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SEROPREVALENCE OF *TRYPANOSOMA CRUZI* IN RACCOONS FROM SOUTH CAROLINA AND GEORGIA

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ABSTRACT: *Trypanosoma cruzi*, the causative agent of American trypanosomiasis or Chagas' disease, is of both medical and veterinary importance as is evidenced by chronic phase myocarditis in humans and dogs. Further, *T. cruzi* has been reported from over 20 species of wildlife reservoir hosts in the USA, with raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) being the most common. Whereas previous studies on *T. cruzi* in raccoons have included only culture and direct examination of blood, the indirect immunofluorescent antibody test (IFAT) was used in the current study to detect anti-*T. cruzi* antibodies in the serum of raccoons. Of 221 raccoons trapped at 13 sites representing the five physiographic regions of South Carolina plus five sites in the Piedmont region of Georgia (from April 1997 to February 2000), 104 (47%) were seropositive. A higher seroprevalence in raccoons was observed in the coastal regions, with seroprevalence in the Lower Coastal Plain South (61%) being significantly higher than that in the Foothills (37%), Piedmont (42%), and Upper Coastal Plain (40%) regions. However, at a seroprevalence of 52%, the Lower Coastal Plain North was not significantly different from any other region. Although more female raccoons were infected than males, no statistical difference in prevalence was observed between sexes. The high seroprevalence of *T. cruzi* in raccoons, together with a few reports of wildlife isolates being infective for other wildlife species and domestic/laboratory animals, suggests that risk of *T. cruzi* infection may be higher than previously suspected.

Key words: Chagas' disease, indirect immunofluorescent antibody test (IFAT), raccoon, serological test, *Trypanosoma cruzi*, zoonosis.

INTRODUCTION

Trypanosoma cruzi, the causative agent of fatal chronic myocarditis (Chagas' disease) in both dogs and humans, is commonly reported from wildlife reservoir hosts. For humans in the USA, autochthonous vector-transmitted *T. cruzi* infection is rare, with cases having been reported only from California, Texas, and Tennessee (Ochs et al., 1996; see Herwaldt et al., 2000). Serological testing of individuals in Texas with a history of bites from *Triatoma gerstaeckeri*, a triatomid bug vector for *T. cruzi*, indicated a prevalence of 2 to 3% (Woody et al., 1961; Woody et al., 1965). Farrar et al. (1972) reported two of 3,883 individuals from Georgia were weakly positive for antibody to *T. cruzi*. For dogs, acute and chronic infections have been reported from the southern states of Texas, Louisiana, Oklahoma, Georgia, South Carolina, and Virginia (see Meurs et al., 1998). Serological testing (direct agglutination) by Tomlinson et al. (1981) indicated that 24 of 365 dogs from Georgia and other south-

eastern states were seropositive for *T. cruzi*; however, investigators failed to indicate prevalence by state.

Reports of *T. cruzi* in wildlife include raccoons (*Procyon lotor*) and a wide variety of other reservoir hosts (see John and Hoppe, 1986). As determined by culture of blood, prevalence of *T. cruzi* in raccoons ranged from as low as 2% in northwestern Florida/southwestern Georgia to as high as 62% in Oklahoma (McKeever et al., 1958; John and Hoppe, 1986). Although no prior study of *T. cruzi* infection in raccoons has been conducted in South Carolina, results of recent culture surveys for raccoons from the adjacent states of Georgia and North Carolina indicated prevalence rates of 22 to 43% and 15%, respectively (Karsten et al., 1992; Pung et al., 1995; Pietrzak and Pung, 1998).

Previous studies of raccoons have included only the culture of whole blood or direct examination of stained blood smears. Such techniques are limited due to detection of the parasite only during the

TABLE 1. Results of IFAT testing for *Trypanosoma cruzi* in 221 raccoons from the five physiographic regions of South Carolina and the Piedmont region of Georgia.

Site (#) ^a	Lat/Long	n	# positive (%) ^b
Foothills—FH		54	20 (37)^c
Clemson area—Pickens Co. (1)	34°41'N 82°50'W	43	18 (42)
18 mile creek—Anderson Co. (2)	34°31'N 82°38'W	11	2 (18)
Piedmont—PD		45	19 (42)^c
South Carolina			
Enoree WMA—Union Co. (3)	34°43'N 81°37'W	1	1 (100)
Tyger WMA—Newberry Co. (4)	34°16'N 81°36'W	4	1 (25)
Georgia			
Elbert WMA—Elber Co. (5)	34°06'N 82°51'W	6	2 (33)
Wilkes WMA—Wilkes Co. (6)	33°44'N 82°44'W	2	1 (50)
University of Georgia—Clarke Co. (7)	33°57'N 83°23'W	22	9 (41)
Rural farm—Oglethorpe Co. (8)	33°52'N 83°06'W	2	1 (50)
Rural farm—Lincoln Co. (9)	33°47'N 82°28'W	8	4 (50)
Upper Coastal Plains—UCP		35	14 (40)^c
Columbia—Richland and Calhoun Co. (10)	34°02'N 80°53'W	6	2 (33)
Savannah River Plant—Aiken Co. (11)	33°32'N 81°43'W	29	12 (41)
Lower Coastal Plains South—LCPS		66	40 (61)^d
Rural Plantations—Jasper Co. (12)	32°20'N 80°56'W	7	1 (14)
Bostick Plantation—Hampton Co. (13)	32°45'N 81°14'W	25	15 (60)
Seabrook Island—Charleston Co. (14)	32°34'N 80°10'W	24	17 (71)
John's Island—Charleston Co. (15)	32°47'N 80°06'W	9	6 (67)
Wadmalaw Island—Charleston Co. (16)	32°40'N 80°14'W	1	1 (100)
Lower Coastal Plains North—LCPN		21	11 (52)^c
Rural farms—Horry Co. (17)	33°56'N 79°08'W	5	2 (40)
Myrtle Beach area—Horry Co. (18)	33°41'N 78°53'W	16	9 (56)
Total		221	104 (47)

^a Number corresponds to map locations on Figure 1.
^b Similar superscript letters indicates no statistical differences in prevalence between physiographic regions (*P* > 0.05).

early acute phase of infection. During the chronic phase of infection, isolation of the parasite is less likely by culture or direct examination due to low circulating parasitemia. Therefore, use of serological tests for detection of anti-*T. cruzi* antibodies increases the sensitivity of reservoir studies. In South America, both the indirect immunofluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) have been used to study the epidemiology of *T. cruzi* in humans and, to a lesser extent, in mammalian reservoir hosts. The sensitivity (96%) and suitability of the IFAT in detecting *T. cruzi* infections in raccoons have been previously reported (Yabsley et al., 2001); therefore, the IFAT was selected as the serological test of choice for the current study. The objective

of this study was to determine the seroprevalence of *T. cruzi* in raccoon populations from the five physiographic regions of South Carolina (SC) and the Piedmont region of Georgia (GA) and to ascertain any differences in prevalence between the regions or study sites within each of these regions.

MATERIALS AND METHODS

From April 1997 through February 2000, 181 raccoons were trapped at 13 sites (Table 1 and Fig. 1) representing the five physiographic regions of SC. Trapping sites within the Foothills region (FH) included the city of Clemson and adjacent housing subdivisions/forests (#1) and a waterfowl management area (WMA, #2). Two sites (#3 and 4) trapped in the Piedmont region (PD) were Enoree WMA and Tyger WMA. An additional 40 raccoons were trapped at five sites in the PD region of GA, which in-

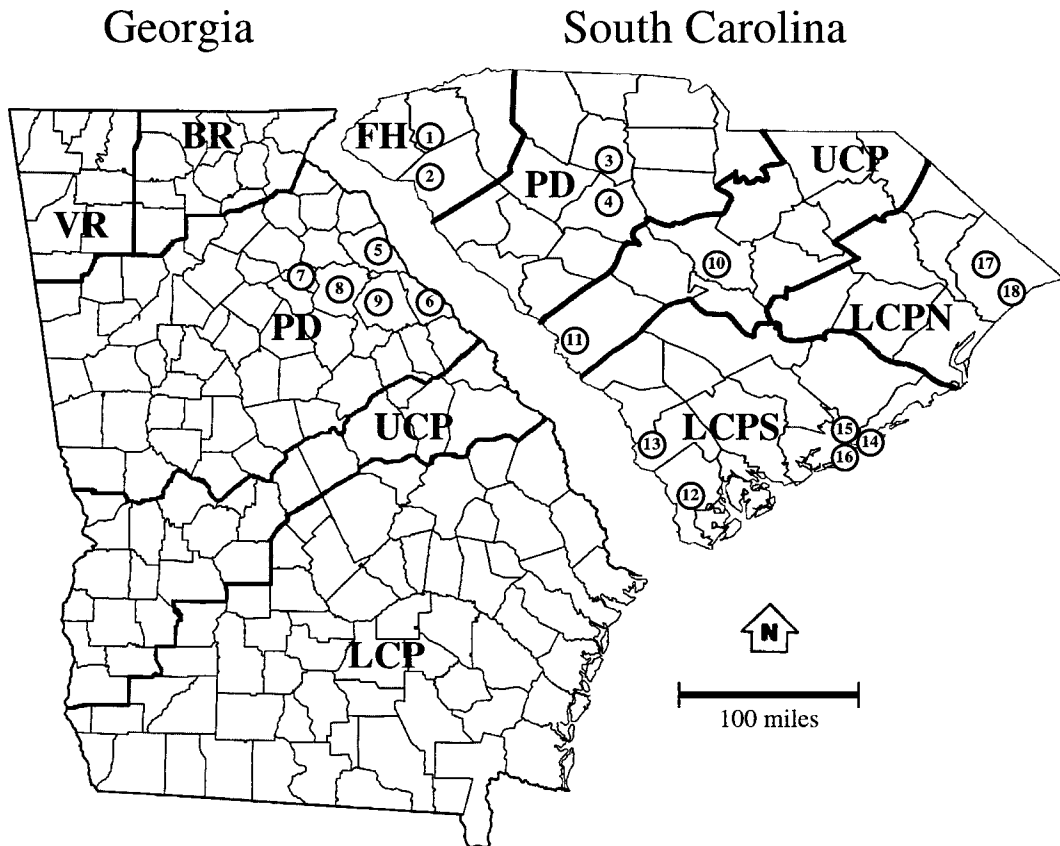


FIGURE 1. South Carolina and Georgia, showing physiographic regions and 18 trapping sites (see Table 1 for site descriptors). FH = Foothills, PD = Piedmont, UCP = Upper Coastal Plain, LCP = Lower Coastal Plain, LCPS = Lower Coastal Plain South, LCPN = Lower Coastal Plain North, VR = Valley and Ridge, and BR = Blue Ridge.

cluded two WMA (#5 and 6), the city of Athens (#7), and rural farms (#8 and 9). In SC, the city of Columbia and surrounding areas (#10) and the rural Savannah River Site (#11) were selected as trapping locations in the Upper Coastal Plain region (UCP). Several sites in the Lower Coastal Plain South (LCPS) were trapped, including rural farming plantations in Jasper County (#12), a large, swampy, hunting plantation (Bostick Plantation) in Hampton County (#13), and three barrier islands located near Charleston (#14–16). In the Lower Coastal Plain North region (LCPN), both a rural area (various inland farms, #17) and an urban setting (beach and wooded areas in the tourist city of Myrtle Beach, #18) were trapped.

Each raccoon trapped for this study was subjected to multiple evaluations. In addition to the current seroprevalence study, tissues from SC raccoons were further utilized for *T. cruzi* culture and DNA studies, the study of gastrointestinal helminth parasites (Yabsley and Nob-

let, 1999), and as museum study specimens. Most animals also were included in a trap-type capture effectiveness study conducted by the International Association of Fish and Wildlife Agencies (Washington, DC) and the SC Department of Natural Resources (SCDNR, Columbia, South Carolina, USA). Georgia raccoon sera ($n = 40$) were obtained as part of ongoing studies by the Southeastern Cooperative Wildlife Disease Study (SCWDS, Athens, Georgia, USA) and the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia, USA).

Both live traps and foot-hold traps were used to capture animals. Live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were baited with canned sardines or commercial cat food in the afternoon and checked the next morning. Raccoons were either euthanized by intramuscular injection of xylazine (0.25 mg/kg body weight; Mobay Corp., Animal Health Division, Shawnee, Kansas, USA) mixed with ketamine hydrochloride (25 mg/kg; Aveco Co.

Inc., Fort Dodge, Iowa, USA) followed by intraperitoneal injection of 1 ml/kg sodium pentobarbital (Fatal Plus, J. A. Webster, Inc., Sterling, Massachusetts, USA), or were hunter-shot. From each animal, 5 to 10 ml of blood was collected and serum frozen at -80°C for serological testing.

For use as antigen, Brazil-strain culture epimastigotes of *T. cruzi* were maintained by bi-weekly passage in liver infusion tryptose (LIT) medium (Powell and Kuhn, 1980). To prepare antigen for the IFAT, 20 ml of 11-day-old culture epimastigotes was centrifuged at 2,450 rpm for 15 min. The supernatant was removed and the pellet washed twice by centrifugation in phosphate buffered saline (PBS). The final washed pellet was resuspended in 20 ml of 1% formalin (10% formalin diluted in PBS). The antigen mixture was allowed to set at room temperature for 24 hr and then was stored at 4°C for up to one year.

The IFAT was used to detect anti-*T. cruzi* antibodies by the method of Camargo (1966) as modified by Yabsley et al. (2001). Antigen was placed in each circle on double-well test slides (Fisher Scientific, Rome, Georgia, USA) and dried in an oven at 50°C for 30 min. The slides were stored in a glass jar with CaCl_2 at -4°C for no longer than one month. A commercial goat anti-raccoon Ig conjugated with fluorescein isothiocyanate (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) diluted with a 1% solution of Evans blue (Sigma Chemical Co., St. Louis, Missouri, USA) in PBS was used for the IFAT. Evans blue counterstained the epimastigotes red, which allowed for easier visualization of epimastigotes under fluorescent microscopy. Slides were examined under a Zeiss (Wetzlar, Germany) microscope equipped with a 50W Hg illuminator (488 nm excitation and a 520 nm band-pass filter). Fluorescence of a positive sample ranged from full bright green fluorescent epimastigotes to bright green outline of red counterstained epimastigotes. If anti-*T. cruzi* antibodies were not present, epimastigotes appeared dim red with no fluorescence. When only weak fluorescence of parasites was observed, the sample was classified as borderline and retested at a later date. If the reaction was still doubtful after additional testing, the result was reported as negative.

Optimal serum (1:40) and conjugate (1:10) concentrations were determined by checkerboard titration, using known positive- and negative-control raccoon serum. Positive controls consisted of pooled serum samples collected from seven culture-positive raccoons trapped in Georgia during a previous study (Pung et al., 1995). Trypanosomes isolated from these seven raccoons were confirmed as *T. cruzi* by analysis

of ribosomal RNA gene polymorphism (Clark and Pung, 1994). Negative controls were pooled sera of 39 raccoons trapped near the Toronto Zoo in Ontario, Canada, which is outside the range of any known vector for *T. cruzi* (Yabsley et al., 2001).

Locality data for appropriate triatomid bug vectors (Hemiptera: Reduviidae) were obtained by searching records of the SC State Arthropod Survey housed in the Clemson University Arthropod Collection (Clemson, South Carolina). Additional data were obtained from The University of Georgia Museum of Natural History Collection of Arthropods (Athens).

Fisher's exact test was used to evaluate infection prevalences for the five physiographic regions, study sites within each region, and sex of host (Sokal and Rohlf, 1981). Values of $P < 0.05$ were considered statistically significant.

RESULTS

When tested with the IFAT, 104 of 221 raccoons (47%) were positive for *T. cruzi* (Table 1). Of the 181 serum samples tested from SC, 87 (48%) were seropositive, with similar prevalences being noted for the three inland physiographic regions. Twenty of 54 raccoons (37%) trapped in the FH region and 14 of 35 raccoons (40%) from the UCP region were positive. Only five raccoons were trapped in the SC PD region, while 40 were sampled from the GA PD. A similar seroprevalence was noted for the PD region of both states, with two of five SC raccoons (40%) and 17 of 40 GA raccoons (43%) being seropositive, for a total 19 of 45 PD raccoons (42%) from both SC and GA being seropositive. In the LCPS and LCPN regions of SC, 40 of 66 raccoons (61%) and 11 of 21 (52%), respectively, were seropositive.

The highest prevalence of 61% in this study for raccoons from the LCPS region of SC was statistically different from those of the three inland physiographic regions (FH, PD and UCP). Although no statistical difference was noted between the LCPS and SC PD regions (probably due to small SC PD sample size, $n = 5$), a significant difference was observed between LCPS and GA PD ($n = 40$) and also the combined GA/SC PD region. Seropreva-

TABLE 2. Seroprevalence of *Trypanosoma cruzi* in wild and nuisance (city-caught) raccoons.

Region ^a	Wild		Nuisance	
	n	# infected (%) ^b	n	# infected (%)
FH	11	2 (18) ^{c,e}	43	18 (42) ^c
PD	23	10 (43) ^c	22	9 (42) ^c
UCP	29	12 (41) ^c	6	2 (33)
LCPS	42	23 (55) ^f	24	17 (71) ^d
LCPN	5	2 (40)	16	9 (56)
Total	110	49 (45)	111	55 (50)

^a FH = Foothills, PD = both GA and SC Piedmont, UCP = Upper Coastal Plain, LCPS = Lower Coastal Plain South, LCPN = Lower Coastal Plain North.

^b Significant differences in prevalence at $P < 0.05$ between superscripts c and d and significant differences at $P < 0.05$ between superscripts e and f.

lence for LCPN was not statistically different from that of any other region.

To assess potential effect of host habitat on *T. cruzi* prevalence, raccoons were classified as either wild or nuisance (city-caught). In Table 2, prevalence data are presented for raccoons by physiographic region and host habitat. Although a few significant differences were detected between LCPS raccoons and habitat within other region locations (Table 2), no overall significant differences were observed between wild and nuisance raccoons, with 49 of 110 wild raccoons (45%) and 55 of 111 nuisance raccoons (50%) being seropositive. Similarly, comparison within each region of wild and nuisance raccoon populations failed to demonstrate any differences.

Relative to prevalence by sex, 11 of 25 GA males (44%) and 8 of 15 GA females (53%) were seropositive. For SC, sex was recorded for only 126 raccoons, with 29 of 77 males (38%) and 32 of 49 females (65%) being seropositive. Although a higher percentage of female raccoons from both SC and GA were seropositive, no significant difference in prevalence was noted between sexes.

Information compiled from examination of locality data for triatomid bugs (*T. sanguisuga* and *T. lecticularia*) indicated that appropriate vectors for *T. cruzi* have been

collected from all five physiographic regions included in the present study. With 38 of 39 bugs in the collections being *T. sanguisuga*, three specimens were collected from the FH, three from the SC PD, seven from the GA PD, two from the UCP, one from the LCPN, and 22 from the LCPS regions. Of these specimens, four were collected while feeding on a person, while 10 of 22 from LCPS were collected from rats' nests on Seabrook Island where a 71% seroprevalence of *T. cruzi* in raccoons was observed. The one specimen of *T. lecticularia* was collected from inside a home on a bed in the UCP region (Kershaw Co.).

DISCUSSION

Data collected for this study provide the first comprehensive serological examination of raccoons for infection with *T. cruzi*. Antibodies to *T. cruzi* were detected in 47% of raccoons tested in this study, which demonstrates a prevalence that is higher than results of culture data for raccoons from the neighboring states of North Carolina and Georgia (Karsten et al., 1992; Pung et al., 1995). Results reported from prior studies have demonstrated the appropriate use of IFAT for detection of *T. cruzi* in wildlife, due to high specificity (Camargo, 1966) and greater sensitivity than blood smears or culture (Yabsley et al., 2001). Only one other IFAT study with wildlife (coyotes) has been completed in the United States, with 19 of 134 coyotes from Texas being seropositive (Grögl et al., 1984). Other serological tests have been used to a limited extent in testing wildlife species (e.g., armadillos, badgers, coyotes) and dogs in the USA. Although Yaeger (1988) used the direct agglutination test for detection of *T. cruzi* infections in armadillos from Louisiana, several factors limit the usefulness of this assay for large surveys, such as expense, an additional technical step (involving use of 2-mercaptoethanol), and difficulty in reading results (see Luquetti, 1990). In a study from Texas, Burkholder et al. (1980) used the in-

direct hemagglutination assay (IHA) to demonstrate the presence of *T. cruzi* in coyotes, dogs, cattle, sheep, and badgers. However, the low sensitivity of IHA has been questioned (see Luquetti, 1990; Lauricella et al., 1993). When dogs from a southern Louisiana rural environment were tested for prevalence of *T. cruzi* by both culture ($n = 188$) and ELISA ($n = 464$), all dogs tested by culture were negative, whereas ELISA testing indicated seroprevalences of 4.7% for roaming rural dogs ($n = 85$), 2.3% for dogs from an animal shelter ($n = 176$), 2% for housed rural dogs ($n = 103$), and 4% for urban housed dogs ($n = 100$) (Barr et al., 1991b).

Although cross-reaction between *T. cruzi* and other closely related kinetoplastid flagellates (*Leishmania* spp. and *Trypanosoma* spp.) has been documented (Dusanic, 1991), few reports have identified *Leishmania* spp. as being present in wildlife in the USA (Arizona and Texas) (Kerr et al., 1995, 1999). In addition, no infections have been reported in wildlife from South Carolina or neighboring states. Most recently, serum samples ($n = 96$) collected from coyotes and red and gray fox trapped in Georgia, South Carolina, North Carolina, Tennessee, and Virginia were all negative for *Leishmania* spp. antibodies when tested by IFAT; an additional 161 animals translocated from central and western states (Illinois, Indiana, Ohio, Wyoming) also tested negative (P. M. Schantz, pers. comm.). Although canine leishmaniasis has been identified by IFAT testing for 21 states and southern Canada, all reports of seroprevalence were from kennel hunting dogs (P. M. Schantz, pers. comm.). Because of potential cross-reactivity, one cannot unequivocally state that positive results of the current study were solely due to *T. cruzi*. However, isolation of *T. cruzi* from South Carolina and Georgia raccoons (unpubl. data; Pung et al., 1995) and current lack of evidence for the presence of cross-reactive organisms in southeastern wildlife lend support to these positive results.

Raccoons from three SC trapping sites

(Bostick Plantation, John's Island and Seabrook Island) located in the LCPS region had the highest prevalences of *T. cruzi*. Bostick Plantation, where 15 of 25 raccoons (60%) were seropositive for *T. cruzi*, is a large hunting plantation located in Hampton County that borders the Savannah River. This plantation is primarily pine/hardwood forest and swamp, both of which are prime raccoon habitats. Interestingly, the highest prevalence found in SC (with 17 of 24 or 71% raccoons infected) was on the heavily populated private barrier island known as Seabrook Island resort. Similarly, for a Georgia barrier island (St. Catherine's Island in Liberty County), a high prevalence of 66% was reported in a prior study (Yabsley et al., 2001). Both large barrier islands are separated from the mainland by water at all times, effectively creating populations of raccoons and bugs that are isolated and possibly increasing the chance for interaction between the two. However, in the current study, a high prevalence of 67% was detected on another large SC barrier island (John's Island) that is connected to the mainland during low tide.

High regional prevalences of 52% and 61% as observed in raccoons from coastal locations may be related to the distribution of bug vectors, which in turn may be influenced by the more temperate climate in humid coastal regions (from Allendale county south for SC). As noted by Pippin (1970), the life cycle of *T. sanguisuga* in Texas averaged 24 to 30 mo, depending upon availability of food and temperature. During winter months, the bugs entered an inactive (nonfeeding) state until warm temperatures returned. In the coastal regions of SC, with few freezing days per year, increased interactions between bug vectors and raccoons may occur over a greater period of the year. Further, higher densities of raccoons in coastal regions may increase the chance of contact between raccoons and infected bugs. For SC raccoon populations, estimates of densities for the FH and PD regions suggest one

raccoon per 8 to 81 h, while densities for coastal regions are estimated to be one raccoon per 2 to 12 ha (South Carolina Department of Natural Resources, 2000). Consequently, over wintering of bugs as adults in animal burrows or nests in coastal regions, plus availability of hosts due to higher densities, may increase survivability of larger numbers of bugs and transmission to raccoons in such areas.

The overall seroprevalence of 47% for raccoons infected with *T. cruzi* as reported in this study is higher than prevalences reported for a variety of wildlife from the southern United States (see John and Hoppe, 1986; Pietrzak and Pung, 1998). Yet one can only speculate as to potential impact on raccoon population dynamics. Although pseudocysts of parasites have rarely been reported from histological examination of wildlife heart tissue, mild myocarditis similar to that found in chronic Chagas' cases in dogs and humans has been reported. In histological studies by Barr et al. (1991a) and Pietrzak and Pung (1998), pseudocysts in hearts were observed in six of 45 opossums and one of 30 raccoons, respectively. Examination of the tongue, diaphragm, intercostals, quadriceps, and brain from 30 of the 45 opossums revealed only one pseudocyst in the tongue of only one animal. Despite low numbers of pseudocysts, both the raccoon and opossums had mild myocarditis. For dogs experimentally infected with an opossum- or armadillo-isolate of *T. cruzi*, acute myocarditis was observed which progressed to chronic bilateral ventricular failure (Barr et al., 1991c). And dogs naturally infected with *T. cruzi* have been reported to exhibit mild multifocal to diffuse severe granulomatous myocarditis (Williams et al., 1977).

In Central and South America, humans and animals become infected when the bug vector passes the infective form of the parasite in feces while feeding on host blood. Parasites then enter the host through mucus membranes or a wound (e.g., the wound made by the feeding

bug). In contrast to the South American species of *Triatoma*, the two species of triatomid bugs in SC and GA (*T. sanguisuga* and *T. lecticularia*) that are known vectors for *T. cruzi* exhibit delayed defecation following ingestion of a blood meal, resulting in a decreased chance of autochthonous human infection (Pippin, 1970). However, ingestion of infected bugs by wildlife or dogs (in response to irritation due to a bug's bite) may result in infection (Yaeger, 1971). Other possible modes of infection for wildlife in the southeastern United States, such as transplacental and milk transmission as reported for humans and a number of experimentally infected animals (see Teixeira, 1987), may contribute to the high prevalence of *T. cruzi* in raccoons.

Despite the high prevalence of *T. cruzi* in the wildlife, humans in the United States apparently are at low risk of infection due to the delayed defecation time of local vectors (Pippin, 1970) and better housing conditions and insect control. However, infections do occur, as was evidenced by the recovery of an infected adult *T. sanguisuga* from the crib of an infant in rural Tennessee who later presented with infection confirmed by polymerase chain reaction (Herwaldt et al., 2000). Interestingly, this family's dog was infected with *T. cruzi* (IFAT titer of 1:1024), which is consistent with a report of an increased number of dog infections in recent years (Meurs et al., 1998). And, as residential housing subdivisions continue to advance into forested wildlife habitats, both humans and their pets (e.g., dogs) may be at a greater risk of infection due to potential exposure to bugs that feed on infected reservoir hosts such as raccoons.

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