

SEROPREVALENCE OF TRYPANOSOMA CRUZI IN MAMMALS OF THE UNITED STATES

by

EMILY LOUISE BROWN

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Trypanosoma cruzi, causative agent of Chagas' disease, is commonly detected in wildlife in North America. Twelve species of mammals from six states were tested for antibodies to *T. cruzi* using indirect immunofluorescent antibody testing. Culture attempts were conducted on animals from Georgia and Florida. In general, the highest antibody prevalence rates were found in raccoons (*Procyon lotor*) (33-68%), followed by Virginia opossums (*Didelphis virginiana*) (28-52%), but antibodies were also detected in small numbers of other animals. Culture-based prevalence rates for raccoons were greater than those for opossums; however, antibody prevalences of raccoons and opossums were not different, indicating similar exposure levels. Several raccoon and opossum population parameters were examined with regards to prevalence and results indicated that *T. cruzi* prevalence varied by host species, host characteristics, and geographic region. The results of this study provide data to guide future studies on the natural history of *T. cruzi* in the United States.

INDEX WORDS: *Trypanosoma cruzi*, Chagas' disease, United States, raccoon, Virginia opossum, indirect immunofluorescent antibody test, serology

SEROPREVALENCE OF TRYPANOSOMA CRUZI IN MAMMALS OF THE UNITED
STATES

by

EMILY LOUISE BROWN

BSFR, University of Georgia, 2006

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2008

© 2008

Emily Louise Brown

All Rights Reserved

SEROPREVALENCE OF TRYPANOSOMA CRUZI IN MAMMALS OF THE UNITED
STATES

by

EMILY LOUISE BROWN

Major Professor: Michael J. Yabsley
Committee: Michael T. Mengak
David E. Stallknecht

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2008

ACKNOWLEDGEMENTS

I would like to begin by thanking my advisor, Dr. Michael Yabsley, for giving me this research opportunity. This project would not have been possible without his direction, advice, and encouragement. I would also like to thank my committee members, Dr. Michael Mengak and Dr. David Stallknecht, for their continued advice and input.

I would like to thank the numerous collaborators who sent me samples, and researchers who allowed me to collect samples during their own research and management endeavors. I would also like to thank the many research technicians at the Southeastern Cooperative Wildlife Disease Study who were of great assistance both in the field and in the laboratory. I would like to thank the National Institutes of Health for financial support.

I would like to thank my family, friends, and lab mates for their patience, companionship, and encouragement. Finally, and most importantly, I would like to thank my parents for their unending love and support.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW	1
INTRODUCTION	1
LITERATURE REVIEW	3
LITERATURE CITED	12
2 SEROPREVALENCE OF TRYPANOSOMA CRUZI AMONG TWELVE	
POTENTIAL RESERVIOR SPECIES FROM SIX STATES	18
ABSTRACT	19
INTRODUCTION	20
MATERIALS AND METHODS	21
RESULTS	24
DISCUSSION	26
ACKNOWLEDGEMENTS	31
REFERENCES	31
3 CONCLUSIONS	42

LIST OF TABLES

	Page
Table 1.1: Wildlife species found to be naturally infected with <i>Trypanosoma cruzi</i> through culture or direct observation of the parasite	10
Table 1.2: Wildlife species found to have antibodies to <i>Trypanosoma cruzi</i> through serologic testing	11
Table 2.1: Results of indirect immunofluorescent antibody testing of mammals from six states for <i>Trypanosoma cruzi</i> antibodies. Similar letters indicate no statistical differences between species within each state (P<0.05)	40
Table 2.2: Exposure rates for <i>Trypanosoma cruzi</i> in Georgia and Florida counties in which at least five raccoons (<i>Procyon lotor</i>) or opossums (<i>Didelphis virginiana</i>) were tested. .	41

LIST OF FIGURES

	Page
Figure 1.1: Life cycle of <i>Trypanosoma cruzi</i> within a human host (CDC 2004)	4

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Trypanosoma cruzi, a hemoflagellate protozoan parasite, is the causative agent of American trypanosomiasis (Chagas' disease) in domestic animals and humans. In Central and South America, the parasite can cause chronic heart disease as well as damage the digestive tract (TDR 2004). *T. cruzi* is very commonly reported in cats and dogs in Latin America (Gurtler et al. 2007), and dogs are considered a domestic reservoir host (Crisante et al. 2006, Estrada-Franco et al. 2006). It is also reported in many wildlife species in Latin America, where the most important reservoir is the common opossum (*Didelphis marsupialis*) (Zeledon et al. 1970). In North America, it is common in several species of mammalian wildlife, and is being increasingly diagnosed in domestic dogs and zoo animals, but is still rare in humans. This parasite is transmitted through the feces of triatomid bugs (Family *Reduviidae*), also known as kissing bugs. The bugs defecate while feeding on the host, and when the host is irritated by and scratches the bite, the parasite enters the bloodstream and infects the host. Several species of triatomid bugs are present in the United States and range from California to Maryland southward.

American trypanosomiasis, although a substantial public health problem in Latin America, is generally not considered a public health threat in the United States due to the lower risk of infection due to the higher standards of living as well as delayed defecation of North American triatomid bugs. In the United States, autochthonous human *T. cruzi* infection has been

reported seven times (Herwaldt et al. 2000, Dorn et al. 2007, CDC, unpublished). Four of the seven cases were in children under the age of two (three in Texas and one in Tennessee), the fifth was a 56-year-old woman in California, the sixth was a 74-year-old woman in Louisiana, and the seventh in a young child from Texas. The two latter cases were diagnosed in 2006. The low number of clinical cases in the United States may be misleading, as the exotic nature of the disease can result in misdiagnosis, as it is not normally included in diagnostic investigations of cardiac problems (Tippit 1978). Serologic studies have shown that asymptomatic and mild acute cases do occur and can go undetected and exposure could be as high as 3% in some populations (Farrar et al. 1963, Woody et al. 1961, Woody et al. 1965, Burkholder et al. 1980). *T. cruzi* is found in a variety of wild mammal species throughout the United States (John and Hoppe 1986). These animals do not often exhibit symptoms of disease and serve as reservoirs of the parasite.

T. cruzi infection has been reported from many species of mammals throughout the southern United States. The most commonly reported mammalian reservoirs are the raccoon (*Procyon lotor*) and the Virginia opossum (*Didelphis virginiana*). Various other mammal species are also known or suspected reservoirs of the parasite, such as armadillos (*Dasypus novemcinctus*), striped skunks (*Mephitis mephitis*), coyotes (*Canis latrans*), gray foxes (*Urocyon cinereoargenteus*), woodrats (*Neotoma* spp.), and various species of mice. The parasite has been reported from California to Florida and north to Maryland, and is present in all states in the southern half of the country (John and Hoppe 1986). Several studies have determined *T. cruzi* prevalence in a variety of species in the past, but the majority of these studies used blood culture to determine prevalence. Another method of *T. cruzi* detection involves testing serum for antibodies to the parasite. While blood culture only detects the parasite during the acute phase of infection when blood parasitemia is high, serological testing will detect exposure to the parasite

during the chronic phase of infection. To date, large scale serological testing has only been done on raccoons and, to a limited extent, armadillos, gray foxes, and coyotes.

LITERATURE REVIEW

T. cruzi Life Cycle and Detection

T. cruzi has a life cycle that includes several different morphological forms (Figure 1.1) (TDR 2004). When a triatomid bug receives a blood meal from an infected host, the bug ingests the trypomastigote form of the parasite that is circulating in the host's blood. These trypomastigotes transform into epimastigotes in the midgut of the bug, where they pass to the hindgut and reproduce asexually. The epimastigotes attach to the cell walls of the hindgut and the rectum where they transform into metacyclic (infective) trypomastigotes. When a triatomid bug bites a mammalian host, it typically defecates on the host, releasing the metacyclic trypomastigotes in the feces. The parasite then enters the body of the host through the bite wound or a mucous membrane. The trypomastigotes invade phagocyte cells at or near the site of entry, then transform into the amastigote form of the parasite. The amastigotes divide within the cell and form a pseudocyst, where they transform back into trypomastigotes. The pseudocyst then ruptures and the trypomastigotes are released into the bloodstream. Transmission can also occur by alternative routes including ingestion of infected bugs, transplacental, transmammary, and via blood transfusion or organ transplant.

T. cruzi exists within the host in three distinct phases: acute, indeterminate, and chronic phases (CDC 2004). During the acute phase, the trypomastigote form is circulating in the blood and the host may exhibit symptoms such as fatigue, fever, swollen lymph glands, or localized swelling around the point of parasite entry. Approximately 5-10% of infected individuals will die from myocarditis or encephalitis during the acute stage. The indeterminate phase begins eight

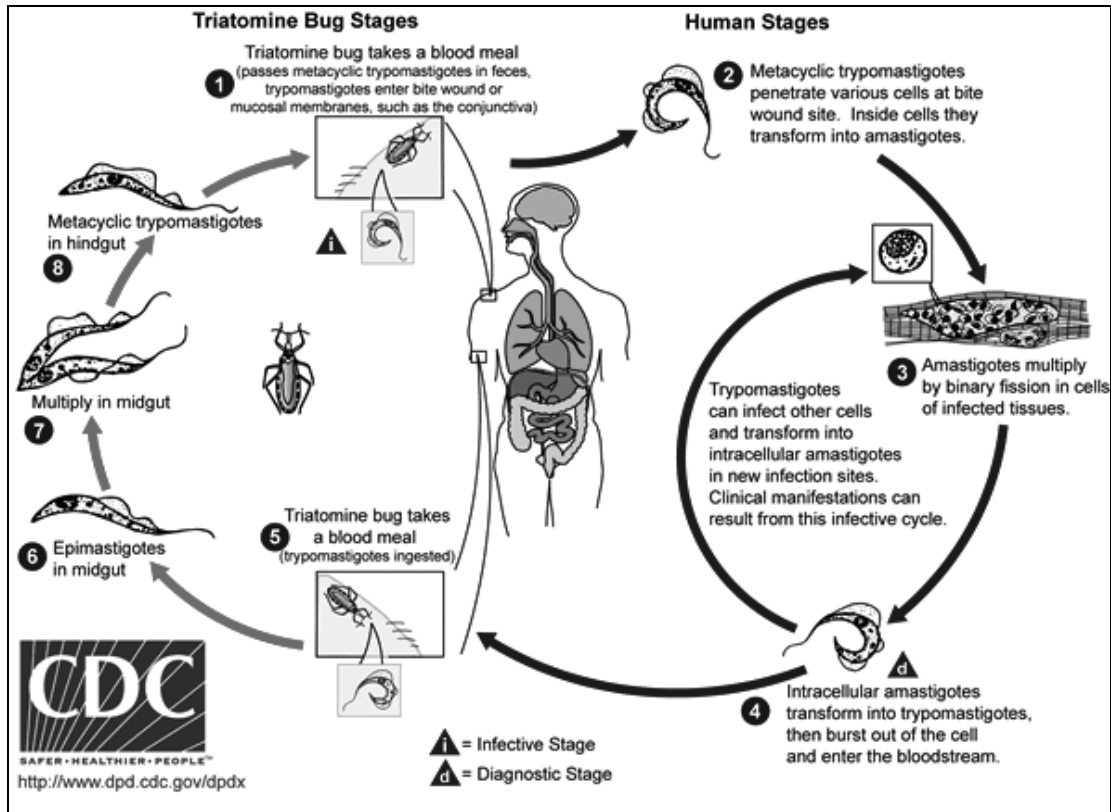


Figure 1.1. Life cycle of *Trypanosoma cruzi* within a human host (CDC 2004).

to ten weeks after infection and is characterized by the presence of antibodies to *T. cruzi* in the host, but with an absence of symptoms of the acute or the chronic phase (Teixiera et al. 2006). About one third of those with indeterminate infections will develop chronic Chagas disease. Chronic Chagas disease is characterized by low parasite counts in the blood, but the possible presence of pseudocysts in the tissues of the host. The chronic stage is characterized by cardiac problems such as an enlarged heart or altered heart rate or rhythm, which can lead to heart failure or cardiac arrest (CDC 2004). Enlargement of the esophagus or bowel can also occur, which can lead to digestive problems.

Several techniques for determining *T. cruzi* prevalence in mammals in the Southeast have been used in the past. These techniques include direct examination of blood on wet mounts or blood films, isolation of the parasite from culture of blood or tissue samples taken from the animal, use of serological testing to detect antibodies for *T. cruzi* in serum samples, and the use of the polymerase chain reaction (PCR) to detect *T. cruzi* specific DNA sequences in fresh or preserved tissue samples (James et al. 2002). The majority of previous studies have used blood culture or the direct examination of blood to determine parasite prevalence. These techniques are limited because they only detect the parasite during the early acute phase of the infection. During the chronic phase of the infection, isolation of the parasite is less likely due to low circulating parasitemia (Yabsley and Noblet 2002). Serological testing, which is widely used in South America to study *T. cruzi* in humans, can detect antibodies to *T. cruzi* from the host during the chronic phase (Yabsley et al. 2001).

Another method of testing has recently been developed that involves rapid testing using immunochromatographic dipstick assays. This type of test has been evaluated for use in dogs (Cardinal et al. 2006) and humans (Luquetti et al. 2003, Ponce et al. 2005) in South America, but

no studies have been conducted on mammalian reservoirs in North America. All of the previous studies found that the dipstick test has a high specificity and sensitivity and is a cost-effective method for large *T. cruzi* surveys; however, this assay must be validated for use in wild species.

T. cruzi in humans and domestic animals of the United States

Seven autochthonous cases of American trypanosomiasis in humans have been reported in the United States. The first autochthonous human case was reported in a 10-month-old infant in Corpus Christi, Texas in July, 1955 (Woody and Woody 1955). The second case was also an infant from Texas in 1955 (Greer 1956). The third case of *T. cruzi* in the United States occurred in a 56-year-old woman from Lake Don Pedro, California (Navin et al. 1985). A subsequent seroprevalence survey of residents living near the patient revealed a prevalence of 2.5%. In the same study, seroprevalence surveys were conducted on residents living in Tuolumne County, California, which is 20 miles away from the infected patient, as well as residents of the San Francisco Bay area. The surveys resulted in prevalence rates of 0.7% for residents of Tuolumne County and 0.2 % for the San Francisco Bay area. The fourth autochthonous case of *T. cruzi* occurred in a seven-month-old child in south Texas, and resulted in fatal myocarditis (Ochs et al. 1996). The fifth reported case occurred in an 18-month old boy from rural Tennessee (Herwaldt et al. 2000). The child tested positive for *T. cruzi* by PCR and DNA hybridization, as did a *Triatoma sanguisuga* bug found in the crib with the child. The sixth case came from a 74 year old woman from New Orleans, Louisiana (Dorn et al. 2007), who reported having been bit many times by triatomid bugs. The seventh case was diagnosed in a young child from near Brownsville, Texas with no history of travel outside of the US (CDC, unpublished).

Several serologic surveys of *T. cruzi* in humans have been conducted in the southern United States. One survey of 500 children from the Corpus Christi, Texas area found 9 (1.8%)

positive for *T. cruzi* antibodies using complement fixation (Woody et al. 1961). Another serologic survey in south Texas tested 117 individuals who had been bitten by a triatomid bug vector and found three (2.5%) positives (Woody et al. 1965). Burkholder et al. (1980) found a seroprevalence rate of 2.4% in humans from the Lower Rio Grande Valley of Texas. A serologic survey in Georgia on 951 individuals found six (0.6%) positives using complement fixation (Farrar et al. 1963). Two of the six seropositive individuals suffered from unexplained chronic heart disease, which may have been due to *T. cruzi* infection. Another serologic study in Georgia found two positive samples from a total of 3,883 serum samples tested, indicating an extremely low seroprevalence (0.05%) (Farrar et al. 1972). A seroprevalence survey of 50 Centers for Disease Control and Prevention employees in Atlanta, GA in 1982 found one positive sample (2%) using the complement fixation test (Navin et al. 1985).

The domestic dog (*Canis lupus*) has been established as a reservoir host for *T. cruzi* in South America, with prevalences of up to 35% (Tippit 1978). Several studies have also detected *T. cruzi* also in domestic dogs throughout the southern United States. Cases of *T. cruzi* in dogs have been reported from central Texas (Nabity et al. 2006, Williams et al. 1977), Louisiana (Snider et al. 1980, Barr et al. 1986, Barr et al. 1989), Oklahoma (Fox et al. 1986), South Carolina (Nissen et al. 1977), and Virginia (Barr et al. 1995). In San Benito, Texas, three dogs died from Chagas cardiomyopathy, and a subsequent serologic survey of stray dogs in the area revealed a seroprevalence rate 28 of 375 (7.5%) (Beard et al. 2003). Duprey et al. (2006) tested 413 foxhounds from 35 states and Canada using the radioimmunoprecipitation assay and found 86 (21%) positive. Although the majority of the positives were from southeastern states, several positive dogs were from northern states and Canadian provinces indicating that travel history was an important factor in whether a dog was positive or not but a lack of detailed travel logs

prohibited the use of this data for estimating geographic prevalences in dogs. A serosurvey of dogs in Houston, Texas revealed a prevalence rate of 2.6% (Shadomy et al., 2004) and an earlier study in Texas indicated that the *T. cruzi* seroprevalence from 1987 and 1996 increased from 1.8% to 17.1% (Meurs et al., 1998). Barr et al. (1995) found a seroprevalence rate of 5.4% in Walker Hounds in Virginia. Tomlinson et al. (1981) tested 365 dogs from several southeastern states, including Georgia, and found seven (1.9%) to be seropositive for antibodies to *T. cruzi*. A study in Louisiana failed to find a correlation of seroprevalence rates in dogs with known contact with wild mammalian reservoirs (4.7% prevalence) and those dogs with no known wild host contact (2.3%) (Barr et al. 1991a).

T. cruzi in wildlife of the United States

T. cruzi has been reported in a variety of mammals in the United States, including opossums, raccoons, striped skunks (*Mephitis mephitis*), gray foxes, armadillos (John and Hoppe 1986), coyotes (Grogl et al. 1984), gray foxes (Rosypal et al. 2007) various woodrat species (*Neotoma* spp.) (Wood 1952, Burkholder et al. 1980), and various species of mice (Wood 1952, John and Hoppe 1986) (See Table 1.1 and Table 1.2).

Raccoons and opossums are the two most important reservoirs for *T. cruzi*. Several ecological factors may be responsible for the connection between *T. cruzi* and these animals (Olsen et al. 1964). These factors include: 1) similarities between triatomid bug habitats and the dens of raccoons and opossums, 2) the use of temporary retreats by these animals, thus increasing the chance of encounter with triatomid bugs, 3) the insectivorous habits of these animals, which may lead to an oral route of contamination with *T. cruzi*, and 4) the high year-round activity level of these animals in the south, which increases their chance of contact with bugs.

Historically, most surveys for *T. cruzi* have been based on culture isolation. Using culture methods, *T. cruzi* has been detected from raccoons and opossums throughout the Southeast, as well as a few reports in other species of mesomammals and rodents (Table 1.1). Serological testing for *T. cruzi* antibodies has been performed on a limited number of species from geographically restricted areas including raccoons from several states, armadillos from Louisiana, one skunk in California, and coyotes and other various species from Texas (Table 2.2).

Urban Wildlife and Disease

Raccoons and opossums, the two most important reservoirs of *T. cruzi*, are readily adaptable to urban and suburban environments. They are tolerant of habitat fragmentation and human presence, and are generalists both in habitat and diet (Prange and Gehrt 2004). Few wildlife species can survive in drastically altered urban environments, but those that can, such as raccoons and opossums, benefit from relatively exclusive use of available resources (Riley et al. 1998). Raccoon populations in urban areas are capable of reaching higher densities than rural raccoon populations (Prange et al. 2003) with urban raccoon densities being 2-400 times greater than rural population densities (Smith and Engeman 2002, Riley et al. 1998). Also, raccoons in urban settings have increased reproduction and survival rates compared to their rural counterparts (Prange et al. 2003). High concentrations in urban areas and willingness to accept food from humans create a much higher probability for human-raccoon contact than other wildlife species (Smith and Engeman 2002).

Dense urban wildlife populations represent a public health threat as reservoirs of parasites and diseases (Prange et al. 2003). These wildlife populations are more likely than their rural counterparts to be impacted by disease and to maintain pathogens (Riley et al. 1998). A high

Table 1.1. Wildlife species found to be naturally infected with *Trypanosoma cruzi* through culture or direct observation of the parasite.

Species	Culture Sample	n	Prevalence (%)	Location and/or state	Reference	
Raccoon (<i>Procyon lotor</i>)	Blood	54	22.1	Southeast Georgia	Pung et al. 1995	
	Blood	30	43	St. Catherines Island, Georgia	Pietrzak and Pung 1998	
	Blood	8	63	Tulsa, Oklahoma	John and Hoppe 1986	
	Heart	472	2	Laurel, Maryland	Herman and Bruce 1962	
	Blood	20	15	North Carolina	Karsten et al. 1992	
	Heart, blood	35	14.3	East-central Alabama	Olsen et al. 1964	
	Kidney	608	1.5	Southwest Georgia and northwest Florida	McKeever et al. 1958	
	Blood	33	12	Florida	Schaffer et al. 1978	
	Blood	10	50	Georgia	Schaffer et al. 1978	
	Blood	25	24	Texas	Schaffer et al. 1978	
Virginia Opossum (<i>Didelphis virginiana</i>)	Blood	5	N/A	Maryland	Walton et al. 1958	
	Blood	3	66	Tennessee	Herwaldt et al., 2000	
	Blood	39	15.4	Southeast Georgia	Pung et al. 1995	
	Heart, blood	126	13.5	Alabama	Olsen et al. 1964	
	Heart	219	0	Laurel, Maryland	Herman and Bruce 1962	
	Blood	12	8.3	North Carolina	Karsten et al. 1992	
	Blood	48	33.3	Southern Louisiana	Barr et al. 1991b	
	Kidney	552	16	Southwest Georgia and northwest Florida	McKeever et al. 1958	
	Armadillo (<i>Dasypos novemcinctus</i>)	Blood	98	1.1	Southern Louisiana	Barr et al. 1991b
		Blood	80	28.8	New Orleans, Louisiana	Yaeger 1988
Striped Skunk (<i>Mephitis mephitis</i>)	Kidney	306	1.0	Southwest Georgia and northwest Florida	McKeever et al. 1958	
Southern Plains Woodrat (<i>Neotoma micropus</i>)	Blood	30	23.3	South Texas	Burkholder et al. 1980	
Pocket mouse (<i>Perognathus hispidus</i>)	Blood	25	16	South Texas	Burkholder et al. 1980	
Mexican spiny pocket mouse (<i>Liomys irrorattus</i>)	Blood	11	9	South Texas	Burkholder et al. 1980	
Grasshoppermouse (<i>Onychomys leucogaster</i>)	Blood	9	11.1	South Texas	Burkholder et al. 1980	
<i>Neotoma</i> spp. and <i>Peromyscus</i> spp.	Blood	410	0.73	California	Wood 1952	
<i>Neotoma</i> spp. and <i>Peromyscus</i> spp.	Blood	86	4.7	Arizona	Wood 1952	
Gray Fox (<i>Urocyon cinereoargenteus</i>)	Kidney	118	1.7	Southwest Georgia and northwest Florida	McKeever et al. 1958	

Table 1. 2. Wildlife species found to have antibodies to *Trypanosoma cruzi* through serologic testing.

Species	Test	<i>n</i>	Prevalence (%)	Location and/or state	Reference
Raccoon (<i>Procyon lotor</i>)	Indirect Immunofluorescence Assay	221	47	South Carolina and Georgia	Yabsley and Noblet 2002
	Indirect Immunofluorescence Assay	464	33	Fairfax County, Virginia	Hancock et al. 2005
	Indirect Hemagglutination Assay	9	0	South Texas	Burkholder et al. 1980
Badger (<i>Taxidea taxus</i>)	Indirect Hemagglutination Assay	8	25	South Texas	Burkholder et al. 1980
Coyote (<i>Canis latrans</i>)	Indirect Immunofluorescent Antibody Test	2	0	South Carolina	Rosypal et al. 2007
	Indirect Immunofluorescence Assay	134	14.2	Central and southeast Texas	Grogl et al. 1984
	Indirect Hemagglutination Assay	156	12.8	South Texas	Burkholder et al. 1980
Striped Skunk (<i>Mephitis mephitis</i>)	Complement Fixation and Direct Agglutination	1	100	Los Angeles, California	Ryan et al. 1985
Gray Fox (<i>Urocyon cinereoargenteus</i>)	Indirect Immunofluorescent Antibody Test	26	8	South Carolina	Rosypal et al. 2007
Nine-banded Armadillo (<i>Dasypus novemcinctus</i>)	Direct Agglutination	80	37.5	New Orleans, Louisiana	Yaeger 1988

prevalence of *T. cruzi* in urban wildlife could have public health implications, since the parasite also infects and can cause disease in humans and domestic dogs.

Only one study to date has researched the difference in *T. cruzi* prevalence between urban and rural wildlife populations. Yabsley and Noblet (2002) surveyed 221 raccoons from South Carolina and Georgia and found *T. cruzi* antibodies in 49 of 110 rural-caught raccoons (45%) versus 55 of 111 nuisance (urban-caught) raccoons (50%). Although more seropositive raccoons were detected in urban areas, no significant difference was observed between the rural and urban raccoons. The study used raccoons trapped from several sites throughout Georgia and South Carolina across several physiographic regions, which makes interpretation of the results difficult. This study also did not investigate the seroprevalence rates in opossums.

Although human infections of *T. cruzi* in the United States have been linked to infected triatomid bugs, it is possible that humans or domestic animals may become infected through contact with wild mammalian reservoirs. Humans that come in contact with the blood, urine, or other fluids from these mammals may become infected through skin abrasions (Olsen et al. 1964). Deane et al. (1986) suggested that direct transmission of *T. cruzi* infection may occur through anal gland secretions of opossums. McKeever et al. (1958) suggested that transmission may occur through animal to animal contact with urine containing live parasites, especially during mating. These alternate routes of transmission make it possible for human or domestic animal infection through contact with an infected animal.

LITERATURE CITED

- Barr, S.C., D. Baker, and J. Markovits. 1986. Trypanosomiasis and laryngeal paralysis in a dog. *Journal of the American Veterinary Medical Association* 188:1307-1309.
- Barr, S.C., R.M. Simpson, S.P. Schmidt, M.M. Bunge, J.M. Authement, and F. Lozano. 1989. Chronic dilatative myocarditis caused by *Trypanosoma cruzi* in two dogs. *Journal of the American Veterinary Medical Association* 195:1237-1241.

- Barr, S.C., V.A. Dennis, and T.R. Klei. 1991a. Serologic and blood culture survey of *Trypanosoma cruzi* infection in four canine populations in southern Louisiana. *American Journal of Veterinary Research* 52: 570-573.
- Barr, S.C, C.C. Brown, V.A. Dennis, and T.R. Klei. 1991b. The lesions and prevalence of *Trypanosoma cruzi* in opossums and armadillos in southern Louisiana. *The Journal of Parasitology* 77:624-627.
- Barr, S.C., O. Van Beek, M.S. Carlisle-Nowack, J.W. Lopez, L.V. Kirchhoff, N. Allison, A. Zajac, A. de Lahunta, D.H. Schlafer, and W.T. Crandall. 1995. *Trypanosoma cruzi* infections in Walker hounds from Virginia. *American Journal of Veterinary Research* 56:1037-1044.
- Beard, C.B., G. Pye, F.J. Steurer, R. Rodriguez, R. Campman, A.T. Peterson, J. Ramsey, R.A. Wirtz, and L.E. Robinson. 2003. Chagas disease in a domestic transmission cycle in southern Texas, USA. *Emerging Infectious Diseases* 9:103-105.
- Burkholder, J.E., T.C. Allison, and V.P. Kelly. 1980. *Trypanosoma cruzi* (Chagas)(Protozoa: Kinetidoplasta) in invertebrate, reservoir, and human hosts of the Lower Rio Grande Valley of Texas. *The Journal of Parasitology* 66:305-311.
- Cardinal, M.V., R. Reithinger, and R.E. Gurtler. 2006. Use of an immunochromatographic dipstick test for rapid detection of *Trypanosoma cruzi* in sera from animal reservoir hosts. *Journal of Clinical Microbiology* 44:3005-3007.
- Centers for Disease Control and Prevention. 2004. Chagas Disease Fact Sheet. Department of Health and Human Services. Atlanta, GA.
- Crisante, G., A. Rojas, M.M.G. Teixeira, and N. Anez 2006. Infected dogs as a risk factor in the transmission of human *Trypanosoma cruzi* infection in western Venezuela. *Acta Tropica* 98: 247-254.
- Deane, M.P., H.L. Lenzi and A.M. Jansen. 1986. Double development cycle of *Trypanosoma cruzi* in the opossum. *Parasitology Today* 2:146-147.
- Dorn, P.L., L. Pernacario, M.J. Yabsley, D.M. Roellig, G. Balsamo, J. Diaz, and D. Wesson. 2007. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. *Emerging Infectious Diseases* 13:605-607.
- Duprey, Z.H., F.J. Steurer, J.A. Rooney, L.V. Kirchhoff, J.E. Jackson, E.D. Rowton, and P.M. Schantz 2006. Canine visceral leishmaniasis, United States and Canada, 2000-2003. *Emerging Infectious Diseases* 12: 440-446.
- Estrada-Franco, J.G., V. Bhatia, H. Diaz-Albiter, L. Ochoa-Garcia, A. Barbabosa, J.C. Vazquez-Chagoyan, M.A. Martinez-Perez, C. Guzman-Bracho, and N. Garg. 2006.

- Human *Trypanosoma cruzi* infection and seropositivity in dogs, Mexico. *Emerging Infectious Diseases* 12: 624-630.
- Farrar, W.E., I.G. Kagan, F.D. Everton, and T.F. Sellers. 1963. Serologic evidence of human infection with *Trypanosoma cruzi* in Georgia. *American Journal of Hygiene* 78:166-172.
- Farrar, W.E., S.D. Gibbins, and S.T. Whitfield. 1972. Low prevalence of antibody to *Trypanosoma cruzi* in Georgia. *The American Journal of Tropical Medicine and Hygiene* 21:404-406.
- Fox, J.C., S.A. Ewing, R.G. Buckner, D. Whitenack, and J.H. Manley. 1986. *Trypanosoma cruzi* infection in a dog from Oklahoma. *Journal of the American Veterinary Medical Association* 189:1583-1584.
- Greer, D.A. 1956. Found: two cases of Chagas disease. *Texas Health Bulletin* 9:11-13.
- Grogl, M., R.E. Kuhn, D.S. Davis, and G.E. Green. 1984. Antibodies to *Trypanosoma cruzi* in coyotes in Texas. *The Journal of Parasitology* 70:189-191.
- Gurtler, R.E., M.C. Cecere, M.A. Lauricella, M.V. Cardinal, U. Kitron and J.E. Cohen 2007. Domestic dogs and cats as sources of *Trypanosoma cruzi* infection in rural northwestern Argentina. *Parasitology* 134: 69-82.
- Hancock, K., A.M. Zajac, O.J. Pung, F. Elvinger, A.C. Rosypal, and D.S. Lindsay. 2005. Prevalence of antibodies to *Trypanosoma cruzi* in raccoons (*Procyon lotor*) from an urban area of Northern Virginia. *The Journal of Parasitology* 91:470-472.
- Herman, C.M. and J.I. Bruce. 1962. Occurrence of *Trypanosoma cruzi* in Maryland. *Proceedings of the Journal of the Helminthological Society* 29:55-58.
- Herwaldt, B.L., A.L. Newsome, C.R. McGhee, M.R. Powell, D.G. Nemeč, F.J. Steurer, and M.L. Eberhard. 2000. Use of polymerase chain reaction to diagnose the fifth reported U.S. case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, in 1998. *The Journal of Infectious Diseases* 181:395-399.
- James, M.J., M.J. Yabsley, O.J. Pung, and M.J. Grijalva. 2002. Amplification of *Trypanosoma cruzi*-specific DNA sequences in formalin-fixed raccoon tissues using polymerase chain reaction. *The Journal of Parasitology* 88:989-993.
- John, D.T., and K.L. Hoppe. 1986. *Trypanosoma cruzi* from wild raccoons in Oklahoma. *American Journal of Veterinary Research* 47:1056-1059.
- Karsten, V., C. Davis, and R. Kuhn. 1992. *Trypanosoma cruzi* in wild raccoons and opossums from North Carolina. *The Journal of Parasitology* 78:547-549.

- Luquetti, A.O., C. Ponce, E. Ponce, J. Esfandiari, A. Schijman, S. Revollo, N. Anez, B. Zingales, R. Rangel-Aldao, A. Gonzalez, M.J. Levin, E.S. Umezawa, and J. Franco da Silveira. 2003. Chagas' disease diagnosis: a multicentric evaluation of Chagas Stat-Pak, a rapid immunochromatographic assay with recombinant proteins of *Trypanosoma cruzi*. *Diagnostic Microbiology and Infectious Disease* 46:265-271.
- McKeever, S., G.W. Gorman, and L. Norman. 1958. Occurrence of a *Trypanosoma cruzi*-like organism from some mammals from southwestern Georgia and northwestern Florida. *The Journal of Parasitology* 44:583-587.
- Meurs, K.M., M.A. Anthony, M. Slater, and M.W. Miller. 1998. Chronic *Trypanosoma cruzi* infection in dogs: 11 cases (1987-1996). *Journal of the American Veterinary Medical Association* 213:497-500.
- Nabity, M.B., K. Barnhart, K.S. Logan, R.L. Santos, A. Kessell, C. Melmed, and K.F. Snowden. 2006. An atypical case of *Trypanosoma cruzi* infection in a young English Mastiff. *Veterinary Parasitology* 140:356-361.
- Navin, T.R., R.R. Roberto, D.D. Juranek, K. Limpakarnjanarat, E.W. Mortenson, J.R. Clover, R.E. Yescott, C. Taclindo, F. Steurer, and D. Allain. 1985. Human and sylvatic *Trypanosoma cruzi* infection in California. *American Journal of Public Health* 75:366-369.
- Nissen, E.E., E.L. Roberson, L.B. Lipham, and W.L. Hanson. 1977. Naturally occurring Chagas' disease in a South Carolina puppy. *Proceedings of the 114th American Association of Veterinary Parasitologists Forum*, p122.
- Ochs, D.E., V.S. Hnilica, D.R. Moser, J.H. Smith, and L.V. Kirchhoff. 1996. Postmortem diagnosis of autochthonous acute chagasic myocarditis by polymerase chain reaction amplification of a species-specific DNA sequence of *Trypanosoma cruzi*. *American Journal of Tropical Medicine and Hygiene* 54:526-529.
- Olsen, P.F., J.P. Shoemaker, H.F. Turner, and K.L. Hays. 1964. Incidence of *Trypanosoma cruzi* (Chagas) in wild vectors and reservoirs in east-central Alabama. *The Journal of Parasitology* 50:599-603.
- Pietrzak, S.M. and O.J. Pung. 1998. Trypanosomiasis in raccoons from Georgia. *Journal of Wildlife Diseases* 34:132-136.
- Ponce, C., E. Ponce, E. Vinelli, A. Montoya, V. de Aguilar, A. Gonzalez, B. Zingales, R. Rangel-Aldao, M.J. Levin, J. Esfandiari, E.S. Umezawa, A.O. Luquetti, and J.F. de Silveira. 2005. Validation of a rapid and reliable test for diagnosis of Chagas' disease by detection of *Trypanosoma cruzi*-specific antibodies in blood of donors and patients in Central America. *Journal of Clinical Microbiology* 43:5065-5068.

- Prange, S. and S.D. Gehrt. 2004. Changes in mesopredator-community structure in response to urbanization. *Canadian Journal of Zoology* 82:1804-1817.
- Prange, S., S.D. Gehrt, and E.P. Wiggers. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. *Journal of Wildlife Management* 67:324-333.
- Pung, O.J., C.W. Banks, D.N. Jones, and M.W. Krissinger. 1995. *Trypanosoma cruzi* in wild raccoons, opossums, and triatomine bugs in southeast Georgia, U.S.A. *The Journal of Parasitology* 81:324-326.
- Riley, S.P., J. Hadidian, and D.A. Manski. 1998. Population density, survival, and rabies in raccoons in an urban national park. *Canadian Journal of Zoology* 76:1153-1164.
- Rosypal, A.C., R.R. Tidwell, and D.S. Lindsay. 2007. Prevalence of Antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in wild canids from South Carolina. *The Journal of Parasitology* 93: 955-957.
- Ryan, C.P., P.E. Hughes, and E.B. Howard. 1985. American Trypanosomiasis (Chagas' disease) in a striped skunk. *Journal of Wildlife Diseases* 21:175-176.
- Schaffer, G.D., W.L. Hanson, W.R. Davidson, and V.F. Nettles. 1978. Hematotropic parasites of translocated raccoons in the Southeast. *Journal of the American Veterinary Medical Association* 173:1148-1151.
- Shadomy, S.V., S.C. Waring, and C.L. Chappell. 2004. Combined use of enzyme-linked immunosorbent assay and flow cytometry to detect antibodies to *Trypanosoma cruzi* in domestic canines in Texas. *Clinical and Diagnostic Laboratory Immunology* 11:313-319.
- Smith, H.T. and R.M. Engeman. 2002. An extraordinary raccoon, *Procyon lotor*, density at an urban park. *Canadian Field-Naturalist* 116:636-639.
- Sinder, T.G. 1980. Myocarditis caused by *Trypanosoma cruzi* in a native Louisiana dog. *Journal of the American Veterinary Medical Association* 177:247-249.
- Special Programme for Research and Training in Tropical Diseases (TDR). 2004. World Health Report 2004. World Health Organization.
- Teixeira, A.R.L. and R.J. Nascimento, and N.R. Sturm. 2006. Evolution and pathology in Chagas disease – a review. *Memorias do Instituto Oswaldo Cruz* 101:463-491.
- Tippit, T.S. 1978. Canine trypanosomiasis (Chagas' disease). *The Southwestern Veterinarian* 31:97-104.

- Tomlinson, M.J., W.L. Chapman, W.L. Hanson, and H.S. Gosser. 1981. Occurrence of antibody to *Trypanosoma cruzi* in dogs in the southeastern United States. *American Journal of Veterinary Research* 42:1444-1446.
- Walton, B.C., P.M. Bauman, L.S. Diamond, and C.M. Herman. 1958. The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. *American Journal of Tropical Medicine and Hygiene* 7:603-610.
- Williams, G.D., L.G. Adams, R.G. Yaeger, R.K. McGrath, W.K. Read, and W.R. Bilderback. 1977. Naturally occurring Trypanosomiasis (Chagas' disease) in dogs. *Journal of the American Veterinary Medical Association* 171:171-177.
- Wood, S.F. 1952. Mammal blood parasite records from southwestern United States and Mexico. *Journal of Parasitology* 38:85-86.
- Woody, N.C., and H.B. Woody. 1955. American trypanosomiasis (Chagas' disease): First indigenous case in the United States. *Journal of the American Medical Association* 159: 676-677.
- Woody, N.C., N. DeDianous, and H.B. Woody. 1961. American trypanosomiasis II. Current serologic studies in Chagas' disease. *Journal of Pediatrics* 58: 738-745.
- Woody, N.C., A. Hernandez, and B. Suchow. 1965. American trypanosomiasis III. The incidence of serologically diagnosed Chagas' disease among persons bitten by the insect vector. *Journal of Pediatrics* 66:107-109.
- Yabsley, M.J., and G.P. Noblet. 2002. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. *Journal of Wildlife Diseases* 38:75-83.
- Yabsley, M.J., G.P. Noblet, and O.J. Pung. 2001. Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). *The Journal of Parasitology* 87:1155-1159.
- Yaeger, R.G. 1988. The prevalence of *Trypanosoma cruzi* infection in armadillos collected at a site near New Orleans, Louisiana. *American Journal of Tropical Medicine and Hygiene* 38:323-326.
- Zeledon, R., G. Solano, G. Saenz S., and J.C. Swartzwelder. 1970. Wild reservoirs of *Trypanosoma cruzi* with special mention of the opossum, *Didelphis marsupialis*, and its role in the epidemiology of Chagas' disease in an endemic area of Costa Rica. *The Journal of Parasitology* 56: 38.

CHAPTER 2

SEROPREVALENCE OF *TRYPANOSOMA CRUZI* AMONG TWELVE POTENTIAL RESERVOIR SPECIES FROM SIX STATES¹

¹Brown, E.L., D.M. Roellig, M. Gabriel, M. Gompper, R. Monello, K.M. Wenning, and M.J.

Yabsley. To be submitted to *Vector Borne and Zoonotic Diseases*.

ABSTRACT

Trypanosoma cruzi, causative agent of Chagas' disease, is a substantial public health concern in Latin America. Although rare in humans and uncommon in domestic animals in the United States, *T. cruzi* is commonly detected in some wildlife species including raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*). Twelve species of mammals from six states were tested for antibodies to *T. cruzi* using indirect immunofluorescent antibody testing and culture isolation attempts were conducted on a limited number of animals from Georgia and Florida. Evidence of *T. cruzi* was found in every state except California. In general, the highest prevalence rates were found in raccoons (33-68%) followed by Virginia opossums (28-52%), but *T. cruzi* were also detected in small numbers of striped skunks (*Mephitis mephitis*) from Arizona and Georgia, bobcats (*Lynx rufus*) from Georgia, two coyotes (*Canis latrans*) from Georgia and Virginia, and a ringtail (*Bassariscus astutus*) from Arizona. Culture-based prevalence rates for raccoons were significantly greater than those for opossums ($X^2 = 5.27$, $P = 0.027$); however, antibody prevalences of raccoons and opossums from several populations in Georgia and Florida were not different indicating exposure rates are similar within a given population. For both raccoons and opossums, adult animals had a higher prevalence rate compared with juveniles, females had a higher prevalence rate compared with males, and no difference was found between animals caught in urban or rural locations. Results indicate that *T. cruzi* prevalence varies by host species, host characteristics, and geographic region and provide data to guide future studies on the natural history of *T. cruzi* in the United States.

INTRODUCTION

Trypanosoma cruzi, a hemoflagellate protozoan parasite, is the causative agent of American trypanosomiasis (Chagas' disease) in domestic animals and humans. *T. cruzi* is an important public health concern in Latin America, where 10-12 million people are estimated to be infected (CDC 2007). In North America, *T. cruzi* is commonly detected in several species of mammalian wildlife, and is being increasingly diagnosed in domestic dogs and exotic animals (Meurs et al. 1998; Kasa et al. 1977, Jaime-Andrade et al. 1997). Autochthonous cases in humans are rare, with only six cases previously reported (Herwaldt et al. 1998, Dorn et al. 2007); however, serologic studies indicate many autochthonous cases may not be diagnosed (Woody et al. 1965, Burkholder 1980, American Red Cross, unpublished data).

The two most commonly reported reservoirs in North America are the raccoon (*Procyon lotor*) and the Virginia opossum (*Didelphis virginiana*). In raccoons, prevalence rates range from 1.5% in southwestern Georgia and northwestern Florida (McKeever et al. 1958) to 63% in Oklahoma (John and Hoppe 1986), with rates varying widely depending on the assay used (e.g., serology, culture, or both) and the geographic location. Reported prevalence rates for opossums have generally been lower, and range from 8% in North Carolina (Karsten et al. 1992) to 33% in southern Louisiana (Barr et al. 1991). Other wildlife species in the United States that are naturally infected with the parasite based on either serology or culture include the armadillo (*Dasypus novemcinctus*) (Yaeger 1988, Barr et al. 1991), badger (*Taxidea taxus*) (Burkholder et al. 1980), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*) (McKeever et al. 1958), striped skunk (*Mephitis mephitis*) (McKeever et al 1958, Ryan et al. 1985), and various rodent species (Burkholder et al. 1980).

The majority of previous studies of *T. cruzi* in wildlife have focused on blood culture as the primary method for determining infection status, but this method has been shown to have a

lower sensitivity than serologic testing (Jansen et al. 1985, Yabsley et al. 2001, Hall et al. 2007). Since culture of the parasite depends on high numbers of circulating parasites, animals in the chronic stage of infection, which are seropositive, are less likely to be culture positive. For example, Hall et al. (2007) tested 50 lemurs from St. Catherine's Island, GA, for *T. cruzi* using culture and serology, and found a 5% prevalence rate with culture and a 50% seroprevalence rate. Yabsley et al. (2001) tested raccoons from Georgia using both serologic and culture techniques and found a 30% prevalence rate using culture but a 51% prevalence rate using serologic testing.

The aim of the current study was to determine the prevalence of *T. cruzi* in several species of mesomammals throughout the United States using serologic testing. In addition, we wanted to assess the exposure rates of raccoons and opossums from several individual populations in Georgia and Florida using both culture and serologic methods. Additionally, several population parameters (e.g., age, sex) of raccoons and opossums were investigated to assess any effect on *T. cruzi* prevalence.

MATERIALS AND METHODS

Sample Collection

Animals were live-trapped in cage traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) baited with sardines. Raccoons were anesthetized by intramuscular injection of either ketamine hydrochloride (25 mg/kg body weight, Aveco Co., Fort Dodge, IA) plus xylazine (0.25 mg/kg body weight, Mobay Corp., Animal Health Division, Shawnee, Kansas) or tiletamine plus zolazepam (Telazol[®], 0.6 mg/kg body weight, Aveco Co., Fort Dodge, IA). Opossums were anesthetized by intramuscular injection of tiletamine plus zolazepam. Approximately 10 ml of blood was collected via cardiac puncture from anesthetized animals.

Whole blood in EDTA was collected for culture and plasma or serum was collected for serological testing. After blood collection, the animals were euthanized with sodium pentobarbital (Beuthanasia[®]-D Special, Schering-Plough Animal Health Corp., Omaha, NE) administered by intracardiac injection. Additionally, serum or plasma samples from animals that had been previously collected for other studies and stored at -20 C were tested. Because serum or plasma was not available from California animals, we conducted serologic testing on frozen whole blood (EDTA).

Age, sex, and land use at the capture site were assessed to determine effects on seroprevalence rates for raccoons and opossums from Georgia and Florida. Opossums and raccoons were classified as juveniles or adults based on weight, tooth wear, and development of reproductive organs (Grau et al. 1970, Kasparian et al. 2004). Only animals caught in Clarke County, GA were used to assess land use effects (i.e. animals captured from urban vs. rural locations) on *T. cruzi* prevalence. Trapping locations within Clarke County were classified as urban or rural based on data from the Georgia Land Use Trends Project (Natural Resources Spatial Analysis Laboratory, Odum School of Ecology, University of Georgia, unpublished data).

Serology

Samples from 12 mammal species from six states (Arizona, California, Florida, Georgia, Missouri, and Virginia) were tested for antibodies to *T. cruzi* (Table 2.1) using the indirect immunofluorescent antibody (IFA) test as described by Yabsley et al. (2001) with the following modifications. Antigen mixture was prepared from epimastigotes grown in liver infusion tryptose (LIT) medium. Antigen was placed onto each circle on 12-well test slides (Fisher Scientific, Rome, Georgia, USA), and allowed to dry at room temperature, and slides were fixed in acetone

for 10 minutes. Samples were tested at a dilution of 1:40. The diluted sera were incubated on the test slides for 30 minutes at 37° C. After incubations, the slides were washed twice with phosphate buffered saline (PBS) and then once with distilled water. A commercial fluorescein-labeled anti-species IgG antibody at a 1:50 dilution was then placed on the test slides and the slides were incubated for 30 minutes at 37° C. After incubation, the slides were washed again, but the last water wash included 1.65% erichrome black that counterstained the epimastigotes red to allow for easier visualization under fluorescent microscopy. Secondary antibodies used include a goat-anti raccoon IgG (Kirkegaard and Perry Laboratories (KPL), Gaithersburg, Maryland, USA), a goat anti-ferret IgG (KPL) for fishers (*Martes pennanti*), ringtails (*Bassariscus astutus*), striped skunks, and hooded skunks (*Mephitis macroura*), a goat-anti dog (KPL) for gray fox, red fox (*Vulpes vulpes*), coyotes, and domestic dogs (*Canis lupus*), a goat anti-pig for feral swine (KPL), and goat-anti cat (KPL) for bobcats (*Lynx rufus*). Opossum serologic testing followed the same procedure, except slides were incubated first with serum samples, then a rabbit anti-opossum IgG (Bethyl Laboratories, Montgomery, Texas, USA), and then a fluorescein-labeled anti-rabbit IgG (KPL). Slides were examined under an Olympus microscope. A sample was positive for *T. cruzi* antibodies if the epimastigotes appeared green under fluorescent microscopy, or red with a green outline. Negative samples appeared red.

Culture

Only blood collected aseptically from live animals was used for *T. cruzi* culture attempts. Blood was placed into culture for *T. cruzi* within 48 hours of collection. Buffy coats were collected from whole blood samples and added to 9 ml of LIT medium and stored at 27 C and/or inoculated on confluent layers of DH82 canine macrophage cells and maintained at 37 C as described (Hall et al. 2007). To minimize bacterial and fungal growth, 0.4 ml of penicillin

(10,000U/ml)-streptomycin (10mg/ml) (Sigma, St. Louis, MO) and 0.4 ml of 5-fluorocytosine (2.5 mg/ml, Sigma, St. Louis, MO) were added to each culture. Cultures were monitored for growth of trypanosomes, and if no parasites were observed after six weeks, the samples were considered negative.

Statistical Analysis

A chi-square analysis was used to assess significant differences between prevalence rates for different species, as well as differences between culture prevalence and seroprevalence for raccoons and opossums. It was also used to assess if significant differences in prevalence were present due to age, sex, or land use for serologic data, as well as if differences in prevalence were present in different geographic areas.

RESULTS

Serology

Results of IFA testing of twelve species of mammals from six states are shown in Table 2.1. Evidence of *T. cruzi* was found in every state except California. In general, the highest prevalence rates were found in raccoons followed by opossums (Table 2.1). Antibodies to *T. cruzi* were also detected in small numbers of striped skunks from Arizona and Georgia, bobcats from Georgia, a coyote from Georgia, and a ringtail from Arizona (Table 2.1). Interestingly, none of 110 feral swine tested from Ossabaw Island (Chatham Co.), Georgia were positive even though 31 of 54 (57%) raccoons from the island were positive (Table 2.1).

Culture

Of the 168 raccoons from Georgia and Florida tested by both culture and serology methods, 50 (30%) were culture positive, and 70 (42%) were seropositive. No difference in raccoon prevalence was found using the two detection methods ($X^2 = 2.46$, $P = 0.1168$). Of the 83 opossums tested, 11 (13%) were culture positive and a significantly higher number were

seropositive (28[34%]; $X^2 = 6.04$, $P = 0.014$). Significantly more raccoons were infected with *T. cruzi* compared with opossums based on culture prevalence ($X^2 = 5.27$, $P = 0.027$); however, no difference was noted based on serologic results between raccoons and opossums ($X^2 = 0.66$, $P = 0.4166$). Interestingly, we observed a marked difference in our ability to isolate *T. cruzi* by the two culture methods, of 10 samples that were ultimately culture-positive for *T. cruzi* and were also cultured in both LIT medium and in DH82 cells, only two were positive by both methods while eight were only positive by the DH82 method.

Exposure rates among raccoons and opossums in Georgia and Florida

Antibody prevalence rates among raccoons and opossums from seven counties in Georgia and across four counties in Florida were not significantly different between the two species, indicating that these species have a similar exposure rate, likely throughout their range in these states (Table 2.2). No geographic difference was noted in prevalence rates among raccoons and opossums from the Piedmont and Lower Coastal Plains physiographic regions of Georgia ($X^2 = 0.45$, $P = 0.5023$; $X^2 = 2.08$, $P = 0.1492$, respectively). Of the 338 raccoons from the Coastal Plain physiographic region, 116 (34%) were seropositive, compared with 50 (30%) of 166 raccoons from the Piedmont physiographic region. Of the 264 opossums from the Coastal Plain, 82 (31%) were seropositive, compared with 34 (22%) of 152 opossums from the Piedmont. Although no differences were noted between prevalences among regions in Georgia, significantly more raccoons and opossums from northern Florida (Leon and Wakulla counties) were seropositive compared to the seven Georgia counties ($X^2 = 5.12$, $P = 0.0237$; $X^2 = 4.24$, $P = 0.0395$, respectively) (Table 2.2).

Population parameters

Although greater numbers of adult animals were infected, no significant difference was noted between adult or juvenile raccoons or opossums from Georgia, Florida, or Missouri. In Georgia and Florida, 40% of 133 adult and 27% of 30 juvenile raccoons ($X^2 = 0.88$, $P = 0.3482$) were seropositive and 33% of 170 adult and 21% of 19 juvenile opossums ($X^2 = 0.62$, $P = 0.431047$) were seropositive. In Missouri, 75% of 85 adult and 43% of 23 juvenile raccoons ($X^2 = 1.79$, $P = 0.1809$) were seropositive. In contrast, significantly more females were seropositive compared with males among 367 raccoons (41% vs. 27%; $X^2 = 3.84$, $P = 0.05$) and 317 opossums (37% vs. 21%; $X^2 = 4.89$, $P = 0.027$) from Georgia and Florida. In Missouri, although more females (44/59, 75%) were seropositive than males (30/49, 61%), this difference was not significant ($X^2 = 1.79$, $P = 0.1809$).

No significant differences were noted between raccoons and opossums from urban or rural areas of Clarke County, Georgia ($X^2 = 0.12$, $P = 0.729$; $X^2 = 0.3$, $P = 0.5839$, respectively). Of 35 raccoons from urban areas, 9 (26%) were seropositive, while 29 of 89 (33%) raccoons from rural areas were seropositive. Of 35 opossums from urban areas, 9 (26%) were seropositive, and of 95 opossums from rural areas, 21 (22%) were seropositive.

DISCUSSION

This study presents a serologic survey of *T. cruzi* in several mammalian species throughout the United States. Evidence of *T. cruzi* was found in all states except California, and found in six of the twelve species tested and represents the first report of antibodies in Virginia opossums, ringtails, and bobcats. Although previously isolated from Virginia opossums, the

finding of *T. cruzi* antibodies suggests that both bobcats and ringtails in North America may be natural hosts.

The finding of *T. cruzi* in Arizona was not surprising; however, we did expect to find a greater number of seropositive animals. A previous study in Arizona detected *T. cruzi* in 4.7% of woodrats and mice (*Neotoma* spp. and *Peromyscus* spp.) using blood smears or xenodiagnosis (Wood 1952), and since serology has been shown to be more sensitive at detecting infections in wild animals (Yabsley et al. 2001), we expected a higher prevalence with serologic testing. The only species tested in relatively high numbers was the striped skunk, of which 9% were seropositive. Antibodies to *T. cruzi* have been detected in a single striped skunk from Los Angeles, California (Ryan et al. 1985) and Georgia (this study) and striped skunks have been implicated as suitable reservoir species through experimental studies (Davis et al. 1980).

Although interesting, the positive ringtail was not surprising, due to their close phylogenetic relationship to raccoons (both Family Procyonidae) and their use of tree cavities and abandoned housing as den sites (Poglayen-Neuwall and Toweill 1988). These cavities are preferred habitats for triatomine bug vectors and other potential reservoirs of *T. cruzi*. The lack of evidence of *T. cruzi* in domestic dogs in Arizona may be due to the small number of dogs tested, since they have been shown to have relatively low prevalence rates (1.9 – 17.1%) in the southern United States (Tomlinson et al. 1981, Meurs et al. 1998).

The lack of evidence of *T. cruzi* in California could be the result of a number of factors. The species most likely to possess antibodies to *T. cruzi*, raccoons and ringtails, were sampled in low numbers, which may have been too low to detect *T. cruzi* in the area. The samples were taken from Humboldt County in northern California, which is possibly outside of the range for *T. cruzi*. The northernmost location where *T. cruzi* has been found in California is in the San

Francisco area (Navin et al. 1985), which is approximately 500 km south of Humboldt County. *Triatoma protracta*, a vector for *T. cruzi* in this area, is known to specialize on woodrats (*Neotoma* spp.) (Peterson et al. 2002), therefore, woodrats may be a more important reservoir for *T. cruzi* in this area than the species tested in the current study.

The prevalence of *T. cruzi* in canids from the southeastern US appears to be very low compared with previous studies in Texas. Two studies conducted in Texas showed that 12.8% and 14.2% of coyotes were seropositive (Burkholder et al. 1980; Grogil et al 1984). Based on blood culture, 1.7% of gray foxes from southwestern Georgia and northwestern Florida were found positive (McKeever et al. 1958) and recently, a serologic survey of wild canids in South Carolina found two of 26 (8%) gray foxes positive for *T. cruzi* antibodies, but no antibodies in two coyotes tested (Rosypal et al. 2007). Although we only detected two positive coyotes and did not find any positive red foxes or gray foxes, the sample sizes were low and more testing is needed to determine an accurate prevalence for these *T. cruzi* hosts in the Southeast.

T. cruzi has been reported sporadically in swine; in a single domestic pig from Mexico by isolation (Fujita et al. 1994), in 4 of 105 (2.8%) domestic pigs in Brazil (Salazar-Schettino et al. 1997), and in 2 of 20 domestic pigs from Paraguay by serology (da Costa Valente 1999). Domestic pigs are also experimentally susceptible to infection with a North American raccoon strain of *T. cruzi* (Diamond and Rubin 1958). In the current study, over 100 feral swine from Ossabaw Island, GA were tested and found to be seronegative which was surprising given the high prevalence of positive raccoons (57%) in the current study.

Raccoons and opossums are considered to be the two major reservoir species in the United States. Although not significant, adult raccoons and opossums in Georgia and Florida had higher prevalence rates compared with juveniles, which could be expected since once a host is

exposed to the parasite, it maintains it for life. A lack of significance suggests that if raccoons become infected, they are infected as young raccoons and only limited numbers of naive adults become infected. Interestingly, we noted prevalence rates in female raccoons and opossums were significantly greater than in males. This is similar to rates found by Yabsley and Noblet (2002), who found a higher seroprevalence in females compared with males, although the results were not significant, perhaps due to a lower sample size. The higher rate of *T. cruzi* in females is possibly due to the denning habits of female animals, which can lead to a higher contact rate with triatomid bugs. The higher infection prevalences in Florida and Missouri could be due to multiple, possibly distinct, factors. Higher densities of triatomid bug vectors and possibly higher densities of animals would be expected in milder climates such as in Florida. The mild climate may also result in more year-round activity by the bug vector as well as the animal reservoirs. In Missouri, increased prevalence could be due to increased densities of raccoons in the area, as well as longer denning periods in colder climates (Stuewer 1943). Although urban wildlife populations are more likely than their rural counterparts to be impacted by disease and to maintain pathogens (Riley et al. 1998), we found that raccoons and opossums in urban and rural areas did not have different prevalence rates. This may be due to differences in transmission dynamics of different pathogens and the ecology of different vectors in urban and rural areas. Animals in urban settings are more likely to live in higher densities than animals in rural habitats (Prange et al. 2003), and are therefore more likely to transmit pathogens directly, but in the case of *T. cruzi*, this trend may be offset by a lower vector density in urban settings.

Depending on the diagnostic test used for identifying infected individuals, prevalence rates in raccoons have ranged from 1.5% - 63% (McKeever et al. 1958, Walton 1958, Schaffer et al. 1978, John and Hoppe 1986, Telford and Forrester 1991, Karsten et al. 1992, Pung et al.

1995, Pietrzak and Pung 1998, Yabsley and Noblet 2002, Hancock et al. 2005). Prevalences in studies based on isolation from blood culture typically are lower compared with those based on serology. The culture prevalence in this study (30%) is similar to previous rates from Georgia that range from 22% (Pung et al. 1995) to 43% (Pietrzak and Pung 1998). These culture rates are higher than previous studies in surrounding states that range from 14% in Alabama (Olsen et al. 1964) to 15% in North Carolina (Karsten et al. 1992), but lower than the 63% (6 of 8 raccoons) prevalence found in Oklahoma (John and Hoppe 1986). The seroprevalences noted in Georgia, Florida, and Missouri were similar to previous studies conducted in South Carolina (37-61%) and northern Virginia (16-41%) (Yabsley and Noblet 2002, Hancock et al. 2005). In the current study there was no difference noted in the seroprevalence between the two tested physiographic regions of Georgia; however we did not test raccoons from the mountainous (Blue Ridge/Ridge and Valley) physiographic regions which had the lowest prevalence in South Carolina (Yabsley and Noblet 2002).

Previous studies on Virginia opossums have been limited to culture based surveillance and have produced prevalences (8-33%) that are lower compared with raccoons (McKeever et al. 1958, Olsen et al. 1964, Barr et al. 1991, Karsten et al. 1992, Pung et al. 1995). In the current study, a similar culture based prevalence (13%) was noted which was significantly less than detected in sympatric raccoons (30%). Importantly however, we did not note a difference in the seroprevalence of sympatric raccoons and opossums indicating exposure rates among the two species is similar. This apparent difference between serology and culture could be due to opossums having lower blood parasitemias at time of sampling which leads to a decreased culture-based prevalence. This may be related to the differences in *T. cruzi* strains, as opossums are infected predominantly with *T. cruzi* I strains, while raccoons are infected predominantly

with *T. cruzi* IIa (Clark and Pung 1994, Barnabe et al. 2001, Roellig et al., in review). These differences in parasitemias may also be due to immunologic factors which allow the opossums to clear the parasite from the bloodstream more quickly than other species. Experimental studies with North American host species and parasite strain are needed to investigate this dynamic.

ACKNOWLEDGEMENTS

This work was supported primarily by the National Institutes of Allergy and Infectious Diseases (R15 AI067304). Further support was provided 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.

The authors would like to thank D. Hughes (USDA Wildlife Services) J. Sleeman, (VA Dept. of Game and Inland Fisheries) and A. Zajac (Virginia Polytechnic Institute and State University) for providing some samples used in this project and J. Wickwire, B. Wilcox, and J. Murdock for field and laboratory assistance.

REFERENCES

Barnabe, C, Yaeger, R, Pung, O, Tibayrenc, M. *Trypanosoma cruzi*: A considerable phylogenetic divergence indicates that the agent of Chagas disease is indigenous to the native fauna of the United States. *Exp Parasit* 2001, 99:73-79.

Barr, SC, Brown, CC, Dennis, VA, Klei, TR. The lesions and prevalence of *Trypanosoma cruzi* in opossums and armadillos in southern Louisiana. *J Parasit* 1991, 77:624-627.

- Burkholder, JE, Allison, TC, Kelly, VP. *Trypanosoma cruzi* (Chagas) (Protozoa: Kinetidoplasta) in invertebrate, reservoir, and human hosts of the Lower Rio Grande Valley of Texas. *J Parasit* 1980, 66:305-311.
- Centers for Disease Control and Prevention. 2004. Chagas Disease Fact Sheet. cdc.gov. Accessed 27 February 2008.
- Clark, CG, Pung, OJ. Host specificity of ribosomal DNA variation in sylvatic *Trypanosoma cruzi* from North America. *Mol Biochem Parasit* 1994, 66:175-179.
- Da Costa Valente, V. Potential for domestication of *Panstrongylus geniculatus* (Latreille, 1811) (Liemiptera, Reduviidae, Triatominae) in the municipality of Muaná, Marajó Island, State of Pará, Brazil. *Mem Inst Oswaldo Cruz* 1999, 94:S399-400.
- Davis, DS, Russell, LH, Adams, LG, Yaeger, RG, Robinson, RM. An experimental infection of *Trypanosoma cruzi* in striped skunks (*Mephitis mephitis*). *J Wild Dis* 1980, 16:403-406.
- Diamond, LS, Rubin, R. Experimental infection of certain farm mammals with a North American strain of *Trypanosoma cruzi* from the raccoon. *Exp Parasit* 1958, 7:383-390.

- Dorn, PL, Pernacario, L, Yabsley, MJ, Roellig DM, Balsamo, G, Diaz, J, Wesson, D.
Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emer
Inf Dis 2007, 13:605-607.
- Fujita, O, Sanabria, L, Inchausti, A, de Arias, AR, Tomizawa, Y, Yuzaburo, O. Animal
reservoirs for *Trypanosoma cruzi* infection in an endemic area in Paraguay. J Vet
Med Sci 1994, 56:305-208.
- Grau, GA, Sanderson, GC, Rogers, JP. Age determination of raccoons. J Wild Mgmt
1970, 34:364-372.
- Grogl, M, Kuhn, RE, Davis, DS, Green, GE. Antibodies to *Trypanosoma cruzi* in
coyotes in Texas. J Parasit 1984, 70:189-191.
- Hall, CA, Polizzi, C, Yabsley, MJ, Norton, TM. *Trypanosoma cruzi* prevalence and
epidemiologic trends in lemurs on St. Catherines Island, Georgia. J Parasit 2007,
93:93-96.
- Hancock, K, Zajac, AM, Pung, OJ, Elvinger, F, Rosypal, AC, Lindsay, DS.
Prevalence of antibodies to *Trypanosoma cruzi* in raccoons (*Procyon lotor*) from
an urban area of Northern Virginia. J Parasit 2005, 91:470-472.

- Herwaldt, BL, Newsome, AL, McGhee, CR, Powell, MR, Nemeč, DG, Steurer, FJ, Eberhard, ML. Use of polymerase chain reaction to diagnose the fifth reported U.S. case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, in 1998. J Infect Dis 2000, 181:395-399.
- Jaime-Andrade, GJ, Avila-Figueroa, D, Lozano-Kasten, FJ, Hernandez-Gutierrez, RJ, Magallon-Gastelum, E, Kasten-Monges, MJ, Lopes, ER. Acute Chagas' cardiopathy in a polar bear (*Ursus maritimus*) in Guadalajara, Mexico. Rev da Soc Bras de Med Trop 1997, 30:337-340.
- Jansen, AM, Morierarty, PL, Galvao Castro, B, Deane, MP. *Trypanosoma cruzi* in the opossum *Didelphis marsupialis*: an indirect fluorescent antibody test for the diagnosis and follow-up of natural and experimental infections. Trans Royal Soc Trop Med Hyg 1985, 79:474-477.
- John, DT, Hoppe, KL. *Trypanosoma cruzi* from wild raccoons in Oklahoma. Am J Vet Res 1986, 47:1056-1059.
- Karsten, V, Davis, C, Kuhn, R. *Trypanosoma cruzi* in wild raccoons and opossums from North Carolina. J Parasit 1992, 78:547-549.

Kasa, TJ, Lathrop, GD, Dupuy, HJ, Bonney, CH, Toft, JD. An endemic focus of *Trypanosoma cruzi* infection in a subhuman primate research colony. J Am Vet Med Assoc 1977, 171:850-854.

Kasparian, MA, Hellgren, EC, Ginger, SM, Levesque, LP, Clark, JE, Winkelman, DL. Population characteristics of Virginia opossum in the Cross Timbers during raccoon reduction. Am Midl Nat 2004, 151:154-163.

McKeever, S, Gorman, GW, Norman, L. Occurrence of a *Trypanosoma cruzi*-like organism from some mammals from southwestern Georgia and northwestern Florida. J Parasit 1958, 44:583-587.

Meurs, KM, Anthony, MA, Slater, M, Miller, MW. Chronic *Trypanosoma cruzi* infection in dogs: 11 cases (1987-1996). J Am Vet Med Assoc 1998, 213:497-500.

Navin, TR., Roberto, RR, Juranek, DD, Limpakarnjanarat, K, Mortenson, EW, Clover, JR, Yescott, RE, Taclindo, C, Steurer, F, Allain, D. Human and sylvatic *Trypanosoma cruzi* infection in California. Am J Pub Health 1985, 75:366-369.

- Olsen, PF, Shoemaker, JP, Turner, HF, Hays, KL. Incidence of *Trypanosoma cruzi* (Chagas) in wild vectors and reservoirs in east-central Alabama. J Parasit 1964, 50:599-603.
- Peterson, AT, Sanchez-Cordero, V, Beard, CB, Ramsey, JM. Ecologic niche modeling and potential reservoirs for Chagas disease, Mexico. Emer Inf Dis 2002, 8:662-666.
- Pietrzak, SM, Pung, OJ. Trypanosomiasis in raccoons from Georgia. J Wild Dis 1998, 34:132-136.
- Poglayen-Neuwall, I, Toweill, DE. *Bassariscus astutus*. Mamm Spec 1988, 327:1-8.
- Prange, S, Gehrt, SD, Wiggers, EP. Demographic factors contributing to high raccoon densities in urban landscapes. J Wild Mgmt 2003, 67:324-333.
- Pung, OJ, Banks, CW, Jones, DN, Krissinger, MW. *Trypanosoma cruzi* in wild raccoons, opossums, and triatomine bugs in southeast Georgia, U.S.A. J Parasit 1995, 81:324-326.
- Riley, SP, Hadidian, J, Manski, DA. Population density, survival, and rabies in raccoons in an urban national park. Can J Zool 1998, 76:1153-1164.

Roellig, DM, Brown, EL, Barnabe, C, Tibayrenc, M, Steurer, FJ, Yabsley, MJ. Molecular typing of human, vector, and reservoir isolates of *Trypanosoma cruzi* from the United States. Emerging Infectious Diseases, in review.

Rosypal, AC, Tidwell, RR, Lindsay, DS. Prevalence of antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in wild canids from South Carolina. J Parasit 2007, 93: 955-957.

Ryan, CP, Hughes, PE, Howard, EB. American Trypanosomiasis (Chagas' disease) in a striped skunk. J Wildl Dis 1985, 21:175-176.

Salazar-Schettino, PM, Bucio, MI, Cabrera, M, Bautista, J. First case of natural infection in pigs. Review of *Trypanosoma cruzi* reservoirs in Mexico. Mem Inst Oswaldo Cruz 1997, 92:499-502.

Schaffer, GD, Hanson, WL, Davidson, WR, Nettles, VF. Hematotropic parasites of translocated raccoons in the Southeast. J Am Vet Med Assoc 1978, 173:1148-1151.

Stuewer, FW. Raccoons: their habits and management in Michigan. Ecological Monographs 1943, 13:203-257.

Telford, SR, Forrester, DJ. Hemoparasites of raccoons (*Procyon lotor*) in Florida. J Wildl Dis 1991, 27:486-490.

Tomlinson, M.J, Chapman, WL, Hanson, WL, Gosser, HS. Occurrence of antibody to *Trypanosoma cruzi* in dogs in the southeastern United States. Am J Vet Res 1981, 42:1444-1446.

Walton, BC, Bauman, PM, Diamond, LS, Herman, CM. The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. Am J Trop Med Hyg 1958, 7:603-610.

Wood, SF. Mammal blood parasite records from southwestern United States and Mexico. J Parasit 1952, 38:85-86.

Woody, NC, Hernandez, A, Suchow, B. American trypanosomiasis III. The incidence of serologically diagnosed Chagas' disease among persons bitten by the insect vector. J Pediatrics 1965, 66:107-109.

Yabsley, MJ, Noblet, GP, Pung, OJ. Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). J Parasit 2001, 87:1155-1159.

Yabsley, MJ, Noblet, GP. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. J Wild Dis 2002, 38:75-83.

Yaeger, RG. The prevalence of *Trypanosoma cruzi* infection in armadillos collected at a site near New Orleans, Louisiana. Am J Trop Med Hyg 1988, 38:323-326.

Table 2.1. Results of indirect immunofluorescent antibody testing of mammals from six states for *Trypanosoma cruzi* antibodies. Similar letters indicate no statistical differences between species within each state ($P < 0.05$).

Location	Species	Total Tested	Positive (%)
Arizona	Domestic Dog (<i>Canis lupus</i>)	20	0
	Hooded Skunk (<i>Mephitis macroura</i>)	1	0
	Raccoon (<i>Procyon lotor</i>)	5	1 (20) ^a
	Ringtail (<i>Bassariscus astutus</i>)	1	1 (100) ^a
	Striped Skunk (<i>Mephitis mephitis</i>)	34	3 (9) ^a
California	Fisher (<i>Martes pennanti</i>)	21	0
	Gray Fox (<i>Urocyon cinereoargenteus</i>)	10	0
	Raccoon	3	0
	Ringtail	10	0
Florida	Striped Skunk	6	0
	Raccoon	70	38 (54) ^a
Georgia	Virginia Opossum (<i>Didelphis virginiana</i>)	27	14 (52) ^a
	Bobcat (<i>Lynx rufus</i>)	62	2(3.2) ^b
Missouri	Coyote (<i>Canis latrans</i>)	23	1(4.4) ^b
	Feral Swine (<i>Sus scrofa</i>)	110	0
	Gray Fox	21	0
	Opossum	421	118(28) ^a
	Raccoon	510	167(33) ^a
	Red Fox (<i>Vulpes vulpes</i>)	5	0
	Striped Skunk	1	1 (100)
Virginia	Raccoon	109	74 (68)
	Coyote	26	1 (3.8) ^a
Virginia	Gray Fox	1	0
	Raccoon	12	0
	Virginia Opossum	6	1(17) ^a

Table 2.2. Exposure rates for *Trypanosoma cruzi* in Georgia and Florida counties in which at least five raccoons (*Procyon lotor*) or Virginia opossums (*Didelphis virginiana*) were tested.

Population	IFA serology results	
	No. Positive/ No. examined	
	(% positive)	
Population	Raccoon	Virginia Opossum
Baker Co., GA	48/177 (27.1)	35/122 (28.7)
Chatham Co., GA	46/105 (43.8)	3/7 (42.8)
Clarke Co., GA	40/128 (31.3)	31/116 (26.7)
Franklin Co., GA	2/7 (28.6)	2/6 (33.3)
Glynn Co., GA	7/10 (70.0)	4/5 (80.0)
Thomas Co., GA	8/22 (36.7)	39/113 (34.5)
Webster Co., GA	1/6 (16.7)	1/9 (11.1)
Hendry Co., FL	6/12 (50.0)	6/17 (35.3)
Leon Co., FL	9/17 (52.9)	4/4 (100.0)
Wakulla Co., FL	16/23 (69.6)	4/6 (66.7)

CHAPTER 3

CONCLUSIONS

Trypanosoma cruzi, causative agent of Chagas' disease, is a substantial public health concern in Latin America. Although rare in humans and uncommon in domestic animals in the United States, *T. cruzi* is commonly detected in several mammalian wildlife species, and is most commonly reported in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*). Twelve species of mammals from six states (Arizona, California, Florida, Georgia, Missouri, and Virginia) were tested for antibodies to *T. cruzi* using indirect immunofluorescent antibody testing and culture isolation attempts were conducted on a limited number of animals from Georgia and Florida. Evidence of *T. cruzi* was found in every state except California. In general, the highest prevalence rates were found in raccoons (33-68%) followed by Virginia opossums (28-52%), but *T. cruzi* were also detected in small numbers of striped skunks (*Mephitis mephitis*) from Arizona and Georgia, bobcats (*Lynx rufus*) from Georgia, two coyotes (*Canis latrans*) from Georgia and Virginia, and a ringtail (*Bassariscus astutus*) from Arizona. Culture-based prevalence rates for raccoons were significantly greater than those for opossums ($X^2 = 5.27$, $P = 0.027$); however, antibody prevalences of raccoons and opossums from several populations in Georgia and Florida were not different indicating exposure rates are similar within a given population. For both raccoons and opossums, adult animals had a higher prevalence rate compared with juveniles, females had a higher prevalence rate compared with males, and no difference was found between animals caught in urban or rural locations. This study represents the first report of antibodies in Virginia opossums, ringtails, and bobcats. Although previously isolated from Virginia

opossums, the finding of *T. cruzi* antibodies suggests that both bobcats and ringtails in North America may be natural hosts. Results indicate that *T. cruzi* prevalence varies by host species, host characteristics, and geographic region and provide data to guide future studies on the natural history of *T. cruzi* in the United States.