160	2.281	+
PT32	1:20480	1:80	2.330	+
PT64	1:20480	1:80	2.475	+

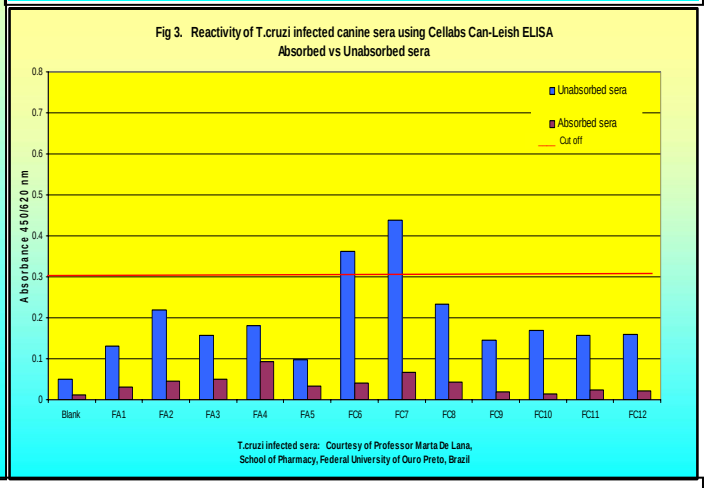
COV: DAT 1:320 Parasitology: + positive
IFA 1:80 - negative
ELISA 0.3
ND Not Done Red text indicates positive for that test

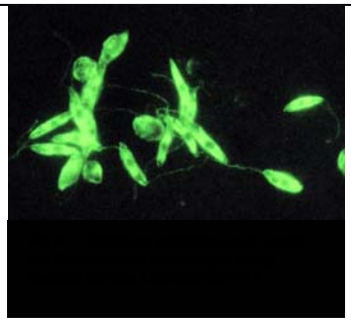
*Samples and results obtained by Luis Cardoso et.al., University of Tras-montes Alto Douro, Vila Real, Portugal.

Cross Reactivity with Chagas. Several reports indicate possible canine co-infection with Leishmania and Chagas in endemic areas of both diseases. Such co-infection can affect the reliability of diagnostic tests such as the ELISA. Any cross-reactive *T.cruzi* antibodies that might interfere with the specificity of the Cellabs Canine Leishmania ELISA assay were investigated by using an absorption technique.

Fig 2. ELISA results of serum samples collected from experimental T.cruzi mono-specific infected canine. The graph indicates the specific reaction to Cellabs T.cruzi ELISA vs. the Canine Leishmania ELISA.

Fig 3. ELISA results showing the comparison of unabsorbed and absorbed T.cruzi infected serum samples. The results performed on the same serum samples to demonstrate the significant decrease in OD value below the Canine Leishmania ELISA assay cut off value of OD 0.3.





Conclusion:

- A specific and sensitive Canine Leishmania IgG ELISA is constructed by using a cocktail of Exo-Ag from *L.donovani* and *L.infantum*.
- Cross reactive antibodies were eliminated by absorbing the serum samples with exo-antigen of *T.cruzi* epimastigotes.
- The ELISA is found to be reliable and equally sensitive with DAT and IFAT.
- IFAT assay is found to be useful in monitoring quarantined dogs for a reference laboratory in Western Australia where dogs are screened before granting exit and entry into the country.