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Genetically different isolates of *Trypanosoma cruzi* elicit different infection dynamics in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*)

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ABSTRACT

Trypanosoma cruzi is a genetically and biologically diverse species. In the current study we determined *T. cruzi* infection dynamics in two common North American reservoirs, Virginia opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*). Based on previous molecular and culture data from naturally-exposed animals, we hypothesised that raccoons would have a longer patent period than opossums, and raccoons would be competent reservoirs for both genotypes *T. cruzi* I (TcI) and TcIIa, while opossums would only serve as hosts for TcI. Individuals ($n = 2$ or 3) of each species were inoculated with 1×10^6 culture-derived *T. cruzi* trypomastigotes of TcIIa (North American (NA) – raccoon), TcI (NA – opossum), TcIIb (South American – human), or both TcI and TcIIa. Parasitemias in opossums gradually increased and declined rapidly, whereas parasitemias peaked sooner in raccoons and they maintained relatively high parasitemia for 5 weeks. Raccoons became infected with all three *T. cruzi* strains, while opossums only became infected with TcI and TcIIb. Although opossums were susceptible to TcIIb, infection dynamics were dramatically different compared with TcI. Opossums inoculated with TcIIb seroconverted, but parasitemia duration was short and only detectable by PCR. In addition, raccoons seroconverted sooner (3–7 days post inoculation) than opossums (10 days post inoculation). These data suggest that infection dynamics of various *T. cruzi* strains can differ considerably in different wildlife hosts.

1. Introduction

Trypanosoma cruzi, the causative agent of Chagas' disease, has a wide host and geographic range. Approximately 200 species or subspecies of wildlife have been identified with *T. cruzi* infection (Barretto and Ribeiro, 1979) in a geographic range encompassing most of the Americas. Within the various host species that can become infected with *T. cruzi*, the genotype of the parasite may vary and an association between host and genotype has been strongly supported by molecular typing of isolates from autochthonously infected wild and domestic animals, humans and vectors (Clark and Pung, 1994; Briones et al., 1999; Yeo et al., 2005; Roellig et al., 2008). Although all six phylogenetic lineages, *T. cruzi* I (TcI) and TcII (a–e), are found in South America, only TcI and TcIIa have been identified in the United States of America (USA) (Clark and Pung, 1994; Barnabé et al., 2001; Hall et al., 2007; Roellig et al., 2008).

Raccoons and Virginia opossums are considered important wildlife reservoirs in the USA with prevalence as high as 63% in raccoons (John and Hoppe, 1986) and 33% in Virginia opossums (Barr et al., 1991a). Lineage typing of *T. cruzi* from these two species has revealed a trend where the majority of *T. cruzi* isolates from raccoons have been TcIIa while all of the Virginia opossum *T. cruzi* isolates have been TcI (Barnabé et al., 2001; Roellig et al., 2008). While there is evidence for a host-genotype dichotomy, the mechanisms driving the strain preference are unknown and experimental evidence of such a preference has not been demonstrated previously in these two wildlife reservoir species.

In addition to differences in *T. cruzi* genotypes isolated from naturally-infected raccoons and Virginia opossums, prevalence based on isolation success varies considerably. Significantly more wild raccoons are culture positive compared with wild Virginia opossums which suggests either different exposure or infection dynamics (Brown et al., in press). The question of different exposure was examined by Brown et al. (in press); sympatric opossums and raccoons from 10 counties in Georgia (USA) were tested for *T. cruzi* exposure and no difference in seroprevalence was noted between opossums and raccoons from the same area. Therefore, differences in prevalence between these two hosts based on culture

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isolation attempts are likely the result of differences in infection dynamics.

In the present study, the infection dynamics of the two major USA reservoirs were determined after inoculation with different genotypes of *T. cruzi*. Our objectives were to determine whether any differences in host susceptibility to different genotypes exists by measuring the duration and magnitude of parasitemias, time to seroconversion, presence of tissue stages, and histopathological lesions. Based on previous genetic studies of field isolates, we hypothesised that raccoons would develop patent infections with TcI and TcII strains, while Virginia opossums would develop patent infections with only TcI. Additionally, because *T. cruzi* is more frequently isolated from raccoons compared with opossums, despite similar exposure rates (Brown et al., in press), we hypothesised that raccoons would develop higher parasitemias that would be maintained for longer periods compared with Virginia opossums.

2. Materials and methods

2.1. Inoculation material

The two North American *T. cruzi* isolates used in this study were originally isolated from a naturally-infected raccoon (FL-RAC9 (TcIIa)) and Virginia opossum (FL-OPO3 (TcI)) from north-western Florida (Roellig et al., 2008). One South American *T. cruzi* strain, Y (TcIIb), was generously provided by Dr. Rick Tarleton (University of Georgia, USA). The FL-OPO3 isolate was used as a representative TcI strain and the FL-RAC9 and Y strains were used as representative TcII strains. Each strain was molecularly typed utilising the mini-exon intergenic spacer gene, 24Sα rDNA D7 divergent domain, and size-variable domain of the 18S rRNA gene (Brisse et al., 2000; Roellig et al., 2008).

Epimastigotes were passaged from liver-infusion tryptose (LIT) medium into DH82 canine macrophage monolayers at 1:5 dilutions to yield the infective culture-derived trypomastigotes. Trypomastigotes were pelleted from culture by centrifugation at 1,620*g* for 15 min and resuspended in minimum essential medium (MEM). The concentration of parasites in suspension was determined with a hemocytometer.

2.2. Animals and experimental design

Ten juvenile raccoons obtained from Ruby Fur Farm, Inc. (New Sharon, IA, USA) were housed individually or in pairs in climate-controlled animal housing at the College of Veterinary Medicine, University of Georgia (Athens, GA, USA). Fourteen juvenile Virginia opossums of two wild-trapped females from Athens, GA were housed with and reared by their respective mothers until weaning at approximately 12 weeks, after which they were individually housed, in climate-controlled animal housing at the College of Veterinary Medicine, University of Georgia. All animals used in this study 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. Animals were given food and water ad libitum. Before use, all raccoons and opossums were determined to be negative for antibodies reactive with *T. cruzi* (as described below). Both opossum mothers were also determined to be negative for *T. cruzi* by PCR, culture and serology.

For both species, animals were randomly separated into four experimental groups and one negative control group. Raccoons ($n = 2$) were inoculated i.v. and Virginia opossums ($n = 3$) were inoculated i.p. with 1×10^6 culture-derived trypomastigotes of one of four inoculums: FL-OPO3 (TcI), FL-RAC9 (TcIIa), Y (TcIIb), or equal parts FL-OPO3 strain and FL-RAC9 strain (5×10^5 of each). Negative controls ($n = 2$) for both species were similarly inoculated with an equivalent volume of culture medium.

For handling and blood collection, raccoons were anaesthetized with an i.m. injection of a mixture of 20 mg/kg ketamine (Fort Dodge Laboratories, Inc., Fort Dodge IA, USA) and 4 mg/kg xylazine (Mobay Corporation, Shawneee, KS, USA). Virginia opossums were anaesthetized with an i.m. injection of tiletamine plus zolazepam (Telazol®, 5 mg/kg body weight, Aveco Co., Fort Dodge, IA, USA). Approximately 1 mL of blood was aseptically collected from the jugular vein of raccoons and 125 μL from the medial saphenous vein of opossums into EDTA tubes at 3, 7, 10, 14, 17, 21, 24, 28, 35, 42, 49, 56, 70, 84 and 112 days post inoculation (PI). One raccoon from each group was euthanised at 28 days PI and 112 days PI as representative acute and chronic infections, respectively. One opossum from each group was euthanised at 28 days PI, 56 days PI and 112 days PI as representative acute, late acute and chronic infections, respectively. Animals were humanely euthanised under anaesthesia by intracardiac injection of sodium pentobarbital (1 mg/kg; Butler Company, Columbus, OH, USA) and exsanguination.

2.3. Direct and molecular detection of T. cruzi

At each sampling time, parasitemias were determined by examining 5 μL of whole blood under an 18 mm cover glass at 400× magnification with a compound microscope. The entire volume of blood was scanned and the number of counted parasites converted to parasites/mL.

DNA was extracted from 100 μl of whole blood using the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol. Extracted DNA was used as template in PCR amplification of the D7 divergent domain of the 24Sα rDNA gene of *T. cruzi* in raccoon infections using a modified nested reaction with primers D75 and D76 (Briones et al., 1999) in the primary reaction and primers D71 and D72 in a secondary reaction (Souto et al., 1996). Because this protocol amplified opossum DNA, opossums were tested for the *T. cruzi* kinetoplast minicircle DNA by using primers S35 and S36 as previously published (Vallejo et al., 1999). The total volume of each reaction mixture was 25 μL and contained 5× buffer, 2 μM of each dNTP, 1 μM of each primer, 2.5 mM $MgCl_2$ and 1.25 U of GoTaq *Taq* polymerase (Promega Corporation, Madison, WI, USA). The temperature and cycling profile was previously described (Souto et al., 1996; Vallejo et al., 1999). Stringent protocols and controls were used in all PCR assays to prevent and to detect contamination. DNA extraction, amplification and product analysis were performed in separate dedicated laboratory areas. A negative water control was included in each set of extractions and PCR reactions as a contamination control. The 330 bp minicircle or 125 or 110 bp 24Sα amplicons were visualised on an ethidium bromide stained 1.5% agarose gel by transillumination.

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, brain, and anal sacs (opossums only)) were collected. One portion of each sample was preserved in 10% neutral buffered formalin for histopathological examination and the remaining portion stored at −20 °C until PCR analysis. Frozen tissues were thawed and one 25-mg section of each was aseptically excised. DNA was isolated from tissue using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's protocol with a 24 h tissue lysation step, and used as template following the same PCR parameters described above.