Short Report: Infectivity, Pathogenicity, and Virulence of *Trypanosoma cruzi* Isolates from Sylvatic Animals and Vectors, and Domestic Dogs from the United States in ICR Strain Mice and SD Strain Rats

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Abstract. *Trypanosoma cruzi*, the causative agent of Chagas disease, is widespread in the southern United States. In addition to detection in numerous wildlife host species, cases have been diagnosed in domestic dogs and humans. In the current investigation, groups of laboratory mice [CrI:CD1 (ICR)] were inoculated with one of 18 United States *T. cruzi* isolates obtained from a wide host range to elucidate their infectivity, pathogenicity, and virulence. In addition, laboratory rats (SD strain) were inoculated with four isolates. Mice and rats were susceptible to infection with all strains, but no morbidity or mortality was noted, which indicates that these *T. cruzi* isolates from the United States had low virulence for laboratory mice and rats.

Trypanosoma cruzi, the causative agent of Chagas disease, infects approximately 10–12 million persons in the Americas; there are approximately 200,000 new cases annually.^{1,2} In the United States, only six autochthonously acquired human infections have been reported. However, > 1,000 seropositive persons have been detected during routine screening of blood donations in the United States since 2007.³ Although few autochthonous human cases in the United States have been reported, reports of domestic dog and captive exotic animal cases are increasing,^{4,5} and the prevalence of *T. cruzi* in wild mammal reservoir species can be as high as in South America.^{6,7} *Trypanosoma cruzi* is currently categorized into one of six discrete typing units (TcI, TcIIa, TcIIb, TcIIc, TcIId, TcIIE). To date, all isolates from humans, vectors, wild mammals, domestic animals, and non-human primates in the United States have been classified as TcI or TcIIa.^{8–10}

Identifying the genotype of a *T. cruzi* strain is often important for characterizing biological differences among isolates, such as virulence, pathogenicity, tissue tropism, geographic locality, and host/reservoir capacity. Previous mouse infection studies using several sylvatic- and domestic-derived isolates from Brazil showed that those from marsupials were generally more infective and generated higher parasitemias than those from vectors or placental mammals.^{11,12} Patent infections were also more frequent in laboratory mice inoculated with TcII strains than TcI strains.¹² In contrast, U.S. isolates rarely cause morbidity and mortality in laboratory rodents,^{13–20} but in one study, a *T. cruzi* isolate from a raccoon caused hind limb paralysis in mice.²¹

Differences in infection outcome in these studies may be caused by different mouse strains, *T. cruzi* inoculum stage, inoculation route and/or dose, and source (host) species of the isolate. Additionally, many studies were conducted with genetically unclassified strains. The goal of the current study was to experimentally infect laboratory rodents with genetically classified *T. cruzi* isolates from the United States from a wide host range to determine infectivity, pathogenicity, and virulence. Based on previous studies on strains from the United

States,^{13–20} we hypothesized that sylvatic isolates would be infective but not virulent to mice.

A total of 18 *T. cruzi* isolates from seven mammalian host species and two vector species was used in the study (Tables 1 and 2). These isolates were chosen to represent both genotypes (TcI and TcIIa) present in the United States and a diverse geographic and host range. Two isolates from Brazil (Y and Brazil strains) were used as positive controls (kindly provided by Dr. Rick Tarleton, University of Georgia, Athens, GA). Parasites stored in liquid nitrogen (first passage for all but the two Brazil strains) were rapidly thawed and established in DH82 canine macrophage monolayers to yield the infective culture-derived trypomastigotes.²²

One hundred eighty-two outbred, eight-week-old male CrI:CD1 (ICR) mice and 16 white CrI:CD (SD) laboratory rats (Charles River Laboratory International, Inc, Wilmington, MA) were housed in microisolator cages in climate-controlled animal facilities at the College of Veterinary Medicine, University of Georgia (Athens, GA). All methods were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Georgia. Mice were weighed and randomly separated into one of 21 groups (18 United States isolate groups, two positive control groups, and one negative control group). Rats were weighed and separated into one of five groups (four United States isolates groups and one negative control). Individuals animals (nine mice and four rats) from each experimental and positive control group were inoculated intraperitoneally with 1×10^6 culture-derived trypomastigotes of one of the representative isolates (Tables 1 and 2). Two negative controls were similarly inoculated with an equivalent volume of culture medium. Any physical or behavioral changes indicative of Chagas clinical signs, such as lethargy, hind limb paralysis, weight loss, or ruffed coat, were noted daily.

At days 3, 7, 10, 14, 17, 21, 24, 28, and 112 post-inoculation (DPI), one mouse from each experimental and positive control group was humanely killed. At days 3, 7, 28, and 112 DPI, one rat from each experimental group was humanely killed. Acutely infected animals were those killed between 3 and 28 DPI; chronically infected animals were killed at 112 DPI. After killing, approximately 1–1.5 mL of whole blood from mice and 3 mL of whole blood from rats were collected by cardiocentesis into tubes containing EDTA.

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TABLE 1
Detection of *Trypanosoma cruzi* in eight acutely-infected and one chronically-infected Crl:CD1 (ICR) mice*

Isolate	Host	Origin	Lineage†	No. PCR-positive acute-stage mice (+, positive chronic mouse)			No. hemoculture positive
				Blood	Heart	Quadriceps muscle	
FL Opo 18	<i>Didelphis virginiana</i>	Wakulla County, FL	I	4 (+)	1	7	1
FL Opo 3	<i>D. virginiana</i>	Wakulla County, FL	I	5	2	6 (+)	1
USA Opossum	<i>D. virginiana</i>	Orleans Parish, LA	I	6 (+)	5 (+)	7 (+)	0
GA Opo 75	<i>D. virginiana</i>	Clarke County, GA	I	6	7 (+)	7	8
GA Opo 43	<i>D. virginiana</i>	Chatham County, GA	I	2	0	0	1
TxTg2	<i>Triatoma gerstaeckeri</i>	TX	I	5	3 (+)	7 (+)	0
Florida C16	<i>T. sanguisuga</i>	Alacua County, FL	I	5	0	6 (+)	3
TX WR 22	<i>Neotoma micropus</i>	Uvalde County, TX	I‡	5	7	6	4
TX WR 30	<i>N. micropus</i>	Uvalde County, TX	IIa‡	5	1 (+)	6 (+)	1
FL Rac 9	<i>Procyon lotor</i>	Liberty County, FL	IIa	2	4	3	1
TX08 Rac 5	<i>P. lotor</i>	Uvalde County, TX	IIa‡	5	2 (+)	7 (+)	2
FL Rac 13	<i>P. lotor</i>	Leon County, FL	I/IIa	5	2 (+)	7 (+)	2
OK Dog	<i>Canis familiaris</i>	Osage and Washington Counties, OK	IIa	3	5	7	1
Griffin Dog	<i>C. familiaris</i>	Coffee County, TN	I/IIa	6	1	8 (+)	4
Clarence RTL	<i>Lemur catta</i>	Liberty County, GA	IIa	2	2	3	0
RTL Meg	<i>L. catta</i>	Liberty County, GA	IIa	3	0	3 (+)	2
GA Sk 1	<i>Mephitis mephitis</i>	Long County, GA	IIa	4	0	6	2
GA Arm 20	<i>Dasypus novemcinctus</i>	Chatham County, GA	IIa	3	1	4	0
Brazil	Human	Brazil	I	8	8	8 (+)	5
Y	Human	Brazil	IIb	6	8	8	2

* PCR = polymerase chain reaction.

† Unless noted, lineage was determined according to methods of Roellig and others.¹⁰

‡ Lineage was determined according to methods of Brisse and others.²³

Infection was determined by detecting *T. cruzi* DNA by polymerase chain reaction (PCR) or culture of blood. For PCR, DNA was extracted from 100 µL of whole blood, and sections of heart and quadriceps muscle were obtained at necropsy. Amplification of the 24Sα ribosomal DNA gene of *T. cruzi* using a modified nested reaction was performed as described.²²⁻²⁴ For culture, remaining whole blood was centrifuged at $1,620 \times g$ for 15 minutes. Plasma was removed and approximately 3 mL of liver infusion tryptose medium was added to the remaining buffy coat and erythrocytes.²⁵ Cultures were evaluated 2–4 months later.

A *t*-test was performed to compare mean number of positive animals between genotype groups for each observed variable ($P < 0.05$ was considered statistically significant), excluding positive controls and results from the FL Rac 13 and Griffin Dog. A *t*-test was also performed to detect mean differences between heart and quadriceps muscle PCR results to determine whether tissue predilection exists ($P < 0.05$).

Based on PCR and culture results, all 18 isolates from the United States caused patent infections in mice (Table 1). Results from the first eight bleeding periods (3–28 DPI) were combined as a measure of infection status during the acute stage. In the groups of isolates from the United States, at least one sample from at least one animal was acutely positive either by hemoculture or PCR. Mice infected with the strain from

Brazil caused infections in each mouse at all time points. Blood samples taken from acute-stage mice infected with TcI from the United States were PCR positive at a significantly higher rate than those infected with TcII isolates ($F = 4.9532$, $P = 0.043$). Additionally, significant differences in detecting *T. cruzi* DNA present in tissue (heart and/or quadriceps) between genotype were noted. More mice infected with TcI isolates from the United States were *T. cruzi* positive in all tissues compared with TcIIa-inoculated mice ($F = 5.1317$, $P = 0.0399$). However, no difference was noted in tissue predilection (heart versus quadriceps muscle) ($F = 1.5217$, $P = 0.2377$).

Two TcI isolates from Virginia opossums (GA Opo 75 and GA Opo 43) yielded dramatically different results; many more mice had detectable infections with the GA Opo 75 strain than with the GA Opo 43 strain (blood: $t = 2.16$, $P = 0.0486$; heart and quadriceps: $t = 4.24$, $P = 0.0008$). Mortality and weight loss were not observed in mice inoculated with any isolates of *T. cruzi* from the United States. However, one Y-strain-inoculated mouse had marked weight loss and lethargy and was humanely killed at 28 DPI. Parasites were detected in the blood and tissues of this animal by PCR.

Similarly, all four isolates caused patent infections in laboratory rats (Table 2). *Trypanosoma cruzi* DNA was detected in the blood and tissues of rats infected with both TcI isolates from the United States at multiple time points, and one rat was

TABLE 2
Detection of *Trypanosoma cruzi* in three acutely infected and one chronically infected white Crl:CD (SD) laboratory rats*

Isolate	Host	Origin	Lineage†	No. PCR-positive acute stage rats (+, positive chronic rat)			No. hemoculture positive
				Blood	Heart	Quadriceps muscle	
USA Opossum	<i>D. virginiana</i>	Orleans Parish, LA	I	3 (+)	3	2	2
Florida C16	<i>T. sanguisuga</i>	Alacua County, FL	I	3	2 (+)	3 (+)	2
TX WR 30	<i>N. micropus</i>	Uvalde County, TX	IIa‡	0	0	0	1
GA Sk 1	<i>Mephitis mephitis</i>	Long County, GA	IIa	0	0	2	1

* PCR = polymerase chain reaction.

† Unless noted, lineage was determined according to methods of Roellig and others.¹⁰

‡ Lineage was determined according to methods of Brisse and others.²³

positive at 112 DPI (USA Opossum). No rats inoculated with TcIIa isolates had detectable parasitemias by PCR, but an animal from each TcIIa group was culture positive on 3 DPI. Although infection differences were noted between genotypes, small sample sizes and few isolates precluded statistical inferences.

In this study, the two *T. cruzi* genotypes caused differential infection dynamics in mice and rats as determined by PCR detection of *T. cruzi* DNA in the blood and tissues. In general, significantly more TcI-inoculated mice had detectable infections than TcIIa-inoculated mice. Additionally, mice and rats inoculated with two TcI isolates (FL Opo 18 and/or USA Opossum) had positive PCR results for blood well into the chronic stage (112 DPI). No TcIIa-inoculated mice or rats were positive beyond the acute stage (28 DPI). These findings suggest that sylvatic TcI isolates from the United States in this study had greater infectivity to laboratory rodents than TcIIa isolates from the United States. Previous studies of *T. cruzi* isolates from the United States have not investigated infectivity differences based on genotype. However, a study of *T. cruzi* isolates from Brazil found TcII strains more infective and virulent than TcI strains to Swiss mice.¹² The population of *T. cruzi* from countries in South America is more diverse than *T. cruzi* from the United States, particularly TcII strains, because all TcII subtypes are found in South America but only TcIIa has been detected in the United States²⁶ (Roellig DM, unpublished data). Additionally, TcIIa strains from the United States are genetically distinct from TcIIa strains from South America at numerous loci²⁷ (Roellig DM, unpublished data). Molecular differences between *T. cruzi* from South America and the United States may account for biological differences, including infectivity to mice and rats.

Interestingly, *T. cruzi* isolates from a wildlife rodent reservoir (*Neotoma micropus*) did not readily infect the laboratory mice or rats. Vectors live within woodrat nests, presumably leading to a continuous transmission cycle where bugs and animals are infected and often reinfected. Therefore, patency would be extended, and *T. cruzi* infections are easily detected in field samples as in previous surveillance studies.^{14,28} Additionally, the isolates may be adapted to woodrats and not infective to all rodent species; host adaptation has been suggested.^{8,10,22,26}

We also noted in this study that none of the laboratory rodents inoculated with strains from the United States resulted in observable clinical signs or mortality. These data support previous studies, which reported that sylvatic isolates from the United States were largely avirulent and did not cause morbidity or mortality in rodent models.^{13–20} In contrast, *T. cruzi* isolates from South America readily infect a wide variety of laboratory mice strains and many cause significant morbidity and mortality.^{11,12} In the current study, one control mouse inoculated with Y strain displayed lethargy and marked weight loss. Although no clinical signs were observed in mice inoculated with the strain from Brazil in this study, this strain has previously been shown to cause disease and mortality.²⁹ A previous study reported mortality in three of four C3H mice inoculated with a *T. cruzi* isolate from a raccoon in North Carolina; these mice were not parasitemic but amastigotes were observed in muscle tissue.²¹ Furthermore, natural infections of captive baboons in Texas have resulted in mortality, indicating that some strains of *T. cruzi* from the United States can cause disease and death.⁵ These data suggest that the biological characteristics of *T. cruzi* isolates from the United States may vary considerably.

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