RESEARCH NOTES

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The Lesions and Prevalence of *Trypanosoma cruzi* in Opossums and Armadillos from Southern Louisiana

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ABSTRACT: The prevalence of *Trypanosoma cruzi* in 48 opossums and 98 armadillos from southern Louisiana was studied. Sixteen opossums (33.3%) and 1 armadillo (1.1%) were positive for *T. cruzi* by blood culture. Hearts from 45 opossums and the tissues from the 1 blood culture-positive armadillo were available for histopathological examination. Although histopathology revealed *T. cruzi* pseudocysts in 6 opossums, 2 were not positive on blood culture. Therefore, 18 opossums (37.5%) were positive for *T. cruzi*. Twenty-two of 45 opossums had histological evidence of myocarditis. No lesion typical of infection with *T. cruzi* was observed in the armadillo tissues. These results substantiate that the opossum is a current reservoir host of *T. cruzi* infection in southern Louisiana and that armadillos may be of relatively minor importance.

Although *Trypanosoma cruzi* has been isolated from Louisiana opossums (Yaeger, 1961), prevalence rates and cardiac lesions associated with infection have not been reported. Based on serology and blood culture a prevalence of 28.8% has been reported in armadillos from a site near New Orleans, Louisiana (Yaeger, 1988). Our study was undertaken to examine the prevalence of *T. cruzi* infection, using blood culture in opossums (*Didelphis virginiana*) and nine-banded armadillos (*Dasypus novemcinctus texanus*) collected from southern Louisiana, and to examine heart tissue histologically to assess the characteristics and extent of lesions present.

Forty-eight adult opossums were live-trapped in the environs of Baton Rouge, Louisiana (East Baton Rouge Parish), over a 2-yr period between May 1985 and May 1987. Opossums were killed with an intraperitoneal injection of 0.5 ml of sodium pentobarbital or a euthanasia solution (T-61®, Hoechst, Somerville, New Jersey), and, using sterile technique, blood was collected by cardiac puncture into heparinized vacutainer blood tubes (Vacutainer®, Becton Dickinson, Sioux City, Iowa). The animals then were exsanguinated by excising the carotid arteries. Ninety-eight armadillos were hand caught at night on river levees in southern Louisiana (Saint Landry Parish). The animals were housed at the Gillis W. Long Hansen's Disease Center. They were anesthetized using ketamine hydrochloride (Ketaset®, Veterinary Products Bristol Laboratories, Syracuse, New York) at a dose rate of 20 mg/kg body weight, and, using sterile technique, 1 ml of blood was collected from the subclavian vein into heparinized-coated tubes and stored at 4 C.

Direct blood examination was done on 24 of 48 opossums. Five microliters of fresh blood were examined for trypomastigotes by phase contrast or bright field microscopy (400×). Blood culture was done on all animals. One milliliter of heparinized blood was added aseptically, within 24 hr of collection, to flasks containing 10 ml of liver infusion tryptose medium supplemented with 10% heat-inactivated (56 C, 30 min) fetal calf serum, 100 IU of penicillin, and 100 μg of streptomycin/ml of medium (Logan and Hanson, 1974). Flasks were kept at room temperature (approximately 25 C). Every 2 wk a drop was examined under a coverslip (400×). Samples were determined to be positive when motile *T. cruzi* epimastigote forms were seen. Subcultures were made when clumps of parasites were seen in culture and of all cultures after 4 wk. Cultures were considered negative if epimastigotes were not seen within 20 wk of culture.

The heart, tongue, diaphragm, intercostal muscle, quadriceps, and brain of 30 opossums were examined histologically. Hearts from an additional 15 opossums were available for gross and histological examination. The heart, thigh muscle, intercostal muscle, lung, kidney, small intestine, bladder, liver, and thymus of the infected armadillo were examined histologically. Two sections each of the right and left ventricle and each atrium from all hearts were examined histologically. All tissues were formalin-fixed,

embedded in glycol methacrylate or paraffin, and stained with hematoxylin and eosin.

Blood culture and histopathological findings are presented in Table I. Of the 48 opossums, 18 (37.5%) were infected with *T. cruzi* as demonstrated either by blood culture or histological examination. *Trypanosoma cruzi* was confirmed by blood culture in 16 (33.3%) of the 48 opossums and by finding pseudocysts in heart tissues of another 2 (4.2%). One of the 24 opossums examined by direct blood smear was positive. Of the 98 armadillo blood samples cultured, 1 was positive 6 wk postinoculation (PI). A second blood culture from the infected animal taken 2 mo after the first also was positive, but a third culture taken 26 mo after the second was negative. During this period, the animal was kept in captivity and isolated from other animals and vectors (subfamily Triatominae).

On the average, positive cultures were identified in opossums and armadillos 5 wk PI (range: 2–12 wk). Positive cultures usually developed clumps of amastigotes prior to the appearance of motile epimastigotes. Four (8.3%) opossum and 2 (2%) armadillo blood cultures were contaminated with bacteria or fungus by 4 wk PI despite stringent sterile techniques used during blood collection.

There was no gross lesion in any of the opossum hearts examined. Of the 45 examined, 22 (49%) had myocarditis of mild severity, consisting of very small aggregates (10–20 cells) of inflammatory cells multifocally expanding the interstitium and occasionally replacing a myofiber (Fig. 1A). In most, macrophages were the predominant cell type with slightly fewer lymphocytes. Plasma cells were present infrequently. Right and left ventricles were affected similarly. The atrial myocardium essentially was normal.

Pseudocysts of *T. cruzi* were present within myofibers of 6 opossums, usually as an isolated cyst except in 1 animal where multiple cysts were found (Fig. 1B). Pseudocysts were present in both right and left ventricles and were not usually associated with inflammation. In all other tissues examined, a single *T. cruzi* cyst was present within the tongue musculature of only 1 opossum. This animal had a positive *T. cruzi* blood culture and a cyst within the right ventricular myocardium but no myocarditis.

Seven of 30 opossums had *Sarcocystis* sp. cysts in 1 or more muscle tissues. Six opossums had *Besnoitia darlingi* cysts within the myocardium and 2 had cysts within other muscles. Degenerating cysts were surrounded by extensive areas of granulomatous inflammation and fibrosis. Multiple small foci of glial cells were identified in the midbrain in 1 animal and in the cerebrocortical gray matter of another.

TABLE I. Results of blood cultures and histopathology in opossums examined for *Trypanosoma cruzi* infection.

Group	Number positive/ number examined	% Positive
Culture positive and/or pseudocysts present	18/48	37.5
LIT blood cultures*	16/48	33.3
Myocarditis	22/45	48.9
Pseudocysts	6/45	13.3
Pseudocysts and myocarditis	5/45	11.1
Culture positive and myocarditis	9/45	20.0

* LIT, liver infusion tryptose medium.

There was no gross lesion in the tissues of the infected armadillo at necropsy. Histologically, apart from 3 *Sarcocystis* sp. cysts in the thigh muscle and a mild focal bronchiolitis obliterans surrounding a fragment of an unidentified nematode, the tissues were normal. Cysts or inflammatory changes typically associated with *T. cruzi* infection were not seen in multiple sections of any tissue examined.

The infection rate in opossums as determined by blood culture of 33.3% reported here is comparable to the level of 32.8% recently reported in Brazil (Lainson et al., 1979). The discovery of only 2 (4.2%) infected animals by histopathological techniques alone suggests that blood culture is one of the more sensitive single measuring methods, although time consuming, of determining *T. cruzi* prevalence in opossums. The fact that some infected animals were culture negative indicates that they either did not have a circulating parasitemia or that it was so low that a more sensitive method, such as xenodiagnosis, would have been needed to detect organisms (Teixeira, 1987). The marked disparity in *T. cruzi* prevalence in armadillos in Louisiana between our data and those reported previously (Yaeger, 1988) suggests that the prevalence of *T. cruzi* in armadillos from different sites within Louisiana is highly variable for reasons unknown.

The mild mononuclear myocarditis, usually unassociated with pseudocysts in opossums, is consistent with findings in chronic Chagas' disease in humans and dogs (Anselmi et al., 1966). Opossums and armadillos are among the group

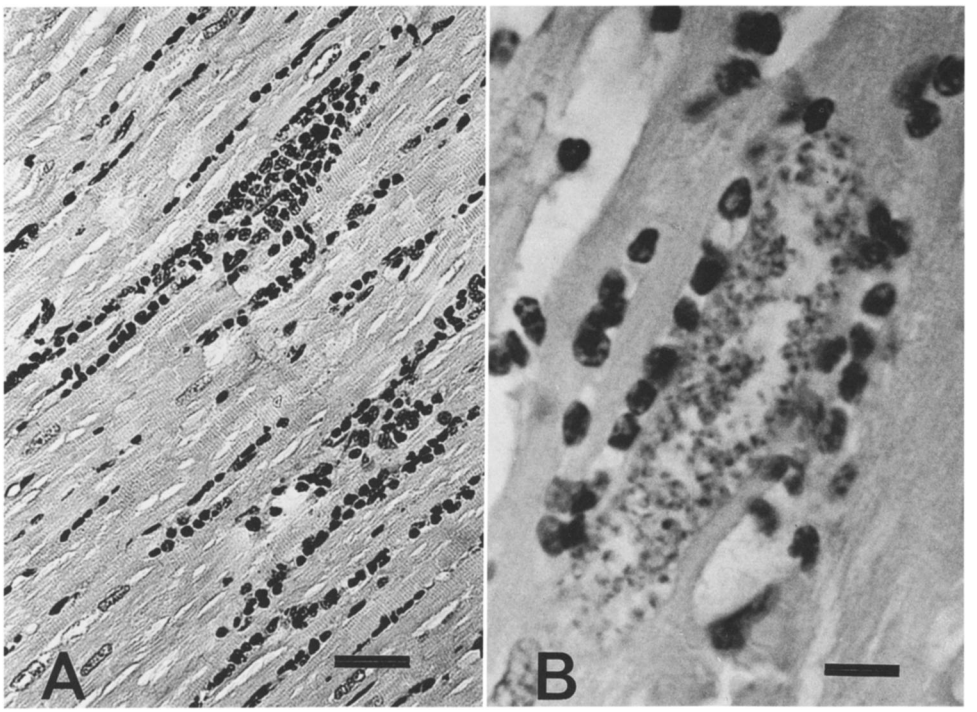

FIGURE 1. **A.** Clusters of inflammatory cells, predominantly lymphocytes present within the interstitium of the myocardium of an opossum. Hematoxylin and eosin stain. **B.** A single *Trypanosoma cruzi* pseudocyst containing amastigotes within a myocardial fiber of an opossum heart. Hematoxylin and eosin stain.

of animals with a considerable degree of adaptation to infection with pathogenic *T. cruzi* isolates of humans and other mammals (Zeledon et al., 1970). Although it was not possible to correlate these lesions to disease in the opossums, such findings in humans and dogs have been associated with severe electrocardiographic disturbances (Anselmi et al., 1966). The lack of lesions and a negative culture after being culture positive 2 yr previously supports the contention that armadillos are well adapted to *T. cruzi* infection (Zeledon et al., 1970).

Six opossums had extensive granulomatous inflammation surrounding degenerating *B. darlingi* cysts typical of previous descriptions (Smith and Frenkel, 1984). This reaction appeared to be distinct from the scattered small aggregates of interstitial macrophages and lymphocytes. Although these small aggregates appeared to be unassociated with tissue stages of *B. darlingi, Sarcocystis* sp., or *T. cruzi,* they were interpreted histologically to be caused by infection with *T. cruzi* as they resembled those around *T. cruzi* pseudocysts.

Within the last 2 decades, the opossum has gained popularity as a laboratory animal. Many animals used in the laboratory are wild-caught. A nonspecific multifocal lymphocytic myocarditis has been reported in experimental (Rowland et al., 1970) and wild-caught opossums (Sherwood et al., 1969). In light of the present findings, it is possible that the myocarditis may have been associated with *T. cruzi* infection.

These results document the high prevalence of *T. cruzi* in opossums from southern Louisiana. In Louisiana, hunting is not only a popular pastime, but a vocation for some groups who trap and skin considerable numbers of animals. Although the virulence of these *T. cruzi* isolates to humans is unknown, care should be exercised when working with wild-caught opossums.

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A Simple Method for Cloning *Giardia duodenalis* from Cultures and Fecal Samples

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ABSTRACT: Using a novel method for cloning *Giardia duodenalis* from cultures and fecal samples, 47 clones from 7 isolates were established in vitro. Average colony-forming efficiency in established cultures was 43.2% compared to 11.2% when cloning directly from excystation. The highest success rate of cloning was found with the Portland (P1, ATCC No. 30888) isolate, with a colony-forming efficiency of 92.7%. Cloned and parent populations were compared over a range of 13 enzymes using starch gel electrophoresis. No genetic difference was found between any of the clones and the parent isolates.

Giardia duodenalis is the most commonly reported intestinal protozoan pathogen in Australia. Isolates of *G. duodenalis,* derived from fecal samples of infected hosts and maintained in axenic culture, have been shown to differ in virulence, infectivity, antigenicity, and susceptibility to drugs (Boreham et al., 1984; Gordts et al., 1985; Gasser et al., 1987; Aggarwal and Nash, 1988; Nash et al., 1988; Capon et al., 1989). Genetic comparisons, using isoenzyme electrophoresis and DNA hybridization, also have shown differences among isolates, but the results are complicated by the possibility of genetic heterogeneity within an isolate (Bertram et al., 1983; Nash et al., 1985; Baveja et al., 1986; Meloni et al., 1988). Genetic homogeneity can be assured only by cloning from a single organism, which is essential for meaningful comparative studies on different isolates. This has been emphasized for *Plasmodium* (Thaithong et al., 1988) and *Trypanosoma* (Goldberg and Pereira, 1983; Tanuri et al., 1985) as well as *Giardia* (Boreham et al., 1987). Furthermore, the availability of a sim-