CLINICAL AND VACCINE IMMUNOLOGY, June 2011, p. 984–989 Vol. 18, No. 6
1556-6811/11/$12.00 doi:10.1128/CVI.00535-10


# *trans*-Sialidase Neutralizing Antibody Detection in *Trypanosoma cruzi*-Infected Domestic Reservoirs[▽]

Paula A. Sartor,[1] Martha V. Cardinal,[2] Marcela M. Orozco,[2] Ricardo E. Gürtler,[2] and M. Susana Leguizamón[1]*

*Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 (1121), Ciudad Autónoma de Buenos Aires, Argentina,[1] and Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Güiraldes 2160 (1428), Ciudad Autónoma de Buenos Aires, Argentina[2]*

Received 1 December 2010/Returned for modification 18 January 2011/Accepted 28 March 2011

**The detection of *Trypanosoma cruzi* infection in domestic dogs and cats is relevant to evaluating human transmission risks and the effectiveness of insecticide spraying campaigns. However, the serological assays routinely used are associated with cross-reactivity in sera from mammals infected with *Leishmania* spp. We used a *trans*-sialidase inhibition assay (TIA) for *T. cruzi* diagnosis in serum samples from 199 dogs and 57 cats from areas where these types of infections are endemic. TIA is based on the antibody neutralization of recombinant *trans*-sialidase, an enzyme that is not detected in the coendemic *Leishmania* species or *Trypanosoma rangeli* parasites. *T. cruzi* infection was also evaluated by conventional serology (CS) (indirect immunofluorescence, indirect hemagglutination, enzyme-linked immunosorbent assay, and immunochromatographic dipstick test) and xenodiagnosis. Sera from 30 dogs and 15 cats from areas where these organisms are not endemic and 5 dogs with visceral leishmaniasis were found to be nonreactive by TIA and CS. Samples from dogs and cats demonstrated 91 and 95% copositivities between TIA and CS, whereas the conegativities were 98 and 97%, respectively. Sera from xenodiagnosis-positive dogs and cats also reacted by TIA (copositivities of 97 and 83%, respectively). TIA was reactive in three CS-negative samples and was able to resolve results in two cat serum samples that were CS inconclusive. Our study is the first to describe the development of *trans*-sialidase neutralizing antibodies in naturally infected dogs and cats. High CS conegativity and the absence of *trans*-sialidase neutralization in dog sera from areas where leishmaniasis is not endemic and from dogs with visceral leishmaniasis support TIA specificity. The TIA may be a useful tool for *T. cruzi* detection in the main domestic reservoirs.**

The parasite protozoan *Trypanosoma cruzi*, the etiological agent of Chagas disease, affects 10 to 18 million people in the Americas (31, 36). The life cycle of *T. cruzi* involves different species of triatomine and mammalian host that maintain sylvatic, peridomestic, and domestic cycles. The vectors adapted to human habitats (mainly *Triatoma infestans* in Argentina and other Southern Cone countries) are responsible for transmission to humans (17, 31). Dogs and cats are important domestic reservoir hosts given their high incidence of infection and infectiousness to bugs (16). *T. infestans* fed preferentially and more frequently on dogs than on other domestic or peridomestic animals (16, 18). Several studies conducted in Latin America have demonstrated that cohabiting with infected dogs and/or cats constitutes a risk factor for the domestic transmission of *T. cruzi* (7, 8, 11, 13, 16).

Current control strategies for Chagas disease include preventive actions directed toward vector-mediated and transfusional transmission. Elimination of vector-mediated transmission could be achieved by effective vector control actions and sustained surveillance in the most affected rural areas (17). In these areas, the detection of *T. cruzi* infection in dogs has been used to monitor the effectiveness of insecticide spraying campaigns in domestic and peridomestic environments (5, 9).

The availability of highly specific and sensitive methods for detecting *T. cruzi* in domestic reservoir hosts would be helpful. Xenodiagnosis (XD) and hemoculture are specific, but their sensitivity is variable depending on the intensity of parasitemia, which differs based on the parasite strain, the duration of infection, and the host nutritional status (27, 35). PCR is a specific test but requires expensive laboratory equipment, and its sensitivity also depends on parasitemia levels. The serological assays routinely used (indirect immunofluorescence [IIF], indirect hemagglutination [IHA], and enzyme-linked immunosorbent assay [ELISA]) present limitations related to the use of *T. cruzi* homogenates that led to cross-reactivity with coendemic parasites such as *Leishmania* spp. and *Trypanosoma rangeli* (4, 14, 34). This issue becomes particularly relevant in countries where dogs (and perhaps cats) are the principal reservoir hosts of the emerging *Leishmania infantum*, the etiological agent of visceral leishmaniasis (6, 12, 28). Many efforts have been made to overcome these drawbacks by using different assay principles with purified and recombinant antigens (6, 33, 34). A rapid immunochromatographic dipstick test has been designed that has advantages for field diagnosis (6).

* Corresponding author. Mailing address: Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 (1121), Ciudad Autónoma de Buenos Aires, Argentina. Phone: 54-11-5950-9500. Fax: 54-11-5950-9577. E-mail: sleguiza@fmed.uba.ar.

▽ Published ahead of print on 6 April 2011.

TABLE 1. Serological tests performed in cat and dog samples collected in areas of endemicity and nonendemicity from Argentina

| Location and host species | Test[a] | Manufacturer[b] | Cutoff |
|---|---|---|---|
| Santiago del Estero | | | |
| Cat ($n$ = 14) | ELISA A | Homemade | 0.2 |
| | IHA | Polychaco | 1/16 |
| | IIF assay | Homemade | 1/16 |
| Dog ($n$ = 107) | ELISA A | Homemade | 0.2 |
| | IHA | Polychaco | 1/16 |
| | IIF assay | Homemade | 1/16 |
| Chaco | | | |
| Cat ($n$ = 43) | ELISA B | Homemade | 0.2 |
| | IHA | Wiener Laboratories | 1/16 |
| Dog ($n$ = 92) | ELISA B | Homemade | 0.17 |
| | IHA | Wiener Laboratories | 1/16 |
| | DT | Inbios | |
| Buenos Aires | | | |
| Cat ($n$ = 15) | ELISA B | Homemade | 0.2 |
| | IHA | Polychaco | 1/16 |
| Dog ($n$ = 30) | ELISA B | Homemade | 0.17 |
| | IHA | Polychaco | 1/16 |

[a] Abbreviations: ELISA, enzyme-linked immunosorbent assay; IIF assay, indirect immunofluorescence assay; IHA, indirect hemagglutination assay; DT, immunochromatographic dipstick test. $n$, Number of samples.

[b] Polychaco, Buenos Aires, Argentina; Wiener Laboratories, SAIC, Buenos Aires, Argentina; Trypanosoma Detect, Inbios, Seattle, WA.

*T. cruzi* expresses a virulence factor named *trans*-sialidase (TS), an enzyme that catalyzes the transference of sialic acid from host glycoconjugate to the parasite surface and among host macromolecules (25). This enzyme was not detected in *Leishmania* spp., *Trypanosoma rangeli*, or *Plasmodium* spp. (10, 15). During the chronic phase of human and murine *T. cruzi* infection, neutralizing antibodies with TS activity are elicited and can be detected by using a TS inhibition assay (TIA) (21, 22, 26). TIA was nonreactive in sera from patients suffering from leishmaniasis, malaria, syphilis, or autoimmune disease and therefore allowed the discrimination of *T. cruzi* and *Leishmania* infections (3). The detection of TS neutralizing antibodies in patients with megasyndromes (previously diagnosed as idiopathic) and in seronegative Amerindians at high risk of vectorial transmission shows the sensitivity of TIA (3, 23). The confirmation of *T. cruzi* infection in patients with inconclusive or borderline conventional serology (CS) results was also achieved by TIA (2, 3). These results encouraged us to assess the validity of TIA in domestic animal hosts of *T. cruzi* residing in rural areas where this parasite is endemic.

## MATERIALS AND METHODS

**Serum samples.** Sera from dogs and cats older than 3 months of age were obtained from two rural areas in northern Argentina where *Trypanosoma* infection is endemic: Amamá in Santiago de Estero Province during 2002 to 2003 and Pampa de Indio in Chaco Province during 2008 (5, 7) (Table 1). Sera from dogs and cats residing in Buenos Aires City, an area where vector-borne *T. cruzi* infection is not endemic, were also included in the study as negative control sera (Table 1). In addition, samples from five dogs suffering from visceral leishmaniasis were also tested. All samples were stored at −20°C.

**Xenodiagnosis.** Xenodiagnosis was performed using 20 laboratory-reared, third- or fourth-instar *T. infestans* nymphs per animal (16). Pools of feces from five bugs that were fed on the same animal for 25 min were microscopically examined for *T. cruzi* infection at 30 and 60 days after feeding. Bugs from each positive pool were reexamined individually (7).

**Serological assays for *T. cruzi* detection.** Samples were tested by indirect hemagglutination assay (IHA) and immunochromatographic dipstick test according to the manufacturer's instructions (Table 1) (6). A homemade IIF test (fluorescein-conjugated anti-gammaglobulin LID; Laboratorio Inmunodiagnóstico, Buenos Aires, Argentina) and an ELISA (ELISA A, anti-IgG-HRP; Santa Cruz Biotechnology, Santa Cruz, CA) were performed according to standardized procedures and criteria reported elsewhere (Table 1) (20). A slightly modified ELISA (ELISA B, Table 1) was also conducted by setting the antigen concentration at 10 mg/ml. Dog and cat serum samples were diluted to 1:500 and 1:800, respectively. Alkaline phosphatase-labeled goat anti-dog IgG (1:12,000) and anti-cat IgG (1:5,000) were used (Kirkegaard & Perry Laboratories, Inc.). ABTS [2,2′azinobis(3-ethylbenzthiazolinesulfonic acid); Kirkegaard & Perry Laboratories] was used as a substrate. Samples determined to be reactive by at least two different serologic tests were considered infected by *T. cruzi.* Sixty-five dog samples and eight cat samples were determined to be reactive by three CS assays. Thirteen dog samples and fourteen cat samples were determined to be reactive by two CS tests.

**TIA.** Recombinant TS was cloned and expressed in *pTrcHis* vector (Invitrogen, San Diego, CA) and purified by immobilized metal ion-affinity chromatography (HiTrap; GE Healthcare) according to the manufacturer's instructions.

In order to detect the presence of TS neutralizing antibodies in infected animals, recombinant TS was incubated with serum samples. The remnant TS activity was assayed by measuring the transfer of sialic acid from sialyl-lactose (1 mM) to [D-glucose-1-$^{14}$C]lactose (12 μM, 54.3 mCi/mmol; GE Healthcare), using 20 mM Tris buffer (pH 7.6) (22). After the interaction with a dense slurry of quaternary aminoethyl-Sepharose A-25 (Sigma, St. Louis, MO), three washes with distilled water were made. Bound material was eluted with 0.5 M NaCl, and the counts per minute (cpm) were quantified in a β-scintillation counter (Rackbeta). The inhibition value was calculated as follows: % inhibition = (1 − [cpm sample/cpm negative control]) × 100. Samples were processed in duplicate in Eppendorf tubes. Pools of sera from dogs and cats infected with *T. cruzi* and not infected were included as positive and negative controls in each assay.

**Ethical statements.** Samples from Santiago del Estero were collected according to Institutional Animal Care and Use Committee protocol 04223 at the University of Illinois at Urbana Champaign (7). Samples from Chaco Province were processed according to the protocol TW-01-004 approved by Comité Etico "Dr. Carlos Barclay" (Buenos Aires, Argentina).

**Statistical analysis.** Categorical variables were analyzed by using the Fisher exact test, whereas a nonparametric test (Wilcoxon) was used to evaluate differences in TIA mean values between CS-positive and -negative samples. The level of significance was set up at $P < 0.01$. Coreactivity between assays was calculated, and the reliability between each pair of methods was measured by using Cohen's kappa coefficient.

## RESULTS

**TS neutralizing antibodies are developed in *T. cruzi*-infected dogs.** To evaluate whether *T. cruzi*-infected dogs are able to develop TS neutralizing antibodies, serum samples from animals previously diagnosed by XD and CS were tested by TIA. The cutoff value for *T. cruzi*-infected and noninfected dogs was selected by constructing a receiver-operator characteristics curve (ROC curve). For this purpose, we used samples from dogs determined to be positive by CS and XD ($n$ = 58) because the isolation of parasites from bugs fed on naturally infected dogs allows the confirmation of *T. cruzi* infection. The selection of truly negative specimens for *T. cruzi* infection can only be achieved by testing samples from dogs native of and residing in areas of nonendemicity because some CS and XD false-negative results may be obtained when samples from areas of endemicity are analyzed. Here, we included samples from dogs residing in Buenos Aires city that were determined to be negative by IHA and ELISA. The cutoff value was achieved at 55%. The accuracy of TIA, summarized by the area under the ROC curve, was 0.98 (95% confidence interval [CI] = 0.9 to 1.0). The sensitivity estimated by the ROC curve was 96.6%

TABLE 2. Results obtained using XD, CS, and TIA for detection of *T. cruzi* infection in dog serum samples from rural areas of endemicity

| TIA result | No. of samples | | | | | |
|---|---|---|---|---|---|---|
| | XD positive | | XD negative | | XD not done | |
| | CS positive | CS negative | CS positive | CS negative | CS positive | CS negative |
| Positive | 56 | 0 | 12 | 1 | 3 | 1 |
| Negative | 2 | 0 | 0 | 47 | 5[a] | 71 |
| Total | 58 | 0 | 12 | 48 | 8 | 72 |

[a] Samples determined to be seroreactive by dipstick test and IHA but not by ELISA.

TABLE 3. Results obtained using XD, CS, and TIA for detection of *T. cruzi* infection in cat serum samples from rural areas of endemicity

| TIA result | No. of samples | | | | | | |
|---|---|---|---|---|---|---|---|
| | XD positive | | XD negative | | XD not done | | |
| | CS positive | CS negative | CS positive | CS negative | CS positive | CS negative | CS inconclusive |
| Positive | 9 | 1 | 9 | 0 | 3 | 0 | 0 |
| Negative | 1 | 1 | 0 | 23 | 0 | 7 | 2[a] |
| Total | 10 | 2 | 9 | 23 | 3 | 7 | 2 |

[a] Samples determined to be reactive by IHA, nonreactive by ELISA, and inconclusive by IIF assay.

(95% CI = 88.1 to 99.6%), and the specificity was 100% (95% CI = 88.4 to 100%). The specificity estimated decreased to 97.9% (95% CI = 88.9 to 99.9%) when CS- and XD-negative dogs from areas of endemicity were included in the uninfected group.

Table 2 summarizes the results obtained by testing serum samples from dogs residing in rural areas of endemicity. The copositivity between TIA and XD was 97% (56/58), while the copositivity between CS and TIA was 91% (71/78). Two samples were determined to be positive by XD and CS (reactive by IHA, dipstick test, and ELISA) but were not reactive by TIA (Table 2). Five serum samples obtained from dogs that were not examined by XD were reactive by the dipstick and IHA tests but not by TIA and ELISA (Table 2, Fig. 1). On the other hand, the TIA and CS conegativity was 98% (118/120). TIA reactivity was observed in two samples determined to be unreactive by all CS methods (Table 2). The concordance between the CS and TIA methods was 0.95 (Cohen's kappa coefficient).

Figure 1 shows the percentages of inhibition in dog sera from areas of endemicity and nonendemicity according to the XD and CS results. The TIA mean values in samples negative by XD or CS (7.25 ± 10.64) were significantly lower than those detected in samples determined to be positive by CS (94.47 ± 8.83; $P < 0.0001$) or XD (94.48 ± 8.57; $P < 0.0001$). Moreover, the inhibition percentages were <10% among most of the TIA-negative samples (92/125) and >90% among most of the TIA-positive samples (59/73) (Fig. 1). All five samples from dogs with visceral leishmaniasis were determined to be negative by TIA (Fig. 1).

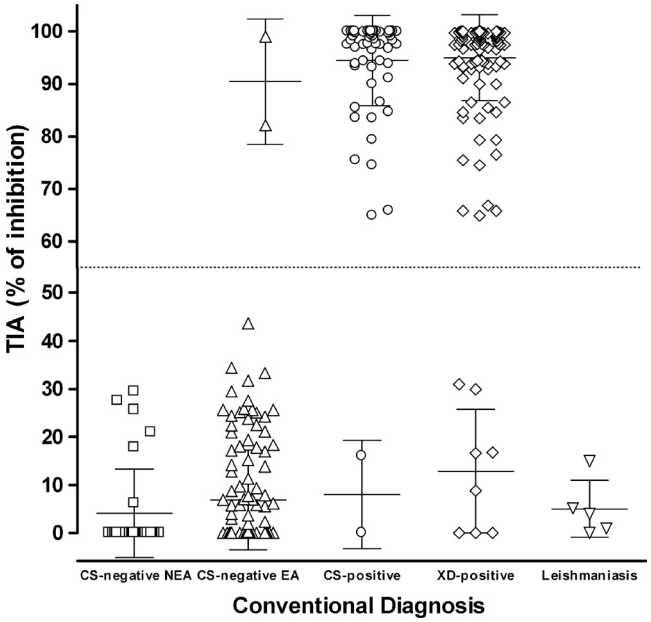


FIG. 1. TIA reactivity in serum samples from dogs classified according to conventional serology (CS) and xenodiagnosis (XD). Symbols: □, CS-negative samples from areas of nonendemicity (NEA); △, CS-negative samples from rural areas of endemicity (EA); ○, CS-positive samples; ◇, XD-positive samples; ▽, samples from dogs with visceral leishmaniasis. The percent inhibition values represent the level of TS neutralizing antibodies in serum samples. The cutoff value is indicated by the dotted horizontal line.

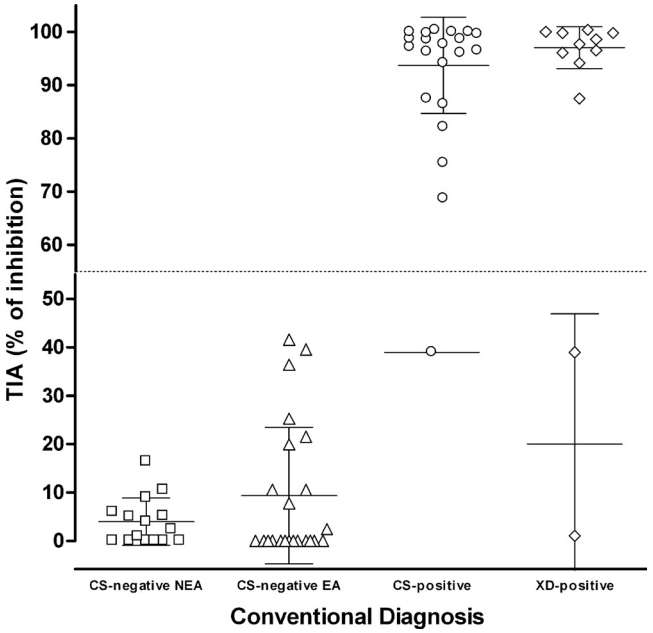


FIG. 2. TIA reactivity in serum samples from cats classified according to conventional serology (CS) and xenodiagnosis (XD). Symbols: □, CS-negative samples from areas of nonendemicity (NEA); △, CS-negative samples from endemic rural areas (EA); ○, CS-positive samples; ◇, XD-positive samples. The percent inhibition values represent the level of TS neutralizing antibodies in serum samples. The cutoff value is indicated by the dotted horizontal line.

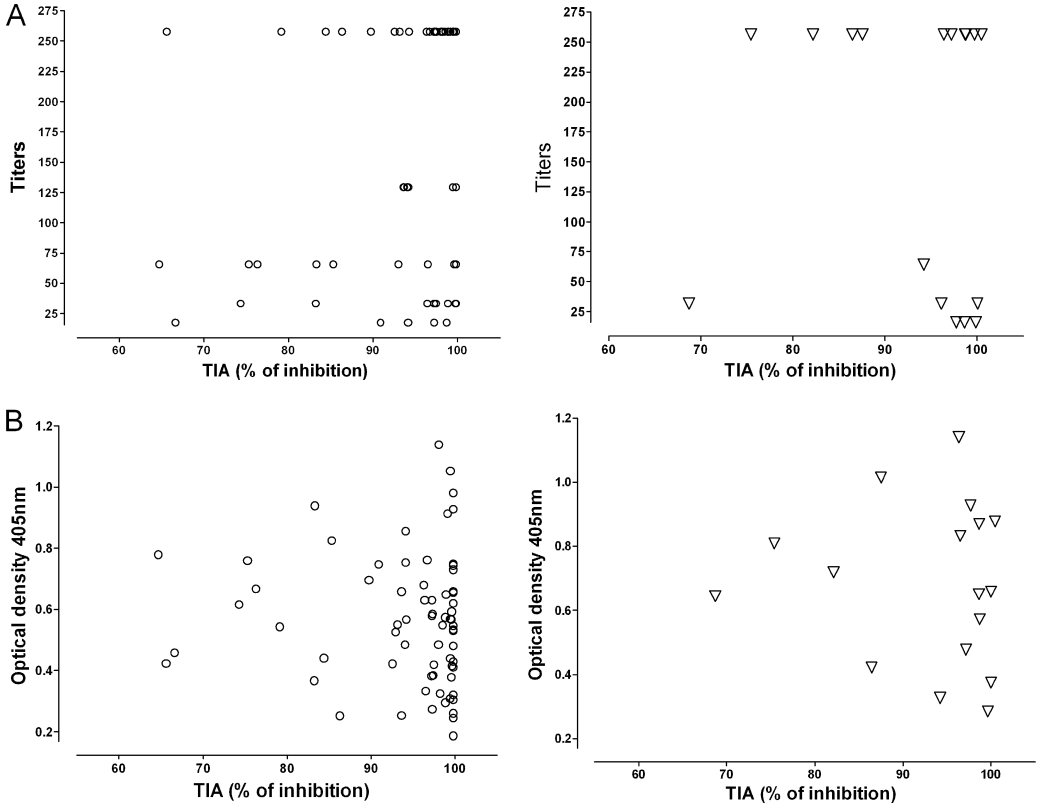


FIG. 3. Relationship between TIA and CS reactivity in positive samples from dogs (○) and cats (▽). (A) Indirect hemagglutination (titers); (B) ELISA (optical density).

A dot plot was design to evaluate the relation between TIA and CS reactivity (see Fig. 3). It can be observed that of the samples with low reactivity (IHA titer, <100; ELISA optical density [OD], <0.4), 44 and 26% were TIA positive, respectively. In samples with intermediate reactivity (IHA titer, 100 to <200; ELISA OD, 0.4 to 0.8), 9 and 62% were TIA positive, respectively. Among sera with high reactivity (IHA titer, >200; ELISA OD, >0.8), 47 and 12% were TIA positive, respectively. The TIA mean values (ranging from 91 to 98%) of these groups showed no significant differences when they were compared.

**TS neutralizing antibodies are developed in *T. cruzi*-infected cats.** To analyze the presence of TS neutralizing antibodies in *T. cruzi*-infected cats, samples from individuals previously diagnosed by XD and CS residing in rural areas of endemicity were studied (Table 3). The cutoff value was set at 60% by analyzing the distribution of TIA results in cat serum samples collected from areas of endemicity and nonendemicity according to the XD and CS results (Fig. 2). For the reasons mentioned above, we also tested samples from CS-negative cats ($n = 15$) residing in Buenos Aires city (Fig. 3). The construction of an ROC curve and the estimation of the test parameters were not possible given the low number of truly positive cat specimens available (XD positive and CS positive) since cats are less abundant than dogs (1:3) in rural areas of endemicity (7, 16).

Table 3 shows the comparative results obtained in cat samples from rural areas of endemicity. Copositivity between TIA and CS was 95% (21/22), conegativity was 97% (31/32), and the Cohen's kappa coefficient was 0.96. Copositivity between TIA or CS and XD was 83% (10/12) in both cases (Table 3). Two samples determined to be XD positive were TIA negative (one of them was also unreactive in all CS tests) (Table 3). TIA was able to resolve as negative two serum samples that were determined to be inconclusive by CS (Table 3).

Most of the TIA-negative cat sera tested (22/34) showed inhibition values of <10%, whereas a high proportion of TIA-positive samples (16/22) presented results that were >90% (Fig. 2). The TIA mean values in samples with positive XD and/or CS results (93.99% ± 7.89%) were significantly higher than in XD- or CS-negative samples (8.32% ± 17.90%; $P < 0.0001$).

The results obtained from the comparison of TIA and CS reactivities are shown in Fig. 3. It can be observed that similar proportions of samples with high (IHA titer, >200; ELISA OD, >0.8), intermediate (ELISA OD, 0.4 to <0.8), and low (IHA titer, <100; ELISA OD, <0.4) reactivities by CS were found to be reactive by TIA. No significant differences were found in comparisons of TIA mean values (ranging from 91 to 98% inhibition) between these groups.

## DISCUSSION

Detection of *T. cruzi* in domestic reservoirs is used to estimate the risk of transmission of infection to human beings and to evaluate the effectiveness of insecticide spraying campaigns.

Since conventional anti-*T. cruzi* serological assays are associated with cross-reactivity with *Leishmania* spp. (4, 11, 14, 34), the use of defined specific antigens is needed (3, 6, 34).

We analyzed the ability of TIA to detect *T. cruzi* infection in domestic reservoirs. This assay is based on the antibody neutralization of recombinant TS, an enzyme that is not detected in coendemic parasites such as *Leishmania* spp. and *T. rangeli* (15).

Our study describes for the first time the development of TS neutralizing antibodies in naturally infected dogs and cats, the main domestic reservoirs of *T. cruzi.* The coreactivity and concordance between TIA and CS demonstrated that both were highly correlated. TIA mean values from infected and noninfected cats and dogs were substantially different, with scarce inhibition values around the cutoff (i.e., high discrimination power). Analysis of TIA versus IHA and of TIA versus ELISA showed that the development of TS neutralizing antibodies is independent of the antibody levels displayed by infected cats or dogs. Moreover, no differences in TIA mean values were obtained in sera from both domestic reservoirs.

TIA was unreactive for all samples obtained from cats and dogs residing in areas of nonendemicity and in dogs suffering from visceral leishmaniasis. Although few dogs with visceral leishmaniasis were tested in our study, this result is consistent with those obtained in humans (3) and strongly suggests that TIA can discriminate between *T. cruzi* and *Leishmania* spp. infection in dogs. Umezawa et al. (34) minimized cross-reactivity with *Leishmania* spp. in dog samples by using a mixture of *T. cruzi* trypomastigote excreted/secreted antigens in a Western blot format (TESA-Blot). However, Amato Neto et al. communicated TESA-Blot reactivity in 2 of 30 human patients with visceral leishmaniasis (1).

The absence of TIA reactivity in samples from two cats and two dogs with positive XD results could be related to a recently acquired infection since infected *T. infestans* was detected at their homes and no *T. cruzi* antibodies were found in a young cat. The development of TS neutralizing antibodies late during infection has been described previously in both human and murine hosts (21, 22, 29).

Our study detected TIA reactivity in 2 of 118 CS- and XD-negative samples from dogs and in 1 sample from an XD-positive cat with a negative CS result. Similar results were obtained using TIA in Amerindians and in patients suffering idiopathic megasyndrome with a history of exposure to *T. cruzi* (3, 15). CS false-negative results in dog samples were also revealed by a dipstick test (6) and PCR (19). Taken together, the evidence shows that TIA detects *T. cruzi* infections in domestic animal reservoirs that are missed by CS. The use of radioactive material hampers its use in the field, but major efforts are under way to replace this substrate with a colorimetric or fluorometric one (24, 30, 32).

The results obtained here demonstrate the ability of the TIA to improve *T. cruzi* diagnosis by performing the differential diagnosis with *Leishmania* spp., as well as resolving inconclusive, false-positive/false-negative results obtained via conventional methods. Our findings support the utility of the TIA as an epidemiological tool for the detection of *T. cruzi* in the main domestic reservoir hosts.

## ACKNOWLEDGMENTS

We thank I. Colaianni, G. Enriquez, and M. Lauricella for laboratory assistance; J. Burgos for critical reading of the manuscript; and Zélia Profeta da Luz (Centro de Pesquiças René Rachou, Fiocruz Minas Gerais) for providing serum samples from dogs with visceral leishmaniasis.

M.S.L., R.E.G., and M.V.C. are researchers and P.A.S. and M.M.O. are fellows of the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina). R.E.G., M.V.C., and M.M.O. were supported by grants from the National Institutes of Health (research grant R01 TW05836 and Fogarty International Center and National Institute of Environmental Health Sciences grants to Uriel Kitron and R.E.G.), the Universidad de Buenos Aires, the International Development Research Center (Canada), and TDR/WHO. P.A.S. and M.S.L. were supported by grants from the National Agency of Science and Technology (to M.S.L.).

## REFERENCES

1. **Amato Neto, V., C. R. De Marchi, C. S. Ferreira, and A. W. Ferreira.** 2005. Observations on the use of TESA-Blot for the serological diagnosis of Chagas' disease. Rev. Soc. Bras. Med. Trop. **38:**534–535.
2. **Blejer, J. L., P. A. Sartor, O. Bottasso, H. J. Salamone, and M. S. Leguizamón.** 2008. *Trans*-sialidase inhibition assay for the detection of *Trypanosoma cruzi* infection in blood donor samples from Argentina. Vox Sang **95:**189–196.
3. **Buchovsky, A. S., et al.** 2001. *Trans*-sialidase inhibition assay, a highly sensitive and specific diagnostic test for Chagas' disease. Clin. Diagn. Lab. Immunol. **8:**187–189.
4. **Caballero, Z. C., O. E. Sousa, W. P. Marques, A. Saez-Alquezar, and E. S. Umezawa.** 2007. Evaluation of serological test to identify *Trypanosoma cruzi* infection in humans and determine cross-reactivity with *Trypanosoma rangeli* and *Leishmania* spp. Clin. Vaccine Immunol. **14:**1045–1049.
5. **Cardinal, M. V., et al.** 2006. A prospective study of the effects of sustained vector surveillance on *Trypanosoma cruzi* infection of dogs and cats in rural northwestern Argentina. Am. J. Trop. Med. Hyg. **75:**753–761.
6. **Cardinal, M. V., R. Reithinger, and R. E. Gürtler.** 2006. Use of an immunochromatographic dipstick test for rapid detection of *Trypanosoma cruzi* in sera from animal reservoir hosts. J. Clin. Microbiol. **44:**3005–3007.
7. **Cardinal, M. V., et al.** 2007. Impact of community-based vector control on house infestation and *Trypanosoma cruzi* infection in *Triatoma infestans*, dogs and cats in the Argentine Chaco. Acta Trop. **103:**201–211.
8. **Cardinal, M. V., et al.** 2008. Molecular epidemiology of domestic and sylvatic *Trypanosoma cruzi* infection in rural northwestern Argentina. Int. J. Parasitol. **38:**1533–1543.
9. **Castañera, M. B., M. A. Lauricella, R. Chuit, and R. E. Gürtler.** 1998. Evaluation of dogs as sentinels of the transmission of *Trypanosoma cruzi* in a rural area of northwestern Argentina. Ann. Trop. Med. Parasitol. **92:**671–683.
10. **Clough, B., et al.** 1996. *Plasmodium falciparum* lacks sialidase and *trans*-sialidase activity. Parasitology **112:**443–449.
11. **Crisante, G., A. Rojas, M. M. Teixeira, and N. Añez.** 2006. Infected dogs as a risk factor in the transmission of human *Trypanosoma cruzi* infection in western Venezuela. Acta Trop. **98:**247–254.
12. **Dantas-Torres, F.** 2009. Canine leishmaniasis in South America. Parasit. Vectors **2:**S1.
13. **Estrada-Franco, J. G., et al.** 2006. Human *Trypanosoma cruzi* infection and seropositivity in dogs, Mexico. Emerg. Infect. Dis. **12:**624–630.
14. **Ferreira, E., et al.** 2007. Comparison of serological assays for the diagnosis of canine visceral leishmaniasis in animals presenting different clinical manifestations. Vet. Parasitol. **146:**235–241.
15. **Frasch, A. A. C.** 2000. Functional diversity in the *trans*-sialidase and mucin families in *Trypanosoma cruzi*. Parasitol. Today **16:**282–286.
16. **Gürtler, R. E., et al.** 2007. Domestic dogs and cats as sources of *Trypanosoma cruzi* infection in rural northwestern Argentina. Parasitology **134:**69–82.
17. **Gürtler, R. E.** 2009. Sustainability of vector control strategies in the Gran Chaco Region: current challenges and possible approaches. Mem. Inst. Oswaldo Cruz **104:**52–59.
18. **Gürtler, R. E., et al.** 2009. Strong host-feeding preferences of the vector *Triatoma infestans* modified by vector density: implications for the epidemiology of Chagas disease. PLoS Negl. Trop. Dis. **3:**e447.
19. **Jimenez-Coello, M., et al.** 2008. American trypanosomiasis in dogs from an urban and rural area of Yucatan, Mexico. Vector-Borne Zoonotic Dis. **8:**755–761.
20. **Lauricella, M. A., M. B. Castañera, R. E. Gürtler, and E. L. Segura.** 1998. Immunodiagnosis of *Trypanosoma cruzi* (Chagas' disease) infection in naturally infected dogs. Mem. Inst. Oswaldo Cruz **93:**501–507.
21. **Leguizamón, M. S., O. Campetella, S. M. González Cappa, and A. C. C. Frasch.** 1994. Mice infected with *Trypanosoma cruzi* produce antibodies

against the enzymatic domain of *trans*-sialidase that inhibit its activity. Infect. Immun. **62:**3441–3446.
22. **Leguizamón, M. S., et al.** 1994. Antibodies inhibiting *Trypanosoma cruzi trans*-sialidase activity in sera from human infections. J. Infect. Dis. **170:** 1570–1574.
23. **Leguizamón, M. S., et al.** 1998. Use of *trans*-sialidase inhibition assay in a population serologically negative for *Trypanosoma cruzi* but at high risk of infection. Clin. Diagn. Lab. Immunol. **5:**254–255.
24. **Neres, J., A. Buschiazzo, P. M. Alzari, L. Walsh, and K. T. Douglas.** 2006. Continuous fluorimetric assay for high-throughput screening of inhibitors of *trans*-sialidase from *Trypanosoma cruzi*. Anal. Biochem. **357:**302–304.
25. **Parodi, A. J., et al.** 1992. Identification of the gene(s) coding for the *trans*-sialidase of *Trypanosoma cruzi*. EMBO J. **11:**1705–1710.
26. **Pereira-Chioccola, V. L., S. Schenkman, and J. Kloetzel.** 1994. Sera from chronic patients and rodents infected with *Trypanosoma cruzi* inhibit *trans*-sialidase by recognizing its amino-terminal and catalytic domain. Infect. Immun. **62:**2973–2978.
27. **Petersen, R. M., et al.** 2001. Association between nutritional indicators and infectivity of dogs seroreactive for *Trypanosoma cruzi* in a rural area of northwestern Argentina. Parasitol. Res. **87:**208–214.
28. **Quinnell, R. J., and O. Courtenay.** 2009. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology **136:**1915–1934.
29. **Risso, M. G., et al.** 2004. Differential expression of a virulence factor, the *trans*-sialidase, by the main *Trypanosoma cruzi* phylogenetic lineages. J. Infect. Dis. **189:**2250–2259.
30. **Sartor, P. A., R. Agusti, M. S. Leguizamón, O. Campetella, and R. M. de Lederkremer.** 2010. Continuous nonradioactive method for screening trypanosomal *trans*-sialidase activity and its inhibitors. Glycobiology **20:**982–990.
31. **Schofield, C. J., J. Jannin, and R. Salvatella.** 2006. The future of Chagas disease control. Trends Parasitol. **22:**583–588.
32. **Schrader, S., E. Tiralongo, G. Paris, T. Yoshino, and R. Schauer.** 2003. A nonradioactive 96-well plate assay for screening of *trans*-sialidase activity. Anal. Biochem. **322:**139–147.
33. **Shadomy, S. V., S. C. Waring, O. A. Martins-Filho, R. C. Oliveira, and C. L. Chappell.** 2004. Combined use of enzyme-linked immunosorbent assay and flow cytometry to detect antibodies to *Trypanosoma cruzi* in domestic canines in Texas. Clin. Diagn. Lab. Immunol. **11:**313–319.
34. **Umezawa, E. S., et al.** 2009. TESA-Blot for the diagnosis of Chagas disease in dogs from co-endemic regions for *Trypanosoma cruzi*, *Trypanosoma evansi*, and *Leishmania chagasi*. Acta Trop. **111:**15–20.
35. **Veloso, V. M., et al.** 2008. *Trypanosoma cruzi:* blood parasitism kinetics and their correlation with heart parasitism intensity during long-term infection of Beagle dogs. Mem. Inst. Oswaldo Cruz **103:**528–534.
36. **World Health Organization.** 2002. Control of Chagas disease. WHO Tech. Rep. Ser. **905:**82–83.