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TRYPANOSOMIASIS IN RACCOONS FROM GEORGIA

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ABSTRACT: *Trypanosoma cruzi* frequently infects wild mammals in the southern United States but little is known about the effect of the parasite on reservoir hosts such as the raccoon (*Procyon lotor*). To investigate this issue, 30 raccoons trapped on St. Catherine's Island (Georgia, USA) during September, 1994 were tested for *T. cruzi* infection by examination of wet mounts of fresh blood and by culturing blood in liver infusion tryptose medium. Thirteen animals (43%) were found to be infected with *T. cruzi*. Heart tissues from 10 of the infected raccoons and 4 uninfected raccoons were fixed, sectioned, stained and examined for the presence of parasites and evidence of tissue damage. One *T. cruzi* pseudocyst was found in cardiac tissue from the left ventricle of a female raccoon. In addition, *Sarcocystis* sp. sarcocysts and schizonts of *Hepatozoon* sp. were observed in heart tissue from seven of the *T. cruzi*-infected raccoons. Mild, multifocal and interstitial inflammation was observed in the heart tissues of all 10 of the infected animals. No evidence of *T. cruzi* pseudocysts or tissue damage was observed in heart tissue from C3H/HeJ mice infected with culture forms of the parasites isolated from raccoons. Our findings suggest that the *T. cruzi* parasites isolated from raccoons in Georgia are not pathogenic to this host or C3H/HeJ mice and may be of low virulence.

Key words: *Hepatozoon* sp., pathogenicity, *Procyon lotor*, raccoon, *Sarcocystis* sp., *Trypanosoma cruzi*, virulence.

INTRODUCTION

Trypanosoma cruzi is a hemoflagellate protozoan parasite and the agent of Chagas' disease. This zoonotic illness is a major cause of myocardiopathy among people living in Latin America where the parasite is most frequently transmitted by triatomine bugs. In the United States, autochthonous *T. cruzi*-infections of people are unusual (Kirchhoff, 1993) but, in the southern one half of the country, the parasite is often found in wild mammals and is occasionally reported in domestic animals (John and Hoppe, 1986). In Georgia (USA), four different species of mammals including raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), gray foxes (*Urocyon cinereoargenteus*), and striped skunks (*Mephitis mephitis*) have been identified as sylvatic reservoir hosts of *T. cruzi* (McKeever et al., 1958; Pung et al., 1995).

The nature of the pathogenic mechanisms responsible for *T. cruzi*-induced myocarditis are not fully understood, but the pathology induced by the parasite in heart tissue of humans infected in Latin America is well documented (Laranja et al., 1956; Rossi and Ramos, 1996). The effect of *T. cruzi* parasites from Latin America on various inbred strains of mice and the opossum (*Didelphis marsupialis*) also has been characterized (Rowland et al., 1992; Carreira, 1996). In contrast, less is known about the effect of *T. cruzi* parasites indigenous to the southeastern United States on wild animal reservoir hosts such as the raccoon. The present study was designed to address this issue by investigating the histologic appearance of lesions in wild raccoons naturally infected with *T. cruzi* and experimentally infected laboratory mice.

MATERIALS AND METHODS

Raccoons were trapped during September, 1994 on St. Catherine's Island; a 5,600 ha barrier island in Liberty County, Georgia (31°39′N, 81°09′W). Live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were baited with canned cat food in the afternoon and checked the next morning. Captured animals were anesthetized with an 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). Blood was drawn by cardiac puncture and stored in heparinized Vacu-

tainer® tubes (Beckton Dickinson, Rutherford, New Jersey, USA) at ambient temperature until examined in the laboratory (usually no more than 2 hr later). The raccoons were then killed with an intravenous injection of sodium pentobarbital (Sleep-A-Way; 2 ml/2.5 kg, Aveco, Fort Dodge, Iowa, USA) and necropsy examination was performed. Tissues were preserved in 10% buffered formalin.

Raccoons were determined to be infected with *T. cruzi* according to the following procedure. A wet mount of fresh blood (4 μl) was systematically scanned for motile trypomastigotes using a light microscope at 400× magnification until the entire slide had been examined (at least 5 min). In addition, blood (1 ml) from each animal was cultured in 9 ml of undefined liver infusion tryptose (LIT) medium (Powell and Kuhn, 1980) at 27 C in 25 cm^2 tissue culture flasks. LIT cultures were monitored for trypanosomatids every 2–3 wk over the course of 15 wk by examination on an inverted microscope at 100× magnification. Analysis of ribosomal RNA gene polymorphism of LIT culture parasites isolated from the raccoons trapped on St. Catherine's Island has confirmed their identification as *T. cruzi* (Clark and Pung, 1994).

Heart tissue and smooth muscle from the urinary bladders of 10 *T. cruzi*-infected raccoons (nine males and one female) and four uninfected raccoons (two males and two females) were processed using standard histological techniques and examined as follows. Briefly, tissues were dehydrated in ethanol after fixation, embedded in paraplast paraffin wax and sectioned at 6 μm thickness. Slides were stained with Harris Hematoxylin and Eosin Y using a Histomatic Slide Stainer (Model 172, Fisher Scientific, Pittsburgh, Pennsylvania, USA). For each tissue, five slides containing two to three tissue sections each were prepared. Each slide was systematically scanned for parasites using a light microscope at 400× and 1,000× (oil immersion) for a minimum of 5 min. To quantify inflammation, the number of inflammatory foci found in five random fields at 100× for each of the four chambers of the heart was recorded (20 fields total per raccoon). Representative slides of parasites from infected raccoons are deposited in the Harold W. Manter Laboratory (University of Nebraska, Lincoln, Nebraska, USA; HWML 39314–39316).

Eight-wk-old inbred female mice (C3H/HeJ; Jackson Laboratory, Bar Harbor, Maine, USA) were infected with *T. cruzi* isolated from seven different raccoons (two mice per isolate) by intraperitoneal injection of 5×10^5 washed, stationary phase LIT culture parasites in 0.5 ml of Hank's balanced salt solution. Control mice included two uninfected animals and two mice infected with Brazil strain *T. cruzi* parasites. Wet mounts of mouse tail blood were examined weekly for motile trypomastigotes as described above. Four months after infection, mice were killed with chloroform and blood from the heart was cultured in LIT medium. Mouse hearts were fixed, processed, stained and examined as described above.

The prevalence of parasites in tissues from different chambers of the raccoon hearts was compared using Cochran's Q test (Siegel, 1956). Student's *t*-test was used to compare the numbers of foci of inflammation in infected and uninfected animals. One way analysis of variance (ANOVA) was used to compare the numbers of inflammatory foci in the different sections of the hearts of *T. cruzi*-infected raccoons. Numerical values are reported as the mean value ± 1 SE.

RESULTS

Twenty five male and 5 female raccoons (average weight = 4.5 kg) were trapped on St. Catherine's Island. Thirteen of these animals (43%) were infected with *T. cruzi*. Trypomastigotes (200–9,400/ml blood) were observed in wet mounts of fresh blood from 4 of the raccoons (13%). Epimastigotes typical of *T. cruzi* appeared in LIT cultures of blood from 11 raccoons (37%). Histological examination of tissues from 10 raccoons infected with *T. cruzi* revealed little evidence of tissue parasitism. Only one small pseudocyst, containing <50 amastigotes, was detected. This pseudocyst was found in striated muscle tissue from the left ventricle of a 3.6 kg female raccoon. Pseudocysts were not found in tissue from the urinary bladders. Two other cardiotrophic parasites also were observed. Sarcocysts of *Sarcocystis* sp. and schizonts of *Hepatozoon* sp. were observed in heart tissue from seven of the *T. cruzi*-infected animals; two raccoons were infected with *Sarcocystis* sp. and *Hepatozoon* sp., two raccoons were infected with *Sarcocystis* sp. alone, and three with *Hepatozoon* sp. alone. Both of these parasites were found in tissue sections from the atria and ventricles of the raccoons. The prevalence of *Hepatozoon* sp. did not differ from one heart chamber to another ($\chi^2 = 3.0$, df = 3, $P > 0.05$) and neither did

the prevalence of *Sarcocystis* sp. ($\chi^2 = 2.0$, df = 3, $P > 0.05$).

Mild, multifocal and interstitial inflammation was the only evidence of pathology in the hearts of the raccoons infected with *T. cruzi*. No gross lesions were observed. The number of inflammatory foci in the hearts of the infected animals (mean number of foci per 20 random 100× microscope fields from four heart chambers of 10 raccoons = 9.5 ± 0.5) was greater than the mean number of foci observed in the uninfected raccoons (0.4 ± 0.8 foci; t = 10.8, df = 54, $P < 0.001$). Two distinct types of inflammatory foci were observed; scattered and clustered. Scattered foci consisted primarily of dispersed lymphocytes and macrophages spreading through large areas of the myocardium. Clustered foci appeared to be smaller in size than scattered foci and were composed of tightly packed lymphocytes and neutrophils. There were no significant differences between numbers of clustered foci of inflammation in tissue from the four chambers of the heart. Similarly there was no difference between the numbers of scattered foci from one chamber of the heart to another. There was no evidence of inflammation in tissues from the urinary bladder.

Parasites were not observed in the blood of C3H/HeJ mice experimentally infected with *T. cruzi* parasites isolated from raccoons. However, parasites did appear in LIT medium cultures of mouse blood obtained 4 mo after infection. Heart tissues taken from these mice 4 mo after infection were essentially normal and there was no evidence of tissue parasitism. In contrast, trypomastigotes ($\leq 1 \times 10^5$/ml) were observed in blood from control mice infected with Brazil strain *T. cruzi* during the first 3 wk of infection. *Trypanosoma cruzi* pseudocysts and severe myocarditis also were observed in the control mice.

DISCUSSION

Trypanosoma cruzi is not uncommon in raccoons in the southern United States. The prevalence of the parasite in raccoons trapped at various locations in this part of the country reportedly ranges from as low as 2% (McKeever et al., 1958) to as high as 62% (John and Hoppe, 1986). Using both LIT cultures and examination, by microscope, of fresh blood we found that at least 22% of raccoons in southeastern Georgia are infected (Pung et al., 1995). In the present study, 43% of the raccoons from St. Catherine's Island that we tested were infected. Direct comparison of our findings with those of other investigators is difficult because the sampling methods and detection methods used from study to study vary.

Based on our observations, it appears that the *T. cruzi* parasites which infect raccoons in Georgia are not pathogenic in this host. This interpretation is supported by the observations that tissue stages of the parasite were rare in the raccoons, raccoon heart tissues were inflamed only mildly, and there was no evidence of other forms of tissue damage typical of Chagas' disease or of *T. cruzi* infection of various laboratory animal models. The possibility that the parasites isolated from the raccoons are not pathogenic in other host species is supported by the fact that no pathology was observed in experimentally infected C3H/HeJ mice. In contrast, strains of *T. cruzi* from Latin America frequently induce severe myocarditis and death in C3H/HeJ mice (Rowland et al., 1992). Other studies suggest that *T. cruzi* isolated in the United States can be extremely pathogenic when injected into a different host species in the laboratory. For example, purebred beagles experimentally infected with *T. cruzi* isolated from wild Virginia opossums and nine-banded armadillos (*Dasypus novemcinctus texanus*) in Louisiana (USA) suffered severe myocarditis and death (Barr et al., 1991b). There is no way to determine how long the raccoons we examined were infected with the parasite. Consequently, the possibility exists that these animals were in the early, asymptomatic stage of the disease, which

may induce greater pathology later in the infection.

In addition to our study, others have examined the effect of autochthonous *T. cruzi* infection on raccoons from the United States. Walton et al. (1958) reported *T. cruzi* pseudocysts in heart tissues, but no clinical signs in naturally and experimentally infected raccoons from Maryland. Schaffer et al. (1978) did not observe tissue stages of the parasite in cardiac muscle of translocated, *T. cruzi*-infected raccoons from the southeastern United States. Neither of these investigations reported on the extent of inflammation in the heart tissues of these animals.

The effect of *T. cruzi* on opossums (*Didelphis marsupialis* and *D. virginiana*) has been examined. The pathology induced by *T. cruzi* parasites from both the United States and Latin America in these reservoir hosts is similar to what we observed in raccoons. For example, mild myocarditis, characterized by small, spreading aggregates of macrophages and lymphocytes, was detected in 22 *T. cruzi*-infected *D. virginiana* trapped in Louisiana (Barr et al., 1991a). Isolated pseudocysts were observed in six of these animals. Similarly, Zeledon et al. (1970) found few pseudocysts in tissues from 19 infected *D. marsupialis* trapped in Costa Rica. Mild inflammation without fibrosis was observed in 10 naturally infected *D. marsupialis* from Brazil (Carreira et al., 1996). Pseudocysts were observed in tissues, including the urinary bladder, from four of these animals.

We found that there were greater numbers of inflammatory foci in the *T. cruzi*-infected raccoons than in the uninfected animals. We can not state with certainty that the inflammation was due to *T. cruzi* because the animals we examined were wild caught and infected with at least two other species of cardiotrophic organisms. However, the scattered foci observed consisted of lymphocytes and macrophages and are typical of *T. cruzi* infection in humans (Laranja et al., 1956) and other wild animal hosts (Barr, 1991a; Carreira et al., 1996). There was no difference between the numbers of inflammatory foci in tissues from the four chambers of the heart in the infected raccoons. In contrast, Barr et al. (1991a) determined that the right and left ventricles of naturally infected *D. virginiana* were similarly affected by inflammation while atria were essentially normal. Carreira et al. (1996) observed foci of inflammation mainly in the atria of naturally infected *D. marsupialis*. Williams et al. (1977) and Tippit (1978) reported that myocarditis was found primarily in the right side of the heart in dogs from Texas.

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