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Experimental infection of two South American reservoirs with four distinct strains of *Trypanosoma cruzi*

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SUMMARY

Trypanosoma cruzi (Tc), the causative agent of Chagas disease, is a diverse species with 2 primary genotypes, TcI and TcII, with TcII further subdivided into 5 subtypes (IIa–e). This study evaluated infection dynamics of 4 genetically and geographically diverse *T. cruzi* strains in 2 South American reservoirs, degus (*Octodon degus*) and grey short-tailed opossums (*Monodelphis domestica*). Based on prior suggestions of a genotype-host association, we hypothesized that degus (placental) would more readily become infected with TcII strains while short-tailed opossums (marsupial) would be a more competent reservoir for a TcI strain. Individuals ($n = 3$) of each species were intraperitoneally inoculated with *T. cruzi* trypomastigotes of TcIIa [North America (NA)-raccoon (*Procyon lotor*) origin], TcI [NA-Virginia opossum (*Didelphis virginiana*)], TcIIb [South America (SA)-human], TcIIe (SA-*Triatoma infestans*), or both TcI and TcIIa. Parasitaemias in experimentally infected degus peaked earlier (7–14 days post-inoculation (p.i.)) compared with short-tailed opossums (21–84 days p.i.). Additionally, peak parasitaemias were higher in degus; however, the duration of detectable parasitaemias for all strains, except TcIIa, was greater in short-tailed opossums. Infections established in both host species with all genotypes, except for TcIIa, which did not establish a detectable infection in short-tailed opossums. These results indicate that both South American reservoirs support infections with these isolates from North and South America; however, infection dynamics differed with host and parasite strain.

Keywords

Trypanosoma cruzi; experimental infection; reservoir host; infection dynamics; degu; grey short-tailed opossum; trypanosome

INTRODUCTION

Trypanosoma cruzi (Tc), the causative agent of American trypanosomiasis (Chagas' disease), is a haemoflagellate protozoan parasite endemic in the Americas. This parasite species has considerable genetic diversity among isolates and the ability to infect a number of mammalian hosts. A significant factor in new *T. cruzi* infection occurrence and prevalence is related to the nearly 200 animal reservoirs that have been identified in the Americas (Barretto and Ribeiro, 1979). This provides an opportunity for development of great genetic variability and distinct transmission cycles, host or habitat speciation among triatomine vectors, and changes among mammal and marsupial community structures.

The genetic structure of the *T. cruzi* population is divided into 2 primary genotypes, TcI or TcII, with type II having 5 subtypes (a–e). In South America, all 6 phylogenetic lineages are present, while only TcI and TcIIa have been identified in the United States (Clark and Pung, 1994; Barnabé *et al.* 2001; Hall *et al.* 2007; Roellig *et al.* 2008). There is increasing evidence that certain reservoir hosts maintain distinct or certain parasite genotypes. In general, it is suggested that marsupial reservoirs more readily harbour TcI, while placental mammals maintain TcII genotypes (Briones *et al.* 1999; Yeo *et al.* 2005; Roellig *et al.* 2008).

In the current study, the infection dynamics of experimental *T. cruzi* infection were studied in 2 natural wild reservoirs from South America, degu (*Octodon degus*) and grey short-tailed opossums (*Monodelphis domestica*). The degu is a diurnal, highly social caviomorph rodent native to Chile. In previous studies, prevalence in wild-captured degus in different geographical regions ranged from 2.2% to 21.4% by haemagglutination or xenodiagnosis and 51%–61% by molecular detection methods (Whiting, 1946; Duran *et al.* 1989; Jimenez and Lorca, 1990; Rozas *et al.* 2005, 2007). The grey short-tailed opossum is a solitary, terrestrial, didelphid opossum that is native to Bolivia, Brazil and Paraguay. A characteristic of short-tailed opossums, as well as some other South American marsupials, is the absence of a pouch for neonates, which may have implications in transmission and maintenance of *T. cruzi*. The underdeveloped young cling to the teats of females during their development. Prevalence data for wild-captured grey short-tailed opossums range from 11.1% to 66.7% in different geographical regions by various detection methods, such as haemoculture, xenodiagnosis, and serology (Herrera *et al.* 2005; Roque *et al.* 2008).

Genotyping of *T. cruzi* isolated from both species has been previously reported, and multiple genotypes have been identified in both species. Only 6 isolates have been characterized from short-tailed opossums; 2 were TcII (undefined subtype), 1 was TcIIc and 3 were TcI (Yeo *et al.* 2005; Roque *et al.* 2008). Multiple genotypes have also been reported from naturally infected degus (Yeo *et al.* 2005; Campos *et al.* 2007; Rozas *et al.* 2007; Spotorno *et al.* 2008; Galuppo *et al.* 2009). One study showed that TcI and TcIIb were more prevalent than other detected genotypes (TcIIc and TcIIe) (Rozas *et al.* 2007), while another found higher prevalence of TcIIc than TcIIb and TcI genotypes (Galuppo *et al.* 2009). These field-based studies suggest that these 2 hosts are susceptible to both major genotypes of *T. cruzi*, which is in contrast to data from 2 major North American reservoirs which exhibit significant predilections for certain *T. cruzi* genotypes (Roellig *et al.* 2008, 2009).

The goals of the present study were to ascertain reservoir potential of *M. domestica* and *O. degus* for 4 different genotypes of *T. cruzi* from the United States and South America. In addition, the infection dynamics of these 4 different strains were studied to determine if there are any associations between parasite genotype and host species.

MATERIALS AND METHODS

The 2 North American isolates used in this study were originally isolated from a naturally infected Florida raccoon, *Procyon lotor* [FL-RAC9 (TcIIa)], and Virginia opossum, *Didelphis virginiana* [FL-OPO3 (TcI)], from northwestern Florida (Roellig *et al.* 2008). Two South American strains, Y (TcIIb) and Tulahuen (TcIIe), were generously provided by Dr Rick Tarleton and Dr Roberto Docampo (The University of Georgia, Athens, GA, USA), respectively. Each strain was molecularly typed as previously described (Brisse *et al.* 2001; Roellig *et al.* 2008). Epimastigotes were passaged from Liver-Infusion Tryptose (LIT) medium into DH82 canine macrophage monolayers as previously described (Roellig *et al.* 2009).

Laboratory raised short-tailed opossums were acquired from the Southwest Foundation for Biomedical Research (San Antonio, TX) and individually housed in large rodent cages in climate-controlled animal housing at the College of Veterinary Medicine, University of Georgia (Athens, GA). Captive-bred degus were purchased from a commercial source (S&S exotics, Houston, TX) and similarly housed at a maximum of 3 individuals per cage. All animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee and under an animal use protocol approved by this committee at the University of Georgia.

For both species, animals were randomly separated into 5 experimental groups and 1 negative control group. Individuals in groups 1, 2, 3 or 4 ($n = 3$) were inoculated intraperitoneally (IP) with 1×10^6 culture-derived trypomastigotes of FL-OPO3 (TcI), FL-RAC9 (TcIIa), Y (TcIIb), or Tulahuen (TcIIe) strain, respectively. Group 5 individuals ($n = 3$) were inoculated with 5×10^5 FL-OPO3 strain and 5×10^5 FL-RAC9 strain trypomastigotes in a single mixed inoculum. Negative controls ($n = 2$) for both species were similarly inoculated with an equivalent volume of MEM.

For handling and blood collection, animals were anaesthetized with subcutaneous administration of a mixture of ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA) and xylazine (Moby Corporation, Shawnee, KS, USA). Degus were given 100 mg/kg of ketamine and 20 mg/kg of xylazine; short-tailed opossums were given 50 mg/kg ketamine and 10 mg/kg xylazine. Approximately 20–100 μ l of blood were aseptically collected at 0, 7, 14, 21, 28, 35, 42, 49, 56, 70, 84, and 112 days p.i. from the medial saphenous vein of degus and lateral tail vein of short-tailed opossums using heparinized capillary tubes. One animal from each group was euthanized at 28, 56, and 112 days p.i., representative of acute, late acute, and chronic infections, respectively. Animals were humanely euthanized under anaesthesia by CO₂. After euthanasia, animals were exsanguinated via cardiac-puncture and blood was collected into 4 ml vol. vacuette ethylenediaminetetraacetic acid (EDTA) tubes (Greiner Bio-one, Monroe, NC, USA).

At each sampling time, parasitaemias were determined by examining 5 μ l of whole blood as previously described (Roellig *et al.* 2009). For each time-point, DNA was extracted from 20–100 μ l of red blood cell/buffy coat homogenate using the GFX genomic blood DNA purification kit (Amersham Biosciences, Piscataway, NJ, USA) or DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturers' protocols. After euthanasia, animals were necropsied and portions of major tissues (retropharyngeal lymph nodes, diaphragm, heart, lungs, liver, spleen, gastrointestinal tract, pancreas, kidney, adrenal glands, reproductive organs, urinary bladder, quadriceps muscle, bone marrow, and brain) were collected. DNA was isolated from tissue using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's protocol with a 24-h tissue digestion step. Extracted DNA was used as template in a modified nested PCR amplification of the *T. cruzi* 24Sa

rDNA D7 divergent domain (Souto *et al.* 1996) as previously published (Roellig *et al.* 2009). Negative samples were verified by amplification of the size-variable domain of the 18S rRNA gene (Clark and Pung, 1994) as previously described (Brisse *et al.* 2001).

Blood (0.5 ml) collected at euthanasia was cultured in DH82 cells (Yabsley *et al.* 2004; Hall *et al.* 2007). Cultures were checked daily for the presence of trypomastigotes. For xenodiagnosis of chronically infected animals, laboratory raised *Rhodnius prolixus* nymphs (4th and 5th instars) ($n = 3$) were fed until repletion on each anaesthetized, chronically infected animal from each group. Bugs were allowed to digest the bloodmeal and moult in an isolated, temperature and humidity controlled environment. The intestinal tracts of resultant 5th instars or adults were removed, added to 700 μ l of PBS, vortexed, and boiled for 15 min. This solution was used for PCR amplification of kinetoplast DNA as described above.

Indirect immunofluorescent antibody assays were performed as previously described (Yabsley *et al.* 2001; Roellig *et al.* 2009) with plasma at a 1 : 40 dilution. Secondary antibody used during degu serology was an FITC-labelled goat anti-mouse IgG (Kirkegaard and Perry Laboratories (KPL), Gaithersburg, Maryland, USA). After the first incubation, short-tailed opossum samples were incubated with a rabbit anti-opossum IgG (Bethyl Laboratories, Montgomery, Texas, USA), and then a FITC-labelled anti-rabbit IgG (KPL). A sample was positive for *T. cruzi* antibodies if epimastigotes appeared green under fluorescent microscopy, or low positive if red with a green outline. Negative samples appeared red.

Formalin-fixed tissues were routinely processed, embedded in paraffin, sectioned at 5 μ m, and stained with haematoxylin and eosin. Slides were examined by light microscopy and blindly scored by a veterinary pathologist. Histological lesions were scored as mild, moderate, or severe for each tissue. The presence of amastigote nests was also noted in tissues after scanning 40 fields at 400 $\times$ magnification.

RESULTS

Degus

Parasitaemias were first detected in all animals at 7 days p.i. (Fig. 1). The highest parasite counts were observed in animals inoculated with TcIIb and TcI, and all animals had a rapid decline in parasitaemia. Significant differences in parasitaemias during the first 28 days of infection were noted between experimental groups ($F = 13.65$, $P < 0.001$) as determined by Greenhouse-Geiser MANOVA methodology. Parasites were not observed in the TcIIb- and TcIIa-inoculated animals after 28 days p.i. ; parasites were undetectable after 56 days p.i. for the TcI, TcIIe, and dual-infected groups. At 28 days p.i., all acutely infected animals still had detectable parasitaemia at euthanasia and were also haemoculture positive (Table 1). At 56 days p.i., late acute-phase animals had variable positive results with TcIIa- and dual-inoculated animals being haemoculture negative, while TcI- and TcIIe-inoculated animals were haemoculture positive. At 112 days p.i., none of the chronically infected degus had detectable parasitaemias; however, haemoculture and xenodiagnosis indicated that TcI-, TcIIe-, and dual (TcI/TcIIa)-inoculated animals were still parasitaemic.

Molecular detection of *T. cruzi* DNA in blood samples was achieved for all experimental groups. Animals became PCR positive by 7 or 14 days p.i., with the exception of 1 degu, which was only positive at 35 days p.i. (Table 1). That individual degu also had a low parasitaemia at 7 and 14 days p.i., and parasites were not observed in blood on other days. PCR amplification was intermittent for many of the animals with no detection on some bleed days; however, trends could be observed. All experimental groups were PCR positive

through the acute phase (28 days p.i.), but *T. cruzi* DNA was only amplified in dual- and TcIIe-chronically infected animals (112 days p.i.). Amplification of *T. cruzi* DNA in tissue samples was achieved for all animals (data not shown). For many of the animals, all tissues were PCR positive but the hearts, quadriceps, and spleens were PCR positive for all animals. Serology revealed seroconversion of all animals by 14 days p.i., and all remained seropositive at the time of euthanasia (Table 1).

Lesions were common in the heart ($n = 14$), skeletal muscle ($n = 13$), brain ($n = 11$), kidney ($n = 9$), and urinary bladder ($n = 9$). Lesions were occasionally noted in liver ($n = 6$), pancreas ($n = 5$), adrenal gland ($n = 4$), testicle ($n = 4$), lung ($n = 2$), and intestine ($n = 2$). In heart and skeletal muscle, lesions consisted of myofibre necrosis and multifocal aggregates of lymphocytes and plasma cells with occasional macrophages or neutrophils. Inflammation in skeletal muscle was mild ($n = 10$) to moderate ($n = 3$) and ranged from mild ($n = 5$) to moderate ($n = 5$) to severe ($n = 4$) in heart. Lesions in brain were mild ($n = 7$) to moderate ($n = 4$) and included lymphoplasmacytic perivascular cuffing, glial nodules, and meningitis. Pseudocysts or amastigotes were observed in multiple tissues including heart ($n = 7$), skeletal muscle ($n = 3$), brain ($n = 1$), testicle ($n = 1$), intestine ($n = 1$), adrenal gland ($n = 1$), and urinary bladder ($n = 1$).

Short-tailed opossums

Parasitaemias were detected in TcIIb-, TcI-, TcIIe-, and dual-infected animals by 7 days p.i., but TcIIa-inoculated animals never developed a detectable parasitaemia (Fig. 2). No significant differences in parasitaemias between experimental groups were detected by Greenhouse-Geiser MANOVA methodology ($F = 7.2716$, $P = 0.1207$). TcIIb-infected animals were parasitaemic until 28 days p.i., TcI- and dual-infected opossums until 84 days p.i., and TcIIe-infected opossums until 112 days p.i.. Haemoculture and xenodiagnosis confirmed that TcIIa-infected animals were either not parasitaemic or had parasitaemias below detection limits (Table 2). The TcIIb-acutely infected opossum was not parasitaemic or haemoculture positive on the day of euthanasia. Interestingly the other 2 animals in this group were haemoculture positive although parasites could not be found in the 5 μ l of blood examined following day 28p.i.. All other groups had circulating parasitaemias that were detected by haemoculture, parasite counts, and/or xenodiagnosis.

Similar to parasitaemia determination, haemoculture, and xenodiagnosis, PCR amplification attempts in TcIIa-inoculated animal blood failed to yield positive results (Table 2). The most consistent detection of *T. cruzi* DNA was accomplished for TcIIe- and TcI-inoculated animals. At least 1 individual for each of these 2 groups was first PCR positive on day 7 p.i. TcIIb and dual-inoculated groups were also PCR positive by 7 day p.i. ; however, *T. cruzi* DNA was only detected intermittently.

PCR amplification in tissues yielded similar results, with tissues from TcIIa-inoculated animals all being negative for *T. cruzi* by PCR while all other groups had at least 1 PCR-positive tissue (data not shown). *T. cruzi* DNA was most commonly amplified from skeletal muscle (quadricep and diaphragm). There were no differences in detection among tissues of animals within an experimental group during different stages of infection. Additionally, no differences in amplification were observed among experimental groups. Serology revealed that all animals seroconverted by 21 days p.i., with animals in TcIIb- and TcIIa-inoculated groups seroconverting after the TcI group.

Histological lesions were uncommon and were usually mild. The heart was the only tissue consistently affected with 10 of 16 animals having myocardial lesions. Lesions were observed rarely in brain ($n = 2$), pancreas ($n = 2$), liver ($n = 2$), adrenal gland ($n = 2$), kidney ($n = 1$), intestine ($n = 1$), urinary bladder ($n = 1$), and skeletal muscle ($n = 1$). In all organs,

inflammation was primarily lymphoplasmacytic with fewer histiocytes and occasional neutrophils and eosinophils. One opossum had a single glial nodule as the only lesion in the brain. Pseudocysts were not observed in any of the tissues examined.

DISCUSSION

The maintenance and continuation of the *T. cruzi* sylvatic cycle is dependent on a competent vector feeding on a parasitaemic animal. Since *T. cruzi* is a genetically and biologically diverse species that can infect a wide range of mammalian hosts, it is reasonable to hypothesize that certain animal species may maintain parasitaemias longer than others and, thus, have differences in their ability to serve as reservoirs. These differences in reservoir potential may be based on the host species or genetic makeup, the genotype of the parasite, or a combination of both. In this preliminary study, the reservoir potential and infection dynamics of experimental *T. cruzi* infections in degus and short-tailed opossums were investigated by inoculating animals with different *T. cruzi* genotypes.

Similar to experimental infections with raccoons, another placental mammal (Roellig *et al.* 2009), the degus, developed patent infections after inoculation with each of the 4 isolates (representing genotypes TcI, TcIIa, TcIIb, and TcIIe) analysed in this study. These data support molecular typing studies conducted on isolates from naturally infected degus from South America that showed natural infection with multiple genotypes including TcI, TcIIa, TcIIb, TcIIc, and TcIIe singly, and some mixed infections (Yeo *et al.* 2005; Campos *et al.* 2007; Rozas *et al.* 2007; Spotorno *et al.* 2008). Interestingly, 2 genotypes, TcI and TcIIe, maintained parasitaemias during chronic infections (112 days p.i.) that were sufficient to infect xenodiagnostics fed *R. prolixus*. Because of their ability to maintain parasitaemias for a long period of time, degus may be considered important reservoirs for the 2 genotypes (TcI and TcIIe). Further experimental studies with additional strains and larger sample sizes would help to understand the infection dynamics of *T. cruzi* in degus and identify infectivity and maintenance differences between genotypes suggested in this study.

In the case of short-tailed opossums, animals inoculated with TcIIa seroconverted, but a patent infection could not be detected by any other means, including molecular and direct examination of blood and tissues. Findings were similar to experimental and field-based molecular studies that found that another marsupial, the Virginia opossum (*Didelphis virginiana*), does not maintain infections with TcIIa (Clark and Pung, 1994; Barnabé *et al.* 2001; Roellig *et al.* 2008, 2009). However, field isolates of other marsupial species from South America, including *D. marsupialis* and *P. frenata*, indicate that based on zymodeme analysis, these species can be infected with TcI, TcIIa, and TcIIc genotypes of South American origin (Miles *et al.* 1981; Póvoa *et al.* 1984; Pinho *et al.* 2000). Additionally, *M. brevicaudata* has been shown to be naturally infected with TcIIa and TcIIc of South American origin (Miles *et al.* 1981; Póvoa *et al.* 1984). Differences in infectivity of TcIIa strains from South versus North America in marsupials may be indicative of biological differences in the parasite and not host susceptibility.

Our findings suggest that short-tailed opossums may serve as reservoirs for multiple strains of *T. cruzi*, including TcI, TcIIb, TcIIe, and mixed infections. These data expand our knowledge on the genotypes to which grey short-tailed opossums are susceptible, which were previously limited to TcIIc and TcI based on natural infections reported from the Gran Chaco of Paraguay and Redenção, Brazil (Yeo *et al.* 2005; Roque *et al.* 2008). The peak in parasitaemia seen in the TcI-inoculated animal at 84 days p.i. is believed to be an artifact of differences between experimental animals in this group as 1 of 2 animals was euthanized at the previous time-point. As all chronically infected experimental animals were parasitaemic at the time of euthanasia, and no differences were statistically detected during acute

infection, short-tailed opossums appear to develop long-term parasitaemias with multiple genotypes, which is in contrast to Virginia opossums that were inoculated with multiple strains (Roellig *et al.* 2009).

The current study also demonstrated that degus and short-tailed opossums are competent hosts for strains of *T. cruzi* from North America. Similar to findings that North American hosts can become infected with South American isolates, no differences in infectivity based on the geographical origin of the isolates were observed (Roellig *et al.* 2009). Field studies have often found that both major lineages (TcI and TcII) can infect vector species (Marcet *et al.* 2006; Falla *et al.* 2009), and experimental studies, including the current one, have described infection in vectors susceptible to multiple genotypes (Perlowagora-Szumlewicz *et al.* 1990; Coronado *et al.* 2006; Campos *et al.* 2007). Combined with the experimental data from the present study, there is a potential for a non-native strain to become established in South America.

This study suggests that different genotypes of *T. cruzi* induce distinct infection dynamics in divergent host species. Further work with additional isolates and genotypes and greater sample sizes will enable a better understanding of parasite genotype-host interactions. Such information would be vital for understanding the epidemiology and epizootiology of Chagas' disease and may lead to better preventative measures in endemic regions.

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Fig. 1.

Parasitaemias of degus (*Octodon degus*) experimentally inoculated with different genotypes of *Trypanosoma cruzi*. Black arrows indicate days when an individual from each experimental group was euthanized. Statistical differences in parasitaemias were detected between experimental groups through the acute stage of infection (MANOVA, $F = 13.65$, $P < 0.0001$).

Fig. 2. Parasitaemias of short-tailed opossums (*Monodelphis domestica*) experimentally inoculated with different genotypes of *Trypanosoma cruzi*. Black arrows indicate days when an individual from each experimental group was euthanized. No statistical differences in parasitaemias were detected between experimental groups through the acute stage of infection (MANOVA, $F = 1.9335$, $P = 0.1207$).

Results* of polymerase chain reaction (PCR) amplification of *Trypanosoma cruzi* 24Sα rDNA D7 divergent domain and indirect immunofluorescence assay (IFA) from experimentally infected degus

Table 1

Group	7 days p.i.	14 days p.i.	21 days p.i.	28 days p.i.	35 days p.i.	42 days p.i.	49 days p.i.	56 days p.i.	84 days p.i.	112 days p.i.
TcIIb										
6752-R	+/-	+/+	+/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.
6756-2R [†]	+/-	+/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.	euth.
6752-L	+/-	-/weak +	-/+	-/n.d.	-/n.d.	+/n.d.	+/n.d.	+/n.d.	-/n.d.	-/+
TcI										
6743-R [‡]	+/-	+/+	+/+	-/n.d.	+/n.d.	+/n.d.	+/n.d.	-/n.d.	-/n.d.	-/+
6743-L [‡]	+/-	+/+	+/+	+/n.d.	-/n.d.	+/n.d.	-/n.d.	+/+	euth.	euth.
6743-B [‡]	+/-	+/+	+/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.
TcIIa										
6749-L	+/-	+/+	-/+	+/n.d.	+/n.d.	+/n.d.	-/n.d.	-/+	euth.	euth.
6749-R	-/-	-/+	-/+	-/n.d.	-/n.d.	+/n.d.	-/n.d.	-/n.d.	-/n.d.	-/+
6749-B [‡]	+/-	+/+	-/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.
TcIIe										
6754-L [‡] ‡	-/-	+/+	+/+	-/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/+
6756-2L [‡]	+/-	+/+	+/+	+/n.d.	+/n.d.	-/n.d.	-/n.d.	-/+	euth.	euth.
6754-R [‡]	-/-	-/weak +	+/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.
Dual (TcIIa & TcI)										
6747-L [‡]	+/-	+/+	+/+	+/+	euth.	euth.	euth.	euth.	euth.	euth.
6747-R	+/-	+/+	+/+	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/+	euth.	euth.
6747-B [‡]	+/-	+/+	+/+	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	-/+

* x/x=PCR result/IFA result, + indicates positive, - indicates negative, n.d., not done, euth., euthanized on previous date.

[†] Haemoculture positive and/or parasitemic on day of euthanasia.

[‡] Positive by xenodiagnosis (determined only for chronic animals).

Results* of polymerase chain reaction amplification (PCR) of *Trypanosoma cruzi* 24Sα rDNA D7 divergent domain and indirect immunofluorescence assay (IFA) of experimentally infected short-tailed opossums

Table 2

Group	7 days p.i.	14 days p.i.	21 days p.i.	28 days p.i.	35 days p.i.	42 days p.i.	49 days p.i.	56 days p.i.	84 days p.i.	112 days p.i.
TcIIb										
6886	+/-	-/-	-/weak +	-/+	euth.	euth.	euth.	euth.	euth.	euth.
6887 [†]	+/-	-/+	-/+	-/n.d.	+/-	+/-	-/n.d.	-/n.d.	-/n.d.	-/+
6888 [†]	+/-	+/+	-/+	-/n.d.	+/-	+/-	+/-	-/+	euth.	euth.
TcI										
6889 [†]	-/-	+/+	-/+	+/-	+/-	+/-	+/-	+/-	+/-	+/-
6890 [†]	+/-	+/-	+/-	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/n.d.
6891 [†]	+/-	+/-	+/-	+/-	euth.	euth.	euth.	euth.	euth.	euth.
TcIIa										
6892	-/-	-/weak +	-/weak +	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/+	euth.	euth.
6893	-/-	-/+	-/+	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/n.d.	-/+
6894	-/-	-/-	-/+	-/+	euth.	euth.	euth.	euth.	euth.	euth.
TcIe										
6898 [†]	+/-	+/+	-/+	+/-	euth.	euth.	euth.	euth.	euth.	euth.
6883 [†]	-/+	-/+	-/n.d.	+/-	+/-	+/-	-/n.d.	+/-	euth.	euth.
6899 [†]	-/-	+/+	+/-	+/-	-/n.d.	+/-	+/-	-/n.d.	+/-	+/-
Dual (TcIIa & TcI)										
6895 ^{†,‡}	+/-	+/+	+/-	+/-	-/n.d.	+/-	+/-	+/-	+/-	+/-
6896 [†]	+/+	+/+	+/-	+/-	+/-	+/-	+/-	+/-	euth.	euth.
6897 [†]	-/-	-/+	+/-	+/-	euth.	euth.	euth.	euth.	euth.	euth.

* x/x=PCR result/IFA result, + indicates positive, - indicates negative, n.d., not done, euth., euthanized on previous date.

[†] Haemoculture positive and/or parasitaemic on day of euthanasia.

[‡] Positive by xenodiagnosis (determined only for chronic animals).