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

DAWN M. ROELLIG[1]*, KATHERINE McMILLAN[1], ANGELA E. ELLIS[2], JOHN L. VANDEBERG[3], DONALD E. CHAMPAGNE[4] *and* MICHAEL J. YABSLEY[1,5]

[1] *Southeastern Cooperative Wildlife Disease Study, Department of Population Health, The University of Georgia, Athens, GA 30602, USA*
[2] *Veterinary Diagnostic Laboratory, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602, USA*
[3] *Southwest Foundation for Biomedical Research and Southwest National Primate Research Center, San Antonio, TX 78245, USA*
[4] *Department of Entomology and Center for Tropical Emerging and Global Diseases, The University of Georgia, Athens, GA 30602, USA*
[5] *D. B. Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, GA 30602, USA*

(*Received 15 September 2009; revised 4 November 2009; accepted 25 November 2009; first published online 4 February 2010*)

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.

Key words: *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, degus (*Octodon degus*) and grey short-tailed opossums (*Monodelphis domestica*). The degu is a diurnal,

* Corresponding author: 589 D.W. Brooks Drive, Wildlife Health Building, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA. E-mail: droellig@uga.edu

Parasitology (2010), **137**, 959–966.
doi:10.1017/S0031182009991995

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 (TcIId and TcIIe) (Rozas *et al*. 2007), while another found higher prevalence of TcIId 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 (Mobay 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 μl 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 CO_2. 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 μl of whole blood as previously described (Roellig *et al*. 2009). For each time-point, DNA was extracted from 20–100 μl of red blood cell/buffy coat homogenate using the GFX genomic blood DNA purification kit (Amersham

https://doi.org/10.1017/S0031182009991995 Published online by Cambridge University Press

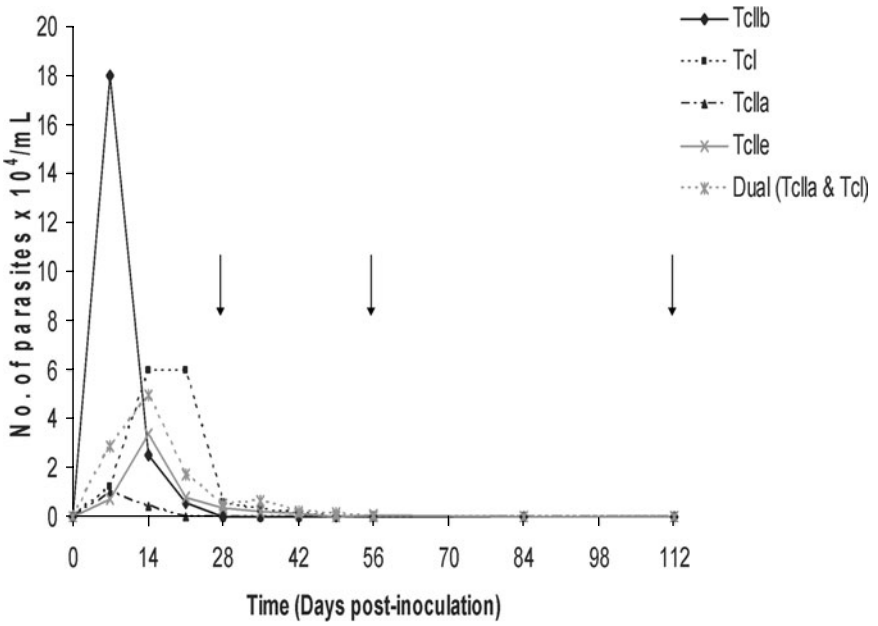

Fig. 1. Parasitaemias of degus (*Octodon degus*) experimentally inoculated with different genotypes of *Trypanosoma cruzi*. Black arrows indicate days when an individual from each experimental group was euthanized. Statistical differences in parasitaemias were detected between experimental groups through the acute stage of infection (MANOVA, F = 13·65, $P < 0{\cdot}0001$).

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* 24Sα 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 μl 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 μm, 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 × 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

https://doi.org/10.1017/S0031182009991995 Published online by Cambridge University Press

Table 1. Results* of polymerase chain reaction (PCR) amplification of *Trypanosoma cruzi* 24Sα rDNA D7 divergent domain and indirect immunofluorescence assay (IFA) from experimentally infected degus

Group	7 days p.i.	14 days p.i.	21days 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).

first 28 days of infection were noted between experimental groups ($F = 13{\cdot}65$, $P < 0{\cdot}001$) 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 ($n = 1$).

https://doi.org/10.1017/S0031182009991995 Published online by Cambridge University Press

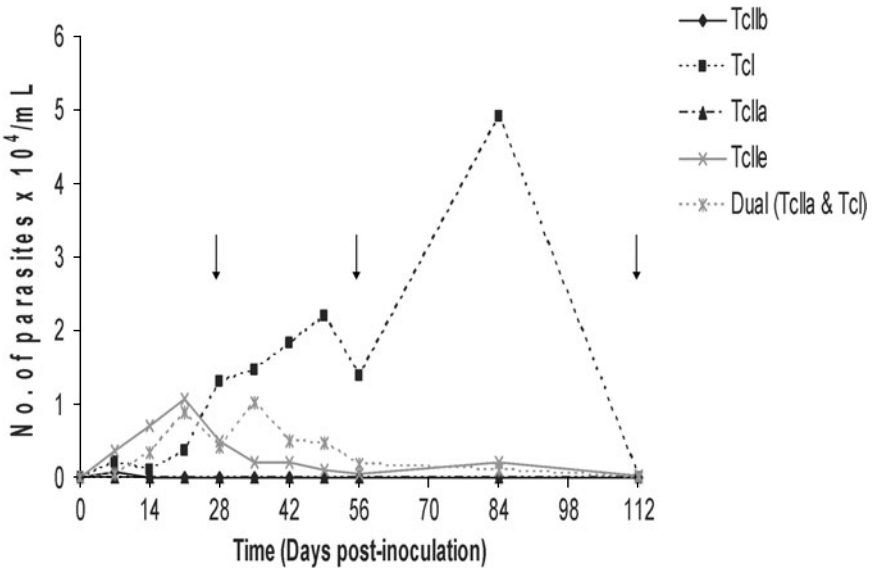

Fig. 2. Parasitaemias of short-tailed opossums (*Monodelphis domestica*) experimentally inoculated with different genotypes of *Trypanosoma cruzi*. Black arrows indicate days when an individual from each experimental group was euthanized. No statistical differences in parasitaemias were detected between experimental groups through the acute stage of infection (MANOVA, F = 1·9335, $P = 0·1207$).

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 μl 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

https://doi.org/10.1017/S0031182009991995 Published online by Cambridge University Press

Table 2. 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

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	+/n.d.	−/−	−/weak+	−/+	*euth.*	*euth.*	*euth.*	*euth.*	*euth.*	*euth.*
6887†	+/−	−/+	−/+	−/n.d.	+/n.d.	+/n.d.	−/n.d.	−/n.d.	−/n.d.	−/+
6888†	+/−	+/+	−/+	−/n.d.	+/n.d.	+/n.d.	+/n.d.	−/+	*euth.*	*euth.*
TcI										
6889†	−/−	+/+	−/+	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/+
6890†	+/weak+	+/weak+	+/+	−/n.d.	−/n.d.	+/n.d.	+/n.d.	+/+	*euth.*	*euth.*
6891†	+/weak+	+/weak+	+/weak+	+/+	*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.*
TcIIe										
6898†	+/−	+/+	−/+	+/+	*euth.*	*euth.*	*euth.*	*euth.*	*euth.*	*euth.*
6883†	−/+	−/+	−/n.d.	+/n.d.	+/n.d.	+/n.d.	−/n.d.	+/+	*euth.*	*euth.*
6899†	−/−	+/+	+/n.d.	+/n.d.	−/n.d.	+/n.d.	+/n.d.	−/n.d.	+/n.d.	+/+
Dual (TcIIa & TcI)										
6895†,‡	+/−	+/+	+/+	+/n.d.	−/n.d.	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/+
6896†	+/+	+/+	+/+	+/n.d.	+/n.d.	+/n.d.	+/n.d.	+/+	*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).

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, TcIId, 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 xenodiagnostically fed *R. prolixis*. 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 Redencão, Brazil

https://doi.org/10.1017/S0031182009991995 Published online by Cambridge University Press

(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.

ACKNOWLEDGEMENTS

The authors thank Mason Savage, Jessica Murdock, Wendy Fujita, and Emily Brown (SCWDS) for laboratory assistance and the Animal Resource staff at The University of Georgia College of Veterinary Medicine for assistance with degu and opossum care.

FINANCIAL SUPPORT

This study was primarily supported by the National Institutes of Health, National Institute of Allergy and Infectious Disease Grant R15 AI067304. K.M. was a Georgia Veterinary Scholar and part of her support was obtained from T35 RR022685-01A1 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. In addition, we thank Merck-Merial Ltd and the Veterinary Medical Experimental Station for their financial support of the students and the Georgia Veterinary Scholars program as a whole. Additional support was through funding provided to John L. VandeBerg by the Robert J. Kleberg, Jr., and Helen C. Kleberg Foundation, and to SCWDS by the Federal Aid to Wildlife Restoration Act (50 Stat. 917) and through sponsorship of the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia.

REFERENCES

Barnabé, C., Yaeger, R., Pung, O. and Tibayrenc, M. (2001). *Trypanosoma cruzi*: A considerable phylogenetic divergence indicates that the agent of Chagas disease is indigenous to the native fauna of the United States. *Experimental Parasitology* **99**, 73–79.

Barretto, M. P. and Ribeiro, R. D. (1979). Reservatorios silvestres do *Trypanosoma cruzi*. *Revista doInstituto de Adolfo Lutz* **39**, 25–26. [In Portugese]

Briones, M. R. S., Souto, R. P., Stolf, B. S. and Zingales, B. (1999). The evolution of two *Trypanosoma cruzi* subgroups inferred from rRNA genes can be correlated with the interchange of American mammalian faunas in the Cenozoic and has implications to pathogenicity and host specificity. *Molecular and Biochemical Parasitology* **104**, 219–232.

Brisse, S., Verhoef, J. and Tibayrenc, M. (2001). Characterization of large and small subunit rRNA and mini-exon genes further support the distinction of six *Trypanosoma cruzi* lineages. *International Journal for Parasitology* **31**, 1218–1226.

Campos, R., Acuña-Retamar, M., Botto-Mahan, C., Ortiz S., Cattan, P. E. and Solari, A. (2007). Susceptibility of *Mepraia spinolai* and *Triatoma infestans* to different *Trypanosoma cruzi* strains from naturally infected rodent hosts. *Acta Tropica* **104**, 25–29.

Clark, C. G. and Pung, O. J. (1994). Host specificity of ribosomal DNA variation in sylvatic *Trypanosoma cruzi* from North America. *Molecular and Biochemical Parasitology* **66**, 175–179.

Coronado, X., Zulantay, I., Albrecht, H., Rozas, M., Apt, W., Ortiz, S., Rodriguez, J., Sanchez, G. and Solari, A. (2006). Variation in *Trypanosoma cruzi* clonal composition detected in blood patients and xenodiagnosis triatomines: implications in the molecular epidemiology of Chile. *American Journal of Tropical Medicine and Hygiene* **74**, 1008–1012.

Duran, J., Videla, M. and Apt, W. (1989). Enfermedad de Chagas en una comunidad de pequeños mamíferos simpátricos de la Reserva Nacional de Las Chinchillas. *Parasitología al Día* **13**, 15–20. [In Spanish]

Falla, A., Herrera, C., Fajardo, A., Montilla, M., Vallejo, G. A. and Guhl, F. (2009). Haplotype identification within *Trypanosoma cruzi* I in Colombian isolates from several reservoirs, vectors and humans. *Acta Tropica* **110**, 15–21.

Galuppo, S., Bacigaluo, A., García, A., Ortiz, A., Coronado, X., Cattan, P. E. and Solari, A. (2009). Predominance of *Trypanosoma cruzi* genotypes in two reservoirs infected by sylvatic *Triatoma infestans* of an endemic area of Chile. *Acta Tropica* **111**, 90–93.

Hall, C. A., Polizzi, C., Yabsley, M. J. and Norton, T. M. (2007). *Trypanosoma cruzi* prevalence

and epidemiologic trends in lemurs on St. Catherines Island, Georgia. *Journal of Parasitology* **93**, 93–96.

Herrera, L., D'Andrea, P. S., Xavier, S. C. C., Mangia, R. H., Fernandes, O. and Jansen, A. M. (2005). *Trypanosoma cruzi* infection in wild mammals of the National Park 'Serra de Capivera' and its surroundings (Piauí, Brazil), an area endemic for Chagas disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 379–388.

Jimenez, J. and Lorca, M. (1990). Trypanosomiasis Americana en vertebrados silvestres y su relación con el vector *Triatoma spinolai*. *Archivos de Medicina Veterinaria* **22**, 179–183.

Marcet, P. L., Duffy, T., Cardinal, M. V., Burgos, J. M., Lauricella, M. A., Levin, M. J., Kitron, U., Gürtler, R. E. and Schijman, A. G. (2006). PCR-based screening and lineage identification of *Trypanosoma cruzi* directly from faecal samples of triatomine bugs from northwestern Argentina. *Parasitology* **132**, 57–65.

Miles, M. A., Póvoa, M. M., de Souza, A. A., Lainson, R., Shaw, J. J. and Ketteridge, D. S. (1981). Chagas disease in the Amazon Basin: II. The distribution of *Trypanosoma cruzi* zymodemes 1 and 3 in Pará state, north Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 667–674.

Perlowagora-Szumlewicz, A., Muller, C. A. and de Carvalho Moreira, C. J. (1990). Studies in search of suitable experimental insect model for xenodiagnosis of hosts with Chagas' disease 4- the reflection of parasite stock in the responsiveness of different vector species to chronic infection with different *Trypanosoma cruzi* stocks. *Revista de Saúde Pública* **24**, 165–177.

Pinho, A. P., Cupolillo, E., Mangia, R. H., Fernandes, O. and Jansen, A. M. (2000). *Trypanosoma cruzi* in the sylvatic environment: distinct transmission cycles involving two sympatric marsupials. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 509–514.

Póvoa, M. M., de Souza, A. A., Naiff, R. D., Arias, J. R., Naiff, M. F., Biancardi, C. B. and Miles, M. A. (1984). Chagas' disease in the Amazon Basin IV: Host records of *Trypanosoma cruzi* zymodemes in the States of Amazonas and Rondonia, Brazil. *Annals of Tropical Medicine and Parasitology* **78**, 479–487.

Roellig, D. M., Brown, E. L., Barnabé, C., Tibayrenc, M., Steurer, F. J. and Yabsley, M. J. (2008). Molecular typing of *Trypanosoma cruzi* isolates, United States. *Emerging Infectious Diseases* **14**, 1123–1125.

Roellig, D. M., Ellis, A. E. and Yabsley, M. J. (2009). Genetically different isolates of *Trypanosoma cruzi* elicit different infection dynamics in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*). *International Journal for Parasitology* **39**, 1603–1610.

Roque, A. L., Xavier, S. C. C., da Rocha, M. G., Duarte, A. C. M., D'Andrea, P. S. and Jansen, A. M. (2008). *Trypanosoma cruzi* transmission cycle among wild and domestic mammals in three areas of orally transmitted Chagas disease outbreaks. *American Journal of Tropical Medicine and Hygiene* **79**, 742–749.

Rozas, M., Botto-Mahan, C., Coronado, X., Ortiz, S., Cattan, P. E. and Solari, A. (2005). Short Report: *Trypanosoma cruzi* infection in wild mammals from a chagasic area of Chile. *American Journal of Tropical Medicine and Hygiene* **73**, 517–519.

Rozas, M., Botto-Mahan, C., Coronado, X., Ortiz, S., Cattan, P. E. and Solari, A. (2007). Coexistence of *Trypanosoma cruzi* genotypes in wild and peridomestic mammals in Chile. *American Journal of Tropical Medicine and Hygiene* **77**, 647–653.

Souto, R. P., Fernandes, O., Macedo, A. M., Campbell, D. A. and Zingales, B. (1996). DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* **83**, 141–152.

Spotorno, A. E., Córdova, L. and Solari, A. (2008). Differentiation of *Trypanosoma cruzi* I subgroups through characterization of cytochrome *b* gene sequences. *Infection, Genetics and Evolution* **8**, 898–900.

Whiting, C. (1946). Contribución al studio de las reservas de parásitos de la enfermedad de Chagas en Chile. Primeros halazgos en Chile de mamíferos silvestres infestados por *Trypanosoma cruzi*. *Revista Chilena de Higiene y Medicina Preventiva* **8**, 69–102. [In Spanish]

Yabsley, M. J., Noblet, G. P. and Pung, O. J. (2001). Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). *Journal of Parasitology* **87**, 1155–1159.

Yabsley, M. J., Norton, T. M., Powell, M. R. and Davidson, W. R. (2004). Molecular and serologic evidence of tick-borne ehrlichiae in three species of lemurs from St. Catherines Island, Georgia, USA. *Journal of Zoo and Wildlife Medicine* **35**, 503–509.

Yeo, M., Acost, N., Llewellyn, M., Sánchez, H., Adamson, S., Miles, G. A., López, E., González, N., Patterson, J. S., Gaunt, M. W., de Arias, A. R. and Miles, M. A. (2005). Origins of Chagas Disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. *International Journal for Parasitology* **35**, 225–233.