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RACCOONS (*PROCYON LOTOR*) SHOW HIGHER *TRYPANOSOMA CRUZI* DETECTION RATES THAN VIRGINIA OPOSSUMS (*DIDELPHIS VIRGINIANA*) IN SOUTH CAROLINA, USA

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ABSTRACT: Chagas disease, a significant public health concern in the Americas, is caused by a protozoan parasite, *Trypanosoma cruzi*. The life cycle of *T. cruzi* involves kissing bugs (*Triatoma* spp.) functioning as vectors and mammalian species serving as hosts. Raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) have been identified as important reservoir species in the life cycle of *T. cruzi*, but prevalence in both species in the southeastern US is currently understudied. We quantified *T. cruzi* prevalence in these two key reservoir species across our study area in South Carolina, US, and identified factors that may influence parasite detection. We collected whole blood from 183 raccoons and 126 opossums and used PCR to detect the presence of *T. cruzi*. We then used generalized linear models with parasite detection status as a binary response variable and predictor variables of land cover, distance to water, sex, season, and species. Our analysis indicated that raccoons experienced significantly higher parasite detection rates than Virginia opossums, with *T. cruzi* prevalence found to be 26.5% (95% confidence interval [CI], 20.0–33.8) in raccoons and 10.5% (95% CI, 5.51–17.5) in opossums. Overall, our results concur with previous studies, in that *T. cruzi* is established in reservoir host populations in natural areas of the southeastern US.

Key words: Chagas disease, disease ecology, reservoir host, wildlife disease.

INTRODUCTION

Trypanosoma cruzi, a flagellate protozoan parasite, has been identified as the etiologic agent that causes Chagas disease in humans. An estimated six million people are currently infected with Chagas disease across the Americas, with the highest disease prevalence reported in Latin America (Kirchhoff 2011; Bern et al. 2019). In the US, an estimated 300,000 people currently have Chagas disease, with the majority of cases probably in immigrants from places with high Chagas disease prevalence, such as Latin America (Busselman and Hamer 2022). Autochthonous human infection in the US is thought to be rare, due to lower vector colonization of housing dwellings; however, there is little medical and research attention on this disease in the US; therefore, cases may be underestimated (Bern et al. 2011; Busselman and Hamer 2022). Individuals with chronic Chagas disease have the potential to develop life-threatening abnormalities of the heart and gastrointestinal tract (Bern et al. 2019).

During its life cycle, *T. cruzi* colonizes host, reservoir, and vector species, while assuming three distinct morphologic forms (Kirchhoff 2011). Kissing bugs (*Triatoma* spp.) are the most common vectors of Chagas disease and expose new hosts to *T. cruzi* when they eat a blood meal and defecate; infected material may then enter the host via mucous membranes

or broken skin (e.g., the bite site). Transmission can also occur vertically, by a blood-borne route, or orally via ingestion of infected kissing bugs or contaminated materials (Klotz et al. 2014; Gürtler and Cardinal 2015; Santana et al. 2020). There are 11 species of kissing bugs in the US, with distributions spanning 27 southern states from the west to the east coast (Busselman and Hamer 2022).

Reservoir hosts are important in the transmission cycle of *T. cruzi* because they maintain persistence of pathogens in the absence of human hosts (Roberts and Heesterbeek 2020). Virtually all mammals are susceptible to *T. cruzi* infection, although some species may have higher reservoir potential: the relative contribution made by a host to the infection potential of a vector (Mather et al. 1989; Hodo and Hamer 2017). Virginia opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*) have been identified as important reservoir species for Chagas disease in the US, inducing concern of pathogen spillover to human hosts due to use of urban habitats (Brown et al. 2010; French et al. 2022). Both species have high seroprevalence rates of *T. cruzi,* with an estimated aggregate prevalence across the southern US of 22.9 and 36.4% for opossums and raccoons, respectively (Hodo and Hamer 2017). Opossums may also serve as a vector for Chagas disease, because anal gland secretions of infected opossums have been found to contain *T. cruzi* parasites (Deane et al. 1984; Bern et al. 2019; Zecca et al. 2020). One study (Lenzi et al. 1984) has suggested that opossum feces could contain parasites, causing concern for oral disease transmission to other mammalian species. The broad distributions of opossums and raccoons across the US and the range expansions northward (Walsh and Tucker 2018; Louppe et al. 2019) may mean that *T. cruzi* is a concern across a greater area of the US than previously recognized.

Landscape factors can influence transmission dynamics of zoonotic pathogens by inducing changes in diversity, abundance, and density of reservoir hosts (Gottdenker et al. 2012). Population densities of raccoons and opossums have been shown to differ among landscapes (Prange et al. 2003; Rosatte et al. 2010; Beatty et al. 2016; Slate et al. 2020; Bernasconi et al. 2022). In natural areas with minimal human influence, higher population densities of both opossums and raccoons are found in bottomland swamps, riparian forests, and habitats with permanent water compared with upland pine habitats (Weckerly et al. 1987; Leberg and Kennedy 1988; Bernasconi et al. 2022). However, few studies have specifically investigated the effect of land cover on *T. cruzi* infection risk in these reservoir species. A study of raccoons in eastern Tennessee, US, from 2005 to 2007 did not find any link between land cover classes and *T. cruzi* seropositivity status, but they did find significantly higher seroprevalence rates in raccoons living in rural compared with urban habitats, possibly due to increased denning habitat in rural areas, where raccoons are likely to encounter kissing bug vectors (Maloney et al. 2010). Raccoons found in coastal regions exhibited higher *T. cruzi* seroprevalence rates than those found in inland regions of South Carolina and Georgia, US, possibly due to the higher raccoon population densities and reduced occurrence of freezing temperatures associated with coastal regions (Yabsley and Noblet 2002). Studies characterizing the influence of land cover on *T. cruzi* infection risk in Virginia opossums appear to be lacking.

Prevalence of *T. cruzi* varies among reservoir species, likely due to differences in infection dynamics (Hodo and Hamer 2017). Although most mammal species are susceptible to *T. cruzi* infection, some species are more prone to infection than others, due to differences in contact rates with vectors, host susceptibility, and strength of parasitemia, or parasite load (Roellig et al. 2009; Gürtler and Cardinal 2015). Within these reservoir species, females have been shown to experience significantly higher infection rates than males (Brown et al. 2010; Bern et al. 2019), with one

study indicating that female adult raccoons were almost 100 times more likely to be seropositive than male adult raccoons (Maloney et al. 2010). Females may experience increased contact rates with triatomine bugs while denning and overwintering with kits, a behavior not exhibited by males (Maloney et al. 2010; Bern et al. 2019).

Our aim was to characterize *T. cruzi* prevalence in raccoons and opossums, two key Chagas disease reservoir species in South Carolina, southeastern US. We investigated whether landscape and demographic factors such as land cover composition, distance to permanent water, sex, season, and species influenced parasite detection in these two species. We hypothesized that land cover type would have the strongest influence on parasite prevalence, specifically that *T. cruzi* prevalence would be higher in raccoons and opossums found in habitats close to a water source or in woody wetland habitats because these features are associated with increased population densities in both species. We also predicted that females of both species would experience higher rates of *T. cruzi* detection than would males.

MATERIALS AND METHODS

Study area

This study was conducted on the Savannah River Site (SRS), a 782-km^2 property managed by the US Department of Energy in Aiken, Barnwell, and Allendale Counties of South Carolina, US (Fig. 1). After this site had been acquired by the US Department of Energy in 1950, pine forests were planted among preexisting deciduous forests. These pine forests mainly consist of longleaf pine (*Pinus palustris*), loblolly pine (*Pinus taeda*), and slash pine (*Pinus elliottii*) and are managed for timber production. During our study, the SRS included 57% pine forest, 21% hardwood forest, 6% mixed forest, and 16% other (industrial use, roads, and lakes). The pine forests were managed with fire, and approximately 100,000 m^3 of timber are harvested annually from the SRS. The landscape has an average elevation of 200 m above sea level and is interspersed with 370 Carolina bays (0.027 ± 0.065 km^2; mean±SD), which are small depressions that are periodically water filled. The SRS has a subtropic climate with winter (December–February), spring (March–May), summer (June–August), and fall (September–November) temperatures averaging 9, 17, 26, and 18 C, respectively. Mean annual rainfall on the SRS is 120 cm. The Savannah River borders the western side of the property and is characterized by a shallow, wide bathymetric profile (White and Gaines 2000).

Animal capture and sample collection

From January 2018 to November 2019, opossums and raccoons were trapped continuously in representative sites within the SRS (Fig. 1). We captured individuals using live-capture box traps (model 108SS, Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) placed in a rectangular grid pattern (5×5 or $6 \times 4+1$) with 100-m spacing between traps. Traps were baited with whole-kernel corn and plaster tabs soaked in fish oil as a scent lure (Webster and Beasley 2019). We replaced plaster tabs after every capture event, following major rainstorms, or after 5 d of inactivity. We replaced corn when it was consumed by target or nontarget species. Because of the logistic difficulty imposed by the size of the combined study area (782 km^2), we divided the number of actively sampled sites into three groups of eight sites equally representing land cover types: bottomland hardwood, upland pine, riparian and isolated wetland. Following trapping sessions, we relocated traps to the next group of eight sites. After we had trapped all 24 sites for 10 d, we repeated the cycle for a second trapping session in each of the three trapping grid groups. The time between the end of the first trapping session and the start of the second trapping session for a given site ranged from 10 d to 50 d (mean 35 d).

All capture and handling methods were conducted as outlined in Beasley et al. (2012) and Beatty et al. (2014). Following capture, we hand injected tiletamine-zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) intramuscularly at a dosage of 5 mg/kg of estimated body mass to immobilize both opossums and raccoons (Gamble 2004; Kreeger and Arnemo 2018). Under anesthesia, we recorded the sex of each individual and collected whole blood from opossums using venipuncture of the caudal vein and from raccoons via venipuncture of the jugular vein and stored samples in lithium heparin blood tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey,

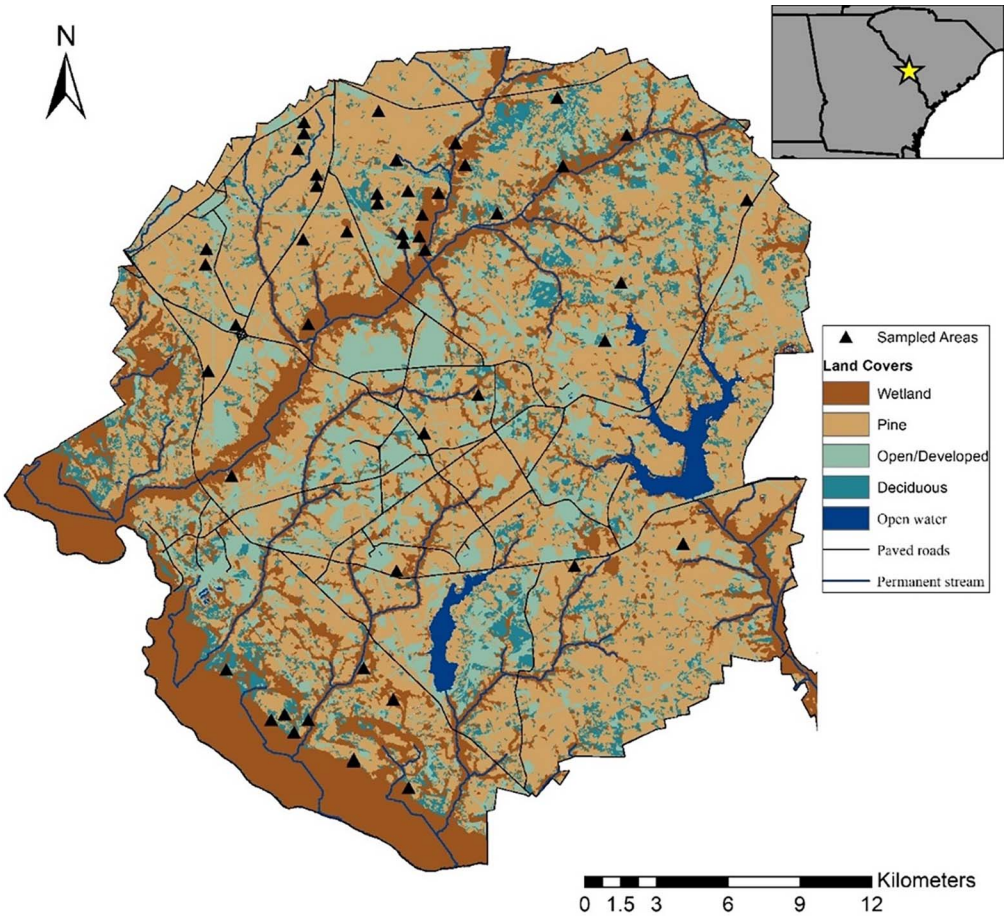

FIGURE 1. Map of the Savannah River Site, Aiken, South Carolina, USA, which served as the site for collection of raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) from January 2018 to November 2019 for the detection of *Trypanosoma cruzi*. The figure exhibits the five grouped National Land Cover database 2016 land cover classes (Dewitz 2019) used in this study, major roads and streams, and capture sites of live-trapped raccoons and opossums. The inset map shows the location of the study site, close to the southern border of South Carolina with Georgia, USA.

USA) at −80 C (Williams-Newkirk et al. 2013). We attached matching numeric ear tags (Monel 3, National Band and Tag Company, Newport, Kentucky, USA) to both ears of each captured animal. In the event of a recapture, we resampled the individual only if its previous capture event was more than 1 mo earlier. All trapping and handling activities were conducted in accordance with the University of Georgia Animal Care and Use Guidelines under Animal Care and Use (protocol A2018 06-024-A12).

Parasite detection

Molecular protocols followed Charles et al. (2013) for detection of the D7 divergent domain of the 24Sα rDNA gene in *T. cruzi*. We extracted DNA from 100 µL of whole blood using the DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, California, USA) according to the manufacturer's protocol. Genomic DNA for each sample was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and DNA concentrations were standardized (20 µg/mL) before further processing. The standardized genomic DNA was used in a nested PCR, which amplified the D7 divergent domain of the 24Sα rDNA gene of *T. cruzi* using D75 5′-GCAGATCTTGGTTGGCGTAG-3′ and D76 5′-GGTTCTCTGTTGCCCCTTTT-3′ primers (Briones et al. 1999) in the primary reaction, followed

by D71 5′-AAGGTGCGTCGACAGTGTGG-3′ and D72 5′-TTTTCAGAATGGCCGAACAGT-3′ primers in a secondary reaction (Souto et al. 1996). We included blanks of deionized water in each set of DNA extractions and PCR reactions as negative controls. As a positive control, we used the reagent *T. cruzi*, strain G (*T.* cruzi, strain G, NR-49382 BEI Resources, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Manassas, Virginia, USA). We sent five samples for sequencing using the primers D72 and D76 (Eurofins Genomics, Louisville, Kentucky, USA) and confirmed that the sequences matched *T. cruzi* with the basic local alignment search tool (Altschul et al. 1990). We then visualized 125- or 110-base pair 24Sα amplicons by transillumination of a SYBR Green (Invitrogen, Carlsbad, California, USA) stained with 2.0% agarose gel for all samples.

Landscape data

To characterize landscape composition around our trapping sites, we used the 2016 National Land Cover database (NLCD) downloaded from the Multi-Resolution Land Characteristics Consortium website (Dewitz 2019) and ArcMap version 10.6 (Esri 2016). Based on predominant land cover types of the SRS and land cover types previously described as biologically important to opossums and raccoons (Weckerly et al. 1987; Leberg and Kennedy 1988; Bernasconi et al. 2022), we grouped the 15 original land cover designations in the NLCD into five categories: open water; woody and herbaceous wetlands; pine; upland deciduous; and open or developed (see Supplementary Material Table S1). We created a 1-km buffer around each capture site and calculated the percentage of land cover for each of the five categories using FRAGSTATS version 4.2 (McGarigal and Marks 1995). In addition, we extracted the distance (meters) from the capture site to the nearest source of permanent water (lakes, ponds, and streams) using the Euclidean distance tool in ArcMap (2016).

Statistical analysis

All analyses were carried out using R version 3.6.2 (R Core Team 2022). We *z*-transformed numeric independent variables to speed convergence and aid interpretation of regression coefficients. We then tested for correlations among all numeric independent variables to determine if we needed to exclude any from future analysis to prevent multicollinearity. We used the Pearson correlation coefficient and a cut-off value of $r=0.70$. Based on our findings, we excluded pine land cover because it had a strong negative correlation ($r=-0.92$) with woody and herbaceous wetlands, a variable that we kept in the analysis due to its biologic importance as preferred habitat of raccoons and opossums (Weckerly et al. 1987; Leberg and Kennedy 1988; Bernasconi et al. 2022).

We modeled *T. cruzi* detection status as a function of landscape and demographic variables using generalized linear models (GLM) with binomial error and a logit link function from the package *stats* (R Core Team 2022). The full model included detection status as a binary response variable and the following as predictor variables: meteorologic season; percentage of woody or herbaceous wetland land cover; percentage of upland deciduous land cover; percentage of open or developed land cover; percentage of open water land cover, distance from permanent water (meters); species; and sex. We included the interaction between species and sex, as well as the interaction between each landscape variable and species to account for any differences in land cover preference between raccoons and opossums.

To quantify goodness of fit, model residuals were plotted with the *DHARMa* package (Hartig 2021) using a QQ residual plot, a plot of standardized residuals against model predictions, a nonparametric dispersion test, and a zero-inflation test. We compared null and predictive models using the dredge function in the package *MuMIn* (Bartoń 2022) and Akaike information criterion (AIC), an information criteria-based relative fit index; the AICc is a fit index adjusted for a small sample size. Specifically, AICc and AIC weights were used as determinants of model fit, and we considered models that were <2 AICc units within the top model to be competitive. If two models were competitive with the top model based on AICc, we followed the principle of parsimony, where the simpler explanation and model is more likely to be correct (Leroux 2019). We also calculated the estimated probability of parasite detection for both species from the top model using the R package *emmeans* (Lenth 2022).

TABLE 1. Results of PCR of 115 Virginia opossum (*Didelphis virginiana*) and 170 raccoon (*Procyon lotor*) unique blood samples to detect the presence of *Trypanosoma cruzi* parasite collected between January 2018 and November 2019 at the Savannah River Site, Aiken, South Carolina, USA.

Species	Year	Sex[a]	No. samples tested	No. positive and unique individuals	Prevalence (%)
Raccoon	2018	F	26	7/25	28
	2018	M	70	17/68	25
	2019	F	18	3/18	17
	2019	M	69	20/69	29
Opossum	2018	F	31	3/27	11
	2018	M	39	2/34	6
	2019	F	20	4/19	21
	2019	M	36	3/35	9

[a] M = male; F = female.

RESULTS

We collected 309 whole blood samples from 285 unique opossums and raccoons between January 2018 and November 2019 at the SRS. Nested PCR assays confirmed the presence of *T. cruzi* in 59 of the 309 samples. Overall, 10.4% (95% [CI], 5.51–17.5) of unique opossums and 26.5% (95% CI, 20.0–33.8) of unique raccoons tested positive at least once throughout the study duration. Within unique raccoons, 26.5% (95% CI, 19.2–34.9) of males and 26% (95% CI, 13–43) of females tested positive for *T. cruzi* at least once (Table 1). Prevalence of *T. cruzi* in unique male opossums was 7% (95% CI, 2–16) and 15% (95% CI, 6–29) in unique female opossums.

We captured 309 adults (age estimated $\geq$1 yr), and raccoons accounted for 170 unique animals (38 females, 132 males) and 183 whole blood samples (44 females, 139 males; Table 1). Opossums accounted for 115 unique animals (45 females, 70 males) and 126 whole blood samples (51 females, 75 males). All opossum resampling events occurred within the same years (n=11); however, most raccoon resampling events (n=13) occurred in subsequent years (n=10) rather than within years (n=3).

Analysis of GLM models examining *T. cruzi* detection status as a function of land cover proportions, habitat type, sex, season, and species produced 2,428 candidate models with eight competing models (Table 2). Parameters of candidate models are listed in Table 3. We chose the top model to be the model, including only species as a predictor variable. All models had nearly identical log likelihoods, and the CIs of additional parameters in the other models overlapped zero, indicating that these additional parameters are likely uninformative (Leroux 2019). The estimated probability of detection ($\pm$SE) for raccoons and opossums from the top model was 0.26$\pm$0.032 and 0.10$\pm$0.026, respectively (Fig. 2).

DISCUSSION

We investigated factors that influence *T. cruzi* prevalence in opossums and raccoons, two key Chagas disease reservoir species in the southeast US. Our findings revealed that only species, and not land cover, sex, or season, was a significant predictor of *T. cruzi* parasite detection in the individuals sampled. The prevalence of *T. cruzi* infection at our study site, 10.5% (95% CI, 5.51–17.5) and 26.5% (95% CI, 20.0–33.8) for opossums and raccoons, respectively, is similar to prevalence found in other states of the southeastern US. For example, hemoculture of blood collected from opossums and raccoons from 1992 to 1994 in six southeast Georgia counties found similar *T. cruzi* prevalence rates of 15 and 22.2%, respectively (Pung et al. 1995). In the Piedmont region of North Carolina, US,

TABLE 2. Set of eight top generalized linear models testing the effect of landscape and demographic variables on *Trypanosoma cruzi* detection in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) at the Savannah River Site, Aiken, South Carolina, USA, between January 2018 and November 2019. Models are ordered by Akaike information criterion with small sample correction (AICc) values, and all models shown are competitive (within 2 AICc units of the top model).

Model rank	Model structure	Df	AICc[a]	ΔAICc[b]	AICcWeight[c]	LogLik[d]
1	Species	2	291.8	0	0.233	−143.9
2	Species+open/developed+open/developed:species	4	292.2	0.427	0.188	−142.1
3	Species+deciduous	3	293.3	1.48	0.111	−143.6
4	Species+season+open/developed+open/developed: species	7	293.5	1.69	0.100	−139.6
5	Species+open/developed	3	293.5	1.74	0.0976	−143.7
6	Species+deciduous+open/developed+open/developed:species	5	293.6	1.80	0.0947	−141.7
7	Species+distance to permanent water	3	293.8	1.96	0.0875	−143.8
8	Species+sex	3	293.8	1.96	0.0874	−143.8

[a] AICc = Akaike information criterion with small sample correction.
[b] ΔAICc = change in AICc.
[c] AICcWeight = AICc weight, the relative likelihood of a model.
[d] LogLik = logarithm of likelihood estimation.

T. cruzi prevalence detected by hemoculture was 8.3% in opossums and 15% in raccoons (Karsten et al. 1992). A comprehensive meta-analysis of Chagas prevalence in reservoir species across 13 southern US states estimated aggregate prevalence of 22.9% for opossums and 36.4% for raccoons, which is slightly higher than in our study (Hodo and Hamer 2017).

Comparing *T. cruzi* prevalence in reservoir species across studies is challenging due to the varying levels of specificity and sensitivity of diagnostic techniques (Bern et al. 2019). Reported seroprevalence of our focal species vary widely in the US, ranging from 15% to 90% in raccoons and from 8% to 33% in opossums (Bern et al. 2019). The combination of diagnostic methods used to generate aggregate *T. cruzi* prevalence in Hodo and Hamer (2017) included blood cultures, serology, blood smear analyses, histopathology, and PCR of tissue collected from multiple organs and resulted in detection of chronic infection, acute infection, and previous infections. Thus, it is possible that the lower prevalence found in our study compared with aggregate prevalence reported in Hodo and Hamer (2017) might be due to our blood PCR diagnosis methods, which detect only acute infections and transient parasitemia, not previous infection or nonviable parasites (Bern et al. 2011; Hodo and Hamer 2017; Zecca et al. 2020).

The difference in *T. cruzi* prevalence between raccoons and opossums found in our study could be driven by species-specific infection dynamics. *Trypanosoma cruzi* currently has seven recognized discrete typing units (DTUs; Bern et al. 2019; Busselman and Hamer 2022). Evidence suggests that opossums are able to be infected by one DTU (TcI), whereas raccoons are able to be infected by three DTUs (TCI, TCII, and TCIV; Roellig et al. 2009; Bern et al. 2019). Most species of the kissing bug vector (*Triatoma* spp.) in the southern US carry *T. cruzi* DTUs with near equal frequency (Curtis-Robles et al. 2018). Although we did not determine the DTU of our positive samples, it is possible that raccoons are exposed to more strains of *T. cruzi*, despite encountering the same number of infected vectors as opossums, providing more opportunities for infection. In addition, raccoons have been associated with more species of kissing bugs than opossums, (n=11 and n=8, respectively), which offers higher chance of contact with an infected vector (Bern et al. 2019). *Triatoma sanguisuga*

TABLE 3. Parameter values for the top generalized linear models (within <2 Akaike information criterion with small sample correction (AICc) units of the top model) used to test the effect of landscape and demographic variables on *Trypanosoma cruzi* detection in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) at the Savannah River Site, Aiken, South Carolina, USA, between January 2018 and November 2019.

Model no.	Model term	Estimate	SE	2.5% lower confidence interval (CI)	97.5% upper CI	Z value	*P*-value[a]
1	Intercept	−2.25	0.303	−2.90	−1.70	−7.42	1.18e-13***
	Species (raccoon)	1.19	0.347	0.537	1.91	3.42	0.000623***
2	Intercept	−1.79	0.427	−2.70	−1.01	−4.20	2.65e-05***
	Open/developed land cover	−0.062	0.0486	−0.171	0.0228	−1.28	0.199
	Species (raccoon)	0.48	0.500	−0.467	1.51	0.965	0.335
	Species: open/developed land cover	0.089	0.00527	−0.00562	0.204	1.70	0.0901
3	Intercept	−2.10	0.366	−2.86	−1.41	−5.73	9.89e-09***
	Species (raccoon)	1.19	0.348	0.540	1.91	3.43	0.000605***
	Deciduous land cover	−0.0141	0.0191	−0.0533	0.0219	−0.738	0.460
4	Intercept	−1.19	0.604	−2.43	−0.426	−1.96	0.0497
	Open/developed land cover	−0.0760	0.0506	−0.188	0.0129	−1.50	0.135
	Species (raccoon)	0.490	0.526	−0.509	1.57	0.931	0.352
	Season (spring)	−0.462	0.493	−1.41	0.543	−0.937	0.350
	Season (summer)	−0.398	0.678	−1.78	0.920	−0.587	0.557
	Season (winter)	−1.01	0.510	−2.00	0.0172	−1.99	0.0467
	Species: open/developed land cover	0.107	0.0550	0.00836	0.226	−1.95	0.0513
5	Intercept	−2.34	0.346	−3.07	−1.70	−6.76	1.4e-11***
	Species (raccoon)	1.19	0.348	0.537	1.91	3.42	0.000626***
	Open/developed land cover	0.00992	0.0181	−0.0263	0.0448	0.549	0.583
6	Intercept	−1.50	0.554	−2.62	−0.437	−2.71	0.000675***
	Deciduous land cover	−0.0171	0.0209	−0.060	0.0225	−0.819	0.413
	Open/developed land cover	−0.0747	0.0521	−0.191	0.0163	−1.43	0.152
	Species (raccoon)	0.428	0.509	−0.543	1.47	0.841	0.400
	Species: open/developed land cover	0.0965	0.0547	−0.00152	0.216	1.76	0.0780
7	Intercept	−2.18	0.390	−2.99	−1.45	−5.59	2.26e-08***
	species (raccoon)	1.19	0.348	0.539	1.91	3.43	0.000611***
	Distance permanent water	−9.48e-05	3.37e-04	−0.000772	0.000552	−0.282	0.778
8	Intercept	−2.20	0.358	−2.95	−1.54	−6.13	8.73e-10***
	Species (raccoon)	1.20	0.352	0.543	1.93	3.42	0.000627***
	Sex	−0.0927	0.330	−0.729	0.572	−0.281	0.779

[a] *** = 0.001.

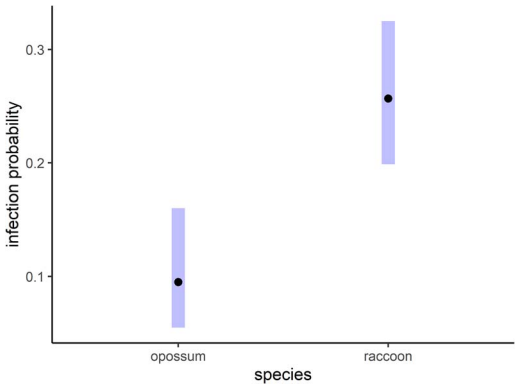

FIGURE 2. Estimated detection probability of *Trypanosoma cruzi* in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) at the Savannah River Site, Aiken, South Carolina, USA, during our study period (January 2018–November 2019). Probabilities are shown as black dots and were generated from our top generalized linear model, which contained only species as a covariate. Blue shading indicates a 95% confidence interval.

and *Triatoma lecticularia* are the two species of kissing bugs that have been reported in South Carolina; however, it is possible that more species are present but have not yet been detected (Klotz et al. 2014). Host population density can influence the risk of *T. cruzi* infection via changes in contact rates with kissing bug vectors (Oda et al. 2014; Botto-Mahan et al. 2020). Throughout our study area at the SRS in Aiken, South Carolina, raccoons occur at higher population densities than opossums (Helton 2021). Thus, it is possible that the higher population densities of raccoons in our study site enabled kissing bug vectors to move more easily and spread *T. cruzi* parasites among raccoon hosts as opposed to the lower population density opossums.

Our analysis did not find that landscape influenced *T. cruzi* prevalence in raccoons or opossums; however, it is possible that sampling in our study occurred at too small of a spatial scale with too few land cover types represented to detect a significant influence of landscape on *T. cruzi* prevalence. For example, Yabsley and Noblet (2002) compared seroprevalence of *T. cruzi* in raccoons across five physiographic regions and two states in the US and found significant differences in detection rates among regions, with the highest prevalence found in coastal regions. Future research examining *T. cruzi* prevalence in reservoir species would benefit from comparing areas where animals are most likely to contact humans, such as urban and agricultural habitats, to natural habitats simultaneously. This will elucidate how anthropogenic factors influence the prevalence of *T. cruzi* in reservoir species and help to identify high-risk areas of parasite transmission to humans. Determining *T. cruzi* prevalence in reservoir species throughout the US will help to identify Chagas disease risk in humans and aid in focusing public health intervention efforts.

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SUPPLEMENTARY MATERIAL

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