|  |  |  |  |
| --- | --- | --- | --- |
| **Dogs** | **Region** | **Diagnosis** | **n** |
| **Symptomatic**  | Croatia | Leishmania Dipstick Rapydtest, Apacor/IFAT | 66 |
| **Asymptomatic** | Croatia | „ | 27 |
| **Healthy endemic controls** | Croatia | „ | 88 |
| **Other infection** | Croatia | parasitological examination | 51 |
| **Symptomatic**  | Brazil | parasitological examination/ DPP® | 13 |
| **Oligosymptomatic** | Brazil | parasitological examination/ DPP® | 12 |
| **Asymptomatic**  | Brazil | parasitological examination/ DPP® | 11 |
| **Healthy endemic controls** | Brazil | parasitological examination/ DPP® | 16 |
| **Healthy vaccinated**  | Brazil | parasitological examination/ DPP® | 20 |
| ***T. cruzi* infected (Chagas)**  | Brazil | parasitological examination | 40 |
| **Total** |  |  | 344 |

**Supplemental Table 1.** Panel of canine sera from Croatia and Brazil that have been pre-examined for CVL by indicated tests and were used for re-evaluation by VetLine® ELISA, rKLi8.3 ELISA and LFT (INgezim® Leishma CROM) tests.



**Supplemental Figure 1.** A)Scheme of the lateral flow assay (INgezim® Leishma CROM, Spain) for the detection of antibodies to *Leishmania*. B) Representative result of LFT with negative and positive CVL sera.

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**Supplemental Figure 2.** A)Scheme of the VetBlot**®** Leishmania Lineblot for the detection of CanL antibodies. Line 1, native *Leishmania* antigen and line 2-4, recombinant antigens of *Leishmania* are printed onto a nitrocellulose membrane.B)Representative result of a lineblot with negative and positive CanL sera.

**Supplemental Table 2.** Comparison of CVL seropositivity by rKLi8.3 based LFT and ELISA and ELISAs based on other recombinant antigens.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Grupo AD** | **Animals** | **LFT** | **rKLi8.3** | **rK28** | **rK39** |
| **AD1** | ˗ | ˗ | ˗ | ˗ |
| **AD2** | ND | ND | ˗ | + |
| **AD3** | ˗ | ˗ | ˗ | ˗ |
| **AD4** | + | + | + | + |
| **AD5** | ˗ | - | + | ˗ |
| **AD6** | + | + | + | + |
| **AD7** | + | + | + | + |
| **AD8** | ND | ND | + | + |
| **AD9** | + | + | + | + |
| **AD10** | ND | ND | + | + |
| **AD11** | + | + | + | + |
| **Grupo OD** | **OD2** | ˗ | ˗ | ˗ | ˗ |
| **OD3** | + | + | + | + |
| **OD4** | + | + | + | + |
| **OD5** | + | + | + | + |
| **OD6** | + | + | + | + |
| **OD7** | ND | ND | + | + |
| **OD8** | ND | ND | + | + |
| **OD9** | + | + | + | + |
| **OD10** | + | + | + | + |
| **OD11** | ND | - | ˗ | ˗ |
| **OD12** | ND | ND | + | + |
| **Grupo SD** | **SD1** | + | + | + | + |
| **SD2** | + | + | + | + |
| **SD3** | + | + | + | + |
| **SD4** | + | + | + | + |
| **SD5** | + | + | + | + |
| **SD6** | + | + | + | + |
| **SD7** | + | + | + | + |
| **SD8** | + | + | + | + |
| **SD9** | + | + | + | ˗ |
| **SD10** | + | + | + | + |
| **SD11** | ND | ND | + | + |
| **SD12** | + | + | + | + |
| **SD13** | + | + | + | + |

IgG seropositivity of dogs divided into asymptomatic (AD), oligosymptomatic (OD) and symptomatic (SD) CVL, based on the cut-off point established for each antigen. Animals whose optical density was greater than the cutoff point were considered positive (+) and those whose optical density was less than or equal to the cutoff point were considered negative (-). LFT = Lateral flow test based on the rKLi8.3 antigen.



**Supplemental Figure 3.** Sera from *T. cruzi* infected dogs with acute and chronic Chagas disease were tested by rKLi8.3 ELISA.

**Supplemental Table 3.** Comparison of CVL seropositivity by rKLi8.3 based LFT and -ELISA in acute and chronic *T.cruzi* infected dogs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **Animals** | **LFT** | **ELISA** |
| **AcuteChagas** | **AC2** | ˗ | ˗ |
| **AC3** | - | - |
| **AC4** | ˗ | ˗ |
| **AC5** | - | - |
| **AC11** | ˗ | - |
| **AC12** | - | - |
| **AC13** | - | - |
| **AC16** | - | - |
| **AC18** | - | - |
| **AC19** | - | - |
| **ChronicChagas** | **CC1** | ˗ | ˗ |
| **CC6** | - | - |
| **CC7** | - | - |
| **CC8** | - | - |
| **CC9** | - | - |
| **CC10** | - | - |
| **CC14** | - | - |
| **CC15** | - | - |
| **CC17** | - | - |
| **CC20** | - | - |

IgG seropositivity of dogs divided into acute and chronic Chagas. Animals whose optical density was greater than the cutoff point were considered positive (+) and those whose optical density was less than or equal to the cutoff point were considered negative (-). LFT = Lateral flow test based on the rKLi8.3 antigen.



**Supplemental Figure 4.** Comparison of anti-Leishmania antibody responses in rK28- rK39- and rLb6H ELISA using symptomatic (SD), oligosymptomatic (OD), asymptomatic (AD) and endemic control sera from Brazil.

**Supplemental Table 4.** Croatian dog sera tested by lineblot*:* reactivity with individual antigens are shown**.**

|  |  |  |
| --- | --- | --- |
| **Groups** | **Animals** | **LineBlot (native, rKLO8, rK39, rKLi8.3)** |
| **SD** | **3280** | 1+, 2+, 3+, 4+  |
| **3285** | 1+, 2+, 3+, 4+ |
| **3286** | 1+, 2+, 3+, 4+ |
| **3288** | 1+, 2+, 3+, 4+ |
| **3292** | 1+, 2+, 3+, 4+ |
| **3293** | 1+, 2+, 3+, 4+ |
| **3297** | 1+, 2+, 3+, 4+ |
| **3298** | 1+, 2+, 3+, 4+ |
| **3299** | 1+, 2+, 3+, 4+ |
| **3302** | 1+, 2+, 3+, 4+  |
| **3303** | 1+, 2+, 3+, 4+  |
| **3304** | 1+, 2+, 3+, 4+  |
| **3308** | 1+, 2+, 3+, 4+ |
| **3309** | 1+, 2+, 3+, 4+ |
| **3310** | 1+, 2+, 3+, 4+ |
| **3334** | 1+, 2+, 3+, 4+  |
| **3341** | 1+, 2+, 3+, 4+ |
| **3347** | 1+, 2+, 3+, 4+ |
| **3348** | 1+, 2+, 3+, 4+ |
| **3357** | 1+, 2+, 3+, 4+ |
| **3358** | 1+, 2+, 3+, 4+ |
| **3361** | 1+, 2+, 3+, 4+ |
| **3365** | 1+, 2+, 3+, 4+ |
| **3371** | 1+, 2+, 3+,4+ |
| **AC** | **3254** | 1-, 2-, 3-, 4- |
| **3289** | 1+, 2+, 3+, 4+ |
| **3307** | 1+, 2+, 3+, 4+ |
| **3312** | 1+, 2+, 3+, 4+ |
| **3336** | 1+, 2+, 3+, 4+ |
| **3337** | 1+, 2+, 3+, 4+ |
| **3338** | 1+, 2+, 3+, 4+ |
| **3354** | 1w+,2+,3+,4+ |
| **3355** | 1+, 2+, 3+, 4+ |
| **3356** | 1+, 2+, 3+, 4+ |
| **3364** | 1+, 2w+, 3+, 4+ |
| **3368** | 1w+, 2+, 3+, 4+  |
| **3373** | 1w+, 2-, 3-, 4- |
| **3375** | 1-, 2-, 3-, 4- |
| **3377** | 1w+, 2w+, 3+, 4+ |
|  **EC****EC** |  |  |
| **3262** | 1w+, 2-, 3-, 4- |
| **3278** | 1w+, 2-, 3-, 4- |
| **3279** | 1w+, 2-, 3-, 4- |
| **3281** | 1w+, 2-, 3-, 4- |
| **3282** | 1-, 2-, 3-, 4-  |
| **3283** | 1-, 2-, 3-, 4- |
| **3284** | 1-, 2-, 3-, 4- |
| **3287** | 1-, 2-, 3-, 4-  |
| **3290** | 1w+, 2-, 3-, 4- |
| **3296** | 1w+, 2-, 3-, 4- |
| **3300** | 1w+, 2-, 3-, 4- |
| **3301** |  1+, 2-, 3-, 4-  |
| **3305** | 1w+, 2-, 3-, 4- |
| **3306** | 1w+, 2-, 3-, 4- |
| **3311** | 1w+, 2-, 3-, 4- |
| **3313** | 1-, 2-, 3-, 4- |
| **3314** | 1-, 2-, 3-, 4-  |
| **3315** | 1w+, 2-, 3-, 4-  |
| **3322** | 1w+, 2-, 3-, 4- |
| **3323** | 1-, 2-, 3-, 4-  |
| **3331** | 1-, 2-, 3-, 4- |
| **3332** | 1w+, 2-, 3-, 4- |
| **3335** | 1-, 2-, 3-, 4-  |
| **3339** | 1-, 2-, 3-, 4-  |
| **3340** | 1w+, 2-, 3-, 4- |
| **3343** | 1-, 2-, 3-, 4- |
| **3346** |  1-, 2-, 3-, 4- |
| **3349** | 1-, 2-, 3-, 4- |
| **3350** | 1-, 2-, 3-, 4- |
| **3353** |  1-, 2-, 3-, 4-  |
| **3359** | 1w+,2-,3-,4- |
| **3360** | 1-, 2-, 3-, 4- |
| **3362** | 1+, 2w+, 3+, 4+ |
| **3363** | 1-, 2-, 3-, 4-  |
| **3366** | 1-, 2-, 3-, 4- |
| **3367** | 1-, 2-, 3-, 4- |
| **3369** | 1+, 2-, 3-, 4-  |
| **3372** | 1w+, 2-, 3-, 4- |
| **3374** | 1+, 2-, 3-, 4-  |
| **3376** | 1+, 2-, 3-, 4- |
| **3378** |  1+, 2-, 3+, 4w+ |
| **3379** | 1w+, 2-, 3-, 4- |
| **3380** | 1w+, 2-, 3w+, 4-  |
| **3381** | 1w+, 2-, 3-, 4- |
| **3382** | 1+, 2-, 3+, 4w+ |
| **3294** | 1w+, 2-, 3-, 4- |
| **3295** | 1w+, 2-, 3-, 4- |
| **3317** | 1-, 2-, 3-, 4-  |
| **3318** | 1-, 2-, 3-, 4-  |
| **3320** | 1w+, 2-, 3-, 4- |
| **3344** | 1w+, 2-, 3-, 4-  |
| **3345** | 1w+, 2-, 3-, 4-  |

**Supplemental Table 5.** Brazilian dog sera tested by lineblot:reactivity with individual antigens are shown**.**

|  |  |  |
| --- | --- | --- |
| **Groups** | **Animals** | **LineBlot (native, rKLO8, rK39, rKLi8.3)** |
| **SD** | **SD1** | 1w+, 2+, 3+, 4+  |
| **SD2** | 1+, 2+, 3+, 4+ |
| **SD3** | 1+, 2+, 3+, 4+ |
| **SD4** | 1+, 2+, 3+, 4+ |
| **SD5** | 1+, 2+, 3+, 4+ |
| **SD6** | 1+, 2+, 3+, 4w+ |
| **SD7** | 1+, 2+, 3+, 4+ |
| **SD8** | 1+, 2+, 3+, 4+ |
| **SD9** | 1+, 2+, 3+, 4+ |
| **SD10** | 1+, 2+, 3+, 4+  |
| **SD12** | 1+, 2w+, 3+, 4+  |
| **SD13** | 1+, 2+, 3+, 4+  |
| **SD14** | 1+, 2+, 3+, 4+ |
| **AD** | **AD1** | 1-, 2-, 3-, 4- |
| **AD2** | 1-, 2-, 3-, 4- |
| **AD3** | 1-, 2-, 3-, 4- |
| **AD4** | 1+, 2+, 3+, 4+ |
| **AD5** | 1w+, 2-, 3-, 4- |
| **AD6** | 1+, 2+, 3+, 4+ |
| **AD7** | 1+, 2w+, 3+, 4+ |
| **AD8** | 1+,2+,3+,4+ |
| **AD9** | 1+, 2+, 3+, 4+ |
| **AD10** | 1+, 2w+, 3w+, 4- |
| **AD11** | 1+, 2+, 3+, 4+ |
|  | **OD1** |  1w+, 2-, 3-, 4- |
|  | **OD2** |  1w+, 2-, 3-, 4- |
|  | **OD3** | 1+, 2+, 3+, 4+ |
|  | **OD4** | 1+, 2+, 3+, 4+ |
| **OD** | **OD5** | 1+, 2+, 3+, 4+ |
|  | **OD6** | 1+, 2+, 3+, 4w+ |
|  | **OD8** | 1+, 2+, 3+, 4+ |
|  | **OD9** | 1+,2+,3+,4+ |
|  | **OD10** | 1+, 2+, 3+, 4+ |
|  | **OD11** |  1w+, 2-, 3-, 4- |
|  | **OD12** | 1+,2+,3+,4+ |
|  | **SAE1** | 1-, 2-, 3-, 4- |
|  | **SAE2** | 1-, 2-, 3-, 4- |
|  | **SAE3** | 1-, 2-, 3-, 4- |
|  | **SAE4** | 1-, 2-, 3-, 4- |
|  | **SAE8** | 1-, 2-, 3-, 4- |
| **EC** | **SAE9** | 1-, 2-, 3-, 4- |
|  | **SAE10** | 1-, 2-, 3-, 4- |
|  | **SAE11** | 1-, 2-, 3-, 4- |
|  | **SAE12** | 1-, 2-, 3-, 4- |
|  | **SAE13** | 1-, 2-, 3-, 4- |
|  | **SAE14** | 1-, 2-, 3-, 4- |
|  | **SAE16** | 1-, 2-, 3-, 4- |
|  | **LEIVT 01** | 1-, 2-, 3-, 4- |
|  | **LEIVT 02** | 1-, 2-, 3-, 4- |
|  | **LEIVT 03**  | 1-, 2-, 3-, 4- |
|  | **LEIVT 04** | 1-, 2-, 3-, 4- |
|  | **LEIVT 05** | 1-, 2-, 3-, 4- |
|  | **LEIVT 05** | 1-, 2-, 3-, 4- |
|  | **LEIVT 06** | 1-, 2-, 3-, 4- |
| **VAC** | **LEIVT 07** | 1-, 2-, 3-, 4- |
|  | **LEIVT 08** | 1-, 2-, 3-, 4- |
|  | **LEIVT 09** | 1-, 2-, 3-, 4- |
|  | **LEIVT 10** | 1-, 2-, 3-, 4- |
|  | **LEIVT 12** | 1-, 2-, 3-, 4- |
|  | **LEIVT 13** | 1-, 2-, 3-, 4- |
|  | **LEIVT 14** | 1-, 2-, 3-, 4- |
|  | **LEIVT 15** | 1-, 2-, 3-, 4- |
|  | **LEIVT 16**  | 1-, 2-, 3-, 4- |
|  |  |  |  |