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Detection of *Trypanosoma cruzi* infection in naturally-infected dogs and cats using serological, parasitological and molecular methods

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Abstract

Domestic dogs and cats are major domestic reservoir hosts of *Trypanosoma cruzi* and a risk factor for parasite transmission. In this study we assessed the relative performance of a polymerase chain reaction assay targeted to minicircle DNA (kDNA-PCR) in reference to conventional serological tests, a rapid dipstick test and xenodiagnosis to detect *T. cruzi* infection in dogs and cats from an endemic rural area in northeastern Argentina. A total of 43 dogs and 13 cats seropositive for *T. cruzi* by an immunosorbent assay (ELISA) and an indirect hemagglutination assay (IHA), which had been examined by xenodiagnosis, were also tested by kDNA-PCR. kDNA-PCR was nearly as sensitive as xenodiagnosis for detecting *T. cruzi*- infectious dogs and cats. kDNA-PCR was slightly more sensitive than xenodiagnosis in seropositive dogs (91% versus 86%, respectively) and cats (77% against 54%, respectively), but failed to detect all of the seropositive individuals. ELISA and IHA detected all xenodiagnosis-positive dogs and both outcomes largely agreed (kappa coefficient, $\kappa = 0.92$), whereas both assays failed to detect all of the xenodiagnosis-positive cats and their agreement was moderate ($\kappa = 0.68$). In dogs, the sensitivity of the dipstick test was 95% and agreed closely with the outcome of conventional serological tests ($\kappa = 0.82$). The high sensitivity of kDNA-PCR to detect *T. cruzi* infections in naturally-infected dogs and cats supports its application as a diagnostic tool complementary to serology and may replace the use of xenodiagnosis or hemoculture.

Keywords

Chagas disease; *Trypanosoma cruzi*; serodiagnosis; xenodiagnosis; polymerase chain reaction; reservoir

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1. Introduction

Domestic dogs (*Canis lupus familiaris*) and cats (*Felis silvestris catus*) are considered major reservoir hosts of *Trypanosoma cruzi* in the domestic environment, and a risk factor for human infection (Beard et al. 2003; Cardinal et al. 2008; Gürtler et al. 2007; 2005; 1991). A reservoir host is capable of indefinite maintenance of a pathogen (Cleaveland and Dye 1995). Pathological alterations found in naturally- and experimentally-infected Beagle dogs are similar to those found in human Chagas disease (Guedes et al. 2009; Kjos et al. 2008). In addition, all the discrete typing units (DTUs) of *T. cruzi* (Zingales et al. 2009) identified in humans were found in domestic dogs from Argentina, Colombia and elsewhere in Latin America (Burgos et al. 2007; Cardinal et al. 2008; Cura et al. 2012; Diosque et al. 2003; Enriquez et al. 2012; Ramirez et al. 2013). Therefore, improved detection of *T. cruzi* infections in dogs or cats is relevant for risk assessment and clinical diagnosis.

Detection of chronic human infections with *T. cruzi* are more appropriately revealed by serological than parasitological methods because of the very low levels of parasitemia during the chronic stage (Luquetti et al. 2009; WHO 2002). Serological tests use crude antigenic preparations, semipurified fractions, or recombinant antigens of *T. cruzi* (Umesawa et al. 2003; Cooley et al. 2008; Longhi et al. 2012). Because none of the currently available serological assays is considered a “gold standard”, seroreactivity by at least two tests has been traditionally used to diagnose *T. cruzi* infection (WHO 2002).

Conventional serological methods used for human serodiagnosis of *T. cruzi* infection standardized for use in dogs achieved high sensitivity and specificity (Cardinal et al. 2006a; Lauricella et al. 1998). Rapid immunocromatographic tests for dogs also showed very good performance (Cardinal et al. 2006b; Nieto et al. 2009; Rosypal et al. 2011). The main limitation of current serological methods is its potential cross-reactivity with other eventually co-endemic, closely related trypanosomatids such as *Trypanosoma rangeli* and *Leishmania sp*. (Caballero et al. 2007; Nieto et al. 2009; Umezawa et al. 2009, Vexenat et al. 1996). The use of parasite amplification methods (xenodiagnosis and hemoculture) mostly resolves specificity issues, but they are less sensitive than conventional serological tests and have other limitations (Cerisola et al. 1974; Junqueira et al. 1996; Luquetti et al. 2009).

The long-term persistence of antibodies to *T. cruzi* during the chronic stage and the low sensitivity of parasitological tests prompted the development of polymerase chain reaction (PCR) strategies targeted to highly repetitive sequences such as a fragment of the minicircle of kinetoplast DNA (kDNA-PCR) or satellite DNA (Sat-DNA-PCR) for follow-up of human patients after treatment. The sensitivity of kDNA-PCR reached 100% among *T. cruzi*-infected infants born to seropositive women (Schijman et al. 2003), and rose from 75.6% to 95.6% in chronic patients when the number of serial blood samples tested increased from one to three (Bisio et al. 2011).

Domestic dogs naturally infected with *T. cruzi* residing in endemic rural areas were much more infectious to xenodiagnosis bugs than local seropositive humans (Gürtler et al. 1996). This suggests that dogs have higher parasitemia, and therefore the sensitivity of kDNA-PCR in dogs would be higher than in humans. In dogs experimentally infected with *T. cruzi*, the sensitivity of kDNA-PCR increased from 67% to 100% when the number of serial blood samples tested increased from 1 to 9, and at least two serial blood samples were required to correctly diagnose all infected dogs (Araújo et al. 2002). Using primers that did not discriminate *T. cruzi* from *T. rangeli*, kDNA-PCR only detected 51% of naturally-infected dogs that tested positive by xenodiagnosis and/or hemoculture (Lucheis et al. 2005). To our knowledge, the molecular diagnosis of *T. cruzi* infection in cats has not been investigated. Because of their relevance as domestic reservoir hosts, we conducted a cross-sectional

survey of dogs and cats in a rural endemic area from northeastern Argentina to assess the performance of a kDNA-PCR assay to detect *T. cruzi* infection in reference to conventional serological methods, a dipstick test and xenodiagnosis.

2. Materials and methods

2.1. Study area

Field work was conducted in the municipality of Pampa del Indio (26° 2′ 0″ S, 59° 55′ 0″ O), Chaco Province, Argentina. The study area was described elsewhere (Gurevitz et al. 2011; see map and photos in doi:10.1371/journal.pntd.0001349.g001). House infestation with the vector *Triatoma infestans* was 45.9% (n = 327 inhabited house) (Gurevitz et al. 2011) and the overall prevalence of bug infection in *T. infestans* was 27.5% (n = 1,869) (Cardinal et al. unpublished results).

2.2. Study design

A combined demographic and sero-parasitological survey targeting all domestic dogs and cats residing in seven contiguous villages (10 de Mayo, Campo Los Toros, El Salvaje, La Loma, Las Chuñas, Los Ciervos and Santos Lugares) with high infestation and bug infection with *T. cruzi* was conducted in August-December 2008. The overall prevalence of *T. cruzi* infection (by serodiagnosis and xenodiagnosis combined) was 26% in dogs (n = 481) and 29% in cats (n = 87) (Cardinal et al. unpublished results). Both host populations were very young, with median ages of 24 months for dogs and 12 months for cats, and composed of mongrel animals under ownership.

Nested within this larger survey, for current purposes we selected two neighboring villages (10 de Mayo and Las Chuñas, totaling 60 inhabited houses) which had high bug prevalence of infection with *T. cruzi* (61%) before initial control interventions. Within these two villages, we selected all houses that had at least one *T. cruzi*-infected *T. infestans* before interventions (n = 38) to increase the chance of detecting a large number of infected dogs and cats, and then examined all the available animals by xenodiagnosis and took blood samples.

Blood samples (up to 7 mL) were drawn by venipuncture and allowed to clot at ambient temperature. Each serum was separated after centrifugation at 3,000 rpm during 15 min., allocated in triplicate vials and preserved at -20 °C at the field laboratory. Blood samples were also drawn (2 mL dogs and 1 mL cats) for DNA extraction and PCR; these were immediately mixed with an equal volume of Guanidine hydrochloride 6 M, EDTA 0.2 M pH 8.00 (GEB), and stored at 4°C. Serum samples were transported in dry ice to the main laboratory at the end of each survey, whereas GEB samples were kept in a cooler.

Each head of household was informed on the objectives and relevance of the study; informed oral consent was requested and obtained in all cases. All animal and blood sample processing were conducted according to the protocol approved by the Argentinean “Dr. Carlos Barclay” Independent Ethical Committee for Clinical Research (IRB No. 00001678, NIH registered, and Protocol N ° TW-01-004).

2.3. Xenodiagnosis

A total of 99 dogs and 29 cats from 10 de Mayo and Las Chuñas villages were examined by xenodiagnosis; in addition, we examined 15 dogs and 11 cats from neighboring villages. For xenodiagnosis we used 10 (small pups and kittens) or 20 uninfected fourth-instar nymphs of *T. infestans* (Gürtler et al. 2007). All insects were mass-reared by the National Coordination of Vector Control (Cordoba, Argentina). Dogs were fitted with a muzzle (except newborn

pups) and handled with the help of their owners when it did not entail any physical risk. Cats were caught by hand or using cage traps and/or nets; when necessary, they were anesthetized prior to venipuncture using one of the following immobilization protocols: a) Tiletamine HCl and Zolazepam HCl (Zelazol®, Fort Dodge Sanidad Animal, La Plata, Argentina) 2-5 mg/kg intramuscular (IM); b) Xilacine HCl (Rompun®, Bayer Argentina, Buenos Aires, Argentina) 0.4-0.6 mg/kg IM, and c) Ketamine HCl (Ketamina 50®, Holliday Scott, Buenos Aires, Argentina) 15 mg/kg IM.

Xenodiagnosis boxes were exposed to the belly of each individual animal during 20 minutes, and a new re-exposure period of 10 minutes followed if most bugs had not blood-fed to repletion. The boxes were held in a cooler to avoid sudden temperature changes during field work, and the bugs were then kept in an insectary with no additional blood-feeding until examination. Initially, feces from two of the insects fed on a given animal were individually examined for *T. cruzi* infection by optic microscopy (OM) at 400× 30 days after exposure; if negative, feces from the rest of the insects were analyzed in pools of 4-5 insects each. When any of the first two bugs or the pool was positive, feces from all insects were examined individually. Bugs negative at 30 days post-exposure were re-examined individually 30 days later. The frequencies of molting and mortality at 30 days post-exposure were used as indices of xenodiagnosis quality (Gürtler et al. 2007). In addition, parasites were isolated from the feces of xenodiagnosis bugs and DTUs identified as described elsewhere (Enriquez et al. 2012).

2.4. Serodiagnosis

Dogs and cats aged 4 months or more were diagnosed serologically whereas younger animals were examined only by xenodiagnosis because maternally-derived antibodies to *T. cruzi* could induce a false-positive result. Of all dogs and cats examined by xenodiagnosis, 85 (75%) dogs and 38 (95%) cats were also examined by serodiagnosis. Sera were tested for antibodies to *T. cruzi* by indirect hemagglutination assay (IHA) following the manufacturer's instructions (Wiener Laboratories S.A.I.C., Buenos Aires, Argentina), and an in-house enzyme-linked immunosorbent assay (ELISA) modified from a standardized protocol (Lauricella et al. 1998) which used a total homogenate of *T. cruzi* strain Tulahuén 2 (*T. cruzi* II) as antigen. Dog and cat serum samples were diluted 1:800 and 1:500, respectively, and tested with ABTS (2.2′-azino-di-[3-ethyl benzthiazoline-6- sulfonate], Kirkegaard & Perry Laboratories (KPL), Gaithersburg, MD) as substrate and alkaline phosphatase-labeled, goat anti-dog heavy and light chains IgG, and goat anti-cat IgG, respectively (KPL, California). Sera with discordant results between IHA and ELISA were tested with an indirect immunofluorescence test (IFAT) (Ififluor Parasitest Chagas, Laboratorio IFI, Buenos Aires, Argentina). Cut-off values were an optical absorbance ≥ 0.17 (i.e., the mean absorbance of negative control sera of dogs and cats from a non-endemic city plus three standard deviations), and titers ≥ 16 (IHA), and ≥ 32 (IFAT). A sample was considered seropositive if it was positive by at least two conventional serological methods among ELISA, IHA and IFAT (i.e., composite serological outcome). Nearly all samples were processed by the same operator.

Sera from 81 dogs examined by IHA and ELISA were also tested by an immunocromatographic dipstick test (*Trypanosoma cruzi* Detect-Canine; Inbios, Seattle, WA) according to the manufacturer's instructions, and using 20 μL of serum (Cardinal et al. 2006b). One seropositive, xenodiagnosis-positive dog could not be tested with the dipstick.

2.5. Polymerase chain reaction

Guanidine-blood samples from all 43 dogs and 13 cats that resulted seropositive for *T. cruzi* (by ELISA and IHA) and had also been examined by xenodiagnosis, were tested by kDNA-

PCR. In addition, we included samples from a xenodiagnosis-positive, seronegative cat (by the two tests assayed) and from 16 dogs and 10 cats negative by ELISA, IHA and xenodiagnosis, all of which resided in the study villages.

Infections with *T. cruzi* were confirmed by kDNA-PCR amplification of the 330 bp fragment from the minicircle DNA of the kinetoplastid genome. GEB samples of 2 and 4 mL were heated in boiling water for 10 and 15 minutes, respectively (Britto et al. 1993). Prior to DNA extraction, 200 pg of *Arabidopsis thaliana* DNA was added to each GEB aliquot as an external control (Duffy et al. 2009). DNA was purified from 400 μL of GEB using a commercial kit (DNeasy Blood & Tissue Kit, QIAGEN Sciences, Maryland, USA). Five μL of extracted DNA were added to 45 μL of reaction mix, which contained buffer, deoxyribonucleotide triphosphate (dNTP), di-chloride magnesium (MgCl2), Platinum Taq DNA polymerase (Invitrogen, USA) and primers 121 (5′-AAATAATGTACGGG(T / G) GAGATGCATGA-3′) and 122 (5′-GGTCGATTGGGGTTGGTGTAATATA-3′). The concentrations of reagents and cycling conditions were taken from Burgos et al. (2005). Aliquots of 12 μL of PCR were analyzed by electrophoresis in 2% agarose gels containing Gel-red (Biotum) and then visualized under UV light. Each PCR run included 5 and 25 fg of *T. cruzi* DNA (CL Brener, TcVI) as a positive control, and sterile distilled water instead of DNA as a negative control. The sensitivity control for the reaction was positive for the minimum dilution used (i.e., 5 fg).

To avoid contamination with foreign DNA we used two separate sets of pipettes, filter tips, microtubes, racks and gloves; one set was used for the preparation of the reaction mixture and the other one for loading the DNA. Both tasks were performed in separate rooms. All instruments were exposed to UV light for 15 minutes before use.

2.6. Data Analysis

We measured the degree of agreement between ELISA and IHA, between serological results and the dipstick test, and between serology and kDNA-PCR by means of Cohen's kappa coefficient (κ); κ is a widely used measure of inter-rater agreement for categorical items despite being conservative. The sensitivity of ELISA, IHA and the dipstick test was computed from the proportion of individual sera reactive to each test relative to the total number of dogs (or cats) with a positive xenodiagnosis, whereas the sensitivity of kDNA-PCR and xenodiagnosis was calculated in reference to the composite serological outcome. To assess the relative quality of xenodiagnosis, the relationship between bug mortality or molting success at 30 days post-exposure (binary response variables) and the host species on which the bugs fed (explanatory variable) was investigated using maximum likelihood logistic regression analysis clustered by subject implemented in Stata 10.1 (StataCorp, College Station, Texas). The probability used for nominal statistical significance was 5%. The Wald test examined the hypothesis that all regression coefficients were 0.

3. Results

3.1. Xenodiagnosis

A total of 43 of 114 dogs and of 10 of 40 cats examined were xenodiagnosis-positive. The bug mortality rate at 30 days post-exposure was 3% in both dogs (n = 2,080 bugs) and cats (n= 792), with no significant effect of host species (OR = 0.9, 95% confidence interval [CI] = 0.55-1.41, Wald χ^2= 0.26, df 1, P = 0.61). More bugs exposed to dogs succeeded in molting after a single blood meal (12%) than bugs that fed on cats (8%), but this difference was marginally significant (OR = 1.5, 95% CI = 0.99-2.26, Wald χ^2= 3.79, df 1, P = 0.052). The fraction of seropositive dogs that was xenodiagnosis-positive was 83% (43 of 52), whereas 50% (8 of 16) of seropositive cats were xenodiagnosis-positive.

3.2. Serodiagnosis

A total of 52 dogs and 16 cats were seropositive by at least two tests. In dogs, ELISA and IHA had a high degree of agreement ($\kappa = 0.92$) (Table 1). Three serologically discordant dogs (IHA-positive only, with titers 16, 64 and 256) were negative by IFAT, the dipstick test, and kDNA-PCR. The sensitivity of ELISA and IHA was 100% (95% CI = 99-100%) among 43 xenodiagnosis-positive dogs. Of 42 xenodiagnosis-negative dogs, 9 were both ELISA- and IHA-reactive.

Among 38 cats examined, the degree of agreement between ELISA and IHA was moderate and lower than for dogs ($\kappa = 0.68$). Of 10 xenodiagnosis-positive cats, 6 were positive by both ELISA and IHA, and 2 cats only reactive by one of the assays were also IFAT-positive and kDNA-PCR-positive (Table 1). The remainder two cats with a positive xenodiagnosis were seronegative by both techniques; one of them was IFAT-positive (not examined by kDNA-PCR), and the other one was kDNA-PCR-positive and indeterminate by IFAT; both were considered to be *T. cruzi*-infected. In five of the six cat sera with discordant results between ELISA and IHA, IFAT agreed with the ELISA outcome. Eight cats with a negative xenodiagnosis were seropositive by ELISA and IHA.

Using the dipstick test, 40 of 42 xenodiagnosis-positive dogs (all seropositive) were dipstick-positive, yielding a sensitivity of 95% (95% CI = 92-96%). All 9 seropositive, xenodiagnosis-negative dogs were dipstick-positive. Of 30 seronegative dogs with a negative xenodiagnosis, 6 (20%) were dipstick-positive and most probably were “false positives”. Three serologically discordant dogs (IHA-positive only) negative by IFAT and kDNA-PCR were also negative by the dipstick test. The kappa coefficient between the dipstick test and the composite results of serodiagnosis was 0.82; with ELISA, 0.78, and with IHA, 0.69.

3.3. kDNA-PCR

Of all dogs and cats seropositive for *T. cruzi* examined by PCR, 39 (91%) and 10 (77%) were kDNA-PCR-positive, respectively (Table 2). In animals that were both seropositive and xenodiagnosis-positive, 36 of 37 dogs and 7 of 7 cats were also positive by kDNA-PCR. The only seropositive, xenodiagnosis-positive dog that was kDNA-PCR-negative had a very low infectiousness (1 infected bug out of 20 bugs exposed), and also was dipstick-negative. Both in dogs and cats, 3 of 6 seropositive, xenodiagnosis-negative individuals were positive by kDNA-PCR (Table 2). All dogs (n = 16) and cats (n = 10) that were both seronegative and xenodiagnosis-negative were also negative by kDNA-PCR, suggesting that this PCR assay was highly specific. The kappa coefficient between kDNA-PCR and ELISA was large for dogs (0.86) and cats (0.82), and weaker with IHA for both host species (0.62).

4. Discussion

Our study documents that kDNA-PCR was slightly more sensitive than xenodiagnosis to detect *T. cruzi*-seropositive dogs (91% versus 86%) and even more so in seropositive cats (77% versus 54%), respectively. The proportion of kDNA-PCR-positive individuals among all seropositive dogs (91%) and cats (77%) was slightly higher or within the range of kDNA-PCR-positive results recorded in seropositive chronic humans (Gomes et al. 1999; Ramírez et al. 2009; Zulantay et al. 2011). In individual dogs or cats with serologically discordant results, molecular diagnosis agreed closely with the composite serological outcome and had a higher concordance with ELISA than with IHA. Nevertheless, both kDNA-PCR and xenodiagnosis failed to detect several animals seropositive by two conventional serological tests. The intensity of parasitemia in these animals most likely fell below the detection threshold of kDNA-PCR and xenodiagnosis.

The chance of detecting *T. cruzi* by xenodiagnosis or by PCR depends on host parasitemia and the volume of blood ingested by the bugs or used for DNA extraction, respectively. In our study, kDNA-PCR performed with higher sensitivity than in other studies that tested a single blood sample (≤ 10 mL) from naturally- or experimentally-infected dogs and used different PCR protocols, primers and DNA polymerase (Araújo et al. 2002; Jimenez-Coello et al. 2008). The dogs and cats we tested were naturally infected and lived in a high-risk rural area; they had different levels of malnutrition, co-infections with other parasites, and exposure to additional vector-mediated infections. The joint effects of these factors, combined with the large variability observed in the sensitivity and specificity of different PCR protocols (Gomes et al. 1999; Kirchhoff et al. 1996; Schijman et al. 2011; Virreira et al. 2003), may explain variations in performance across studies.

Assuming that each fourth-instar nymph of *T. infestans* ingests 0.09 mL of blood when feeding to repletion (Cerisola et al. 1974), approximately 1.8 mL were ingested by the 20 xenodiagnosis bugs –similar to the volume of blood used for molecular diagnosis. The sensitivity of xenodiagnosis based on the microscopical examination of bugs' feces may be increased by further testing the negative bugs by kDNA-PCR (Zulantay et al. 2007). The average proportion of *T. cruzi*-seropositive individuals that were infectious to bugs was substantially higher in dogs (83%) than in cats (50%). Such differences between host species may be related to the fact that the xenodiagnosis bugs exposed to dogs apparently ingested more blood than the bugs exposed to cats, as suggested by differential bug molting rates (i.e., a surrogate indicator of bloodmeal size).

An additional source of PCR-based diagnostic variability relates to the different *T. cruzi* DTUs circulating; for example, SAT-DNA-PCR has less sensitivity to detect *T. cruzi* I infections (Schijman et al. 2011). However, kDNA-PCR based strategies were highly sensitive for detecting reference stocks of TcI (Silvio X10), TcVI (CL-Brener) and TcIV (CAN III) (Schijman et al. 2011). *T. cruzi* VI and TcV predominated in the study dogs (Enriquez et al. 2012), which is consistent with the high sensitivity of kDNA-PCR.

Previous studies have shown the usefulness of molecular tools to discriminate between infecting agents in multiple infections with related protozoan species (Botero et al. 2010; Pavia et al. 2007; Troncarelli et al. 2009). *Trypanosoma rangeli* has not been detected in triatomine bugs or hosts in Argentina (D'Alessandro and del Prado 1977), nor has *Rhodnius sp.* been detected so far. Conversely, *Leishmania braziliensis* circulates in the region (Salomon et al. 2008) and therefore serological cross-reactivity may be a potential problem in some places.

We found a high degree of agreement between ELISA and IHA results for detecting *T. cruzi* infection in dogs, though in general the ELISA had much better performance than IHA. The sensitivity of ELISA was as high as in other dog populations (Araújo et al. 2002; Gürtler et al. 1996; 2007; Lauricella et al. 1998), whereas the sensitivity of IHA was similar or greater than with other commercial kits (Cardinal et al. 2007, Lauricella et al. 1998; 1993). The three dogs negative by xenodiagnosis, ELISA, IFAT and the dipstick test that also were IHA-reactive most likely were “false positive” results related to the subjective nature of IHA readings. We selected IHA for mass processing of serum samples because it is less laborious than IFAT and we had prior experience with its use in dogs and cats; therefore, we restricted the use of IFAT for serologically discordant cases.

For cats, the degree of agreement between ELISA and IHA was moderate and weaker than in dogs. Both methods were non-reactive in 2 of 10 cats with a positive xenodiagnosis. The two cats were one year of age and cohabited with other dogs or cats seropositive for *T. cruzi* in houses found infested after the community-wide insecticide spraying. The evidence is not

sufficient to distinguish whether these infected cats were acute cases or were immunologically depressed or unresponsive. The occurrence of *T. cruzi*-seronegative dogs with a positive xenodiagnosis has also been documented in another endemic rural area (Lauricella et al. 1998).

The dipstick test was as sensitive as in a previous study in which its specificity was 100% (Cardinal et al. 2006), and agreed closely with the outcome of conventional serodiagnosis. These findings support the suitability of the dipstick test for mass-screening surveys of *T. cruzi* infection in dogs. However, 6 (20%) seronegative dogs with a negative xenodiagnosis were dipstick-positive; these samples showed a weak red line (indicative of *T. cruzi* antibodies) open to subjective interpretations. In theory, cross-reactivity with other closely related protozoa such as *Leishmania infantum* (Mettler et al. 2005) might occur, though it has not been recorded in our study area yet. Recently, a western blotting technique that uses a trypomastigote excreted–secreted antigen of *T. cruzi* (TESA-blot) achieved 100% sensitivity and specificity in dogs (Umezawa et al. 2009). High sensitivity was also obtained with a trans-sialidase inhibition assay (TIA) applied to naturally-infected dogs, in which no cross-reactivity was found with canine visceral leishmaniasis (Sartor et al. 2011).

Xenodiagnosis has several limitations and its use in clinical diagnosis is increasingly considered obsolete (Gomes et al. 2009), yet it still plays a unique role for assessment of infectiousness to the vector. When parasite isolation is required, parasite culture from feces of xenodiagnosis bugs is a highly productive method. Xenodiagnosis is easier to use than hemoculture under field conditions and usually performed better in humans (Luquetti and Rassi 2000). kDNA-PCR was nearly as sensitive as xenodiagnosis for detecting *T. cruzi*-infectious dogs and cats, and may complement serodiagnosis to decide the final outcome of serologically discordant cases. Furthermore, kDNA-based amplification allows downstream applications in molecular epidemiology, such as the identification of minicircle signatures using LSSP-PCR (Andrade et al. 1999; Vago et al. 2000; 1996), RFLP-PCR (Burgos et al. 2005) and hybridization with kDNA-specific probes (Bosseno et al. 2000; Rozas et al. 2007), to map transmission routes.

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Highlights

We assessed the performance of kDNA-PCR to detect *T. cruzi* infection in dogs and cats.

kDNA-PCR was slightly more sensitive than xenodiagnosis in seropositive dogs and cats.

kDNA-PCR and xenodiagnosis failed to detect all the seropositive individuals in both host species.

kDNA-PCR may be used as a complementary method to serodiagnosis to detect infectious hosts.

Table 1

Comparison of serological and xenodiagnosis results in dogs and cats from Pampa del Indio, Chaco, 2008.

Host	Xenodiagnosis	No. of hosts examined	Seroreactivity to ELISA and IHA				
			Both positive	Positive by IHA only	Positive by ELISA only	Both negative	Kappa coefficient (κ)
Dogs	Positive	43	43	0	0	0	-
	Negative	42	9	3[a]	0	30	-
	Total	85	52	3	0	30	0.92
Cats	Positive	10	6	1[b]	1[b]	2[c]	-
	Negative	28	8	4[d]	0	16	-
	Total	38	14	5	1	18	0.68

[a] All were IFAT-, dipstick-, and kDNA-PCR-negative.

[b] IFAT-positive and kDNA-PCR-positive.

[c] One serum IFAT-positive, and the other indeterminate by IFAT and kDNA- PCR-positive .

[d] All sera were IFAT-negative and were not tested by kDNA-PCR.

Table 2

Relationship between serodiagnosis, xenodiagnosis and kDNA-PCR for *T. cruzi* infection in dogs and cats of Pampa del Indio, Chaco, 2008.

			kDNA-PCR		
Host	Seroreactivity	Xenodiagnosis	No. examined	No. positive	% positive
Dogs	Positive	Positive	37	36	97
	Positive	Negative	6	3	50
	Total		43	39	91
Cats[a]	Positive	Positive	7	7	100
	Positive	Negative	6	3	50
	Total		13	10	77

[a] A seronegative cat with a positive xenodiagnosis was also positive by kDNA-PCR.