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POSITIVE DOGS AND CATS FOR *TRYPANOSOMA CRUZI*, FROM BOTUCATU, SÃO PAULO, BRAZIL

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Comunicación

Objectives of the study

To evaluate the occurrence of *T. cruzi* in dogs and cats from region of Botucatu, São Paulo, Brazil, which is considered not endemic to Chagas disease (CD)

Material and Methods

Blood samples were collected of 50 dogs and 50 cats, coming from Municipal Kennel. Each sample was inoculated into three tubes containing 5mL of medium LIT (Liver Infusion Tryptose), separating out the portions of plasma, leukocyte and sediment of red cells. The first reading was made after ten days of inoculation of the sample, and then twice a week, for four months. In the end of the readings, the blood cultures, were processed to DNA extraction of the parasite, using the IllustraTM blood genomicPrep Mini Spin Kit (GE Healthcare ®). The reactions were performed in duplicate, containing 2.5µL of the PCR buffer, 0.2mM of each deoxynucleotides triphosphate, 1.0U of Taq-polymerase, 10pmol of each primer, 2µL DNA and 17.8 µL of ultra pure water, to a final volume of 25µL. The conditions of amplification in thermocycler occurred with one cycle to denaturation starting at 96ºC for 2 minutes; denaturation, annexation of primers and elongation at 30 cycles for one minute each at 94ºC, 60ºC and 72ºC, respectively, and a cycle of 72ºC for ten minutes. For amplification of fragments of kDNA minicircles, were used P35 (5 AATAATGTACGGGGGAGAT-GCATGA 3') and P36 (5 'GGGTTCGATTGGGGTT-GGTGT 3') primers. The Y strain of *T. cruzi* was used as positive control and the MIX-PCR as negative control. The identification of amplified products was performed by agarose gel electrophoresis at 1.0%, stained with GelredTM. The race was held at 80 volts for 90 minutes. The bands were visualized in UV transilluminator. Mc Nemar test was performed, resulting in $p = 0.01$ for populations of dogs and cats (i.e. < 0.05), showing significance in difference of positivity between the two techniques employed.

Results

From the 50 dogs, four samples (8%) and from the 50 cats, three of them (6%) had viable parasites in culture. Among the dogs, a sample was diagnosed in the first reading, two with 25 days of inoculation and one after 40 days. In the group of cats, the positive samples were diagnosed in the first reading. For positive dogs, a sample was related to leukocyte portion and the other three to red cells´sediment portion. Among the positive cats, all samples were of the red cells´sediment. For the PCR technique, from 50 dogs evaluated, ten (20%) were positive, as well as from 50 cats, ten (20%) presented amplified products of 330 base pairs (bp) in length, corresponding to amplification of the fragment containing a specific region of *T. cruzi* kDNA minicircle (1). From 100 animals examined, only one cat (1%) presented positivity to both blood culture and PCR.

Conclusions

The parasitic forms were best viewed in blood culture of the red cells´sediment. Forty days was the maximum period for the visualization of the parasite, which may mean a low efficiency of the LIT medium to keep it viable after this period (2). The difference in

positivity between the two techniques is characterized by higher sensitivity and specificity of PCR (ranging from 90 to 100%) in relation to the blood culture, which has low sensitivity and specificity for trypanosomatids in general, as to *T. cruzi* (3). The detection of this parasite in dogs and cats from non endemic area for CD, shows the importance of investigating these domestic animals as reservoirs of *T. cruzi* and sources of infection for humans. It is concluded that the use of the PCR technique is an excellent diagnostic method for detection of CD in dogs and cats.

Bibliography

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