

TRYPANOSOMA CRUZI IN TRIATOMES FROM AN URBAN AND A DOMESTIC SETTING IN MIDDLE TENNESSEE

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ABSTRACT—The presence of *Trypanosoma cruzi* in the southern United States continues to be an emerging issue. Clearly wildlife reservoirs exist and on occasion domestic canines have been infected with this protozoan parasite. The risk to human infection likely would be promoted if infected triatomines were present in populated areas and invade residences. Tennessee most recently (2000) was added to the list of states known to be endemic for *T. cruzi*. This first dispatch was accompanied by the report of a human autochthonous infection and a seropositive family canine. The current report describes the recovery of infected triatomines from counties adjacent to the initial report site in middle Tennessee. The triatomines (*Triatoma sanguisuga*) were captured in an urban and domestic setting. Informed individuals among the general public were responsible for capture of the insects and simple laboratory techniques were subsequently used to identify *T. cruzi* in the intestinal contents of the triatomines. If a well-defined understanding of *T. cruzi* in the southern region of the United States is to be established, it may be prudent to consider that public awareness and knowledge can contribute to capture of infected insects.

Trypanosoma cruzi is the etiological agent of Chagas' disease. The disease is transmitted to humans through the bite wound of reduviid bugs (triatomids, kissing bugs, or conenose bugs). Metacyclic trypanomastigotes are expelled in feces when the insect is taking a blood meal. The trypanomastigotes may be rubbed or scratched into the bite wound or onto mucosal surfaces such as the eyes or mouth. *Trypanosoma cruzi* also may be transmitted to humans as congenital infections, by blood transfusions, or by organ transplantations. Once in the body, protozoa invade local tissue and multiply within cells. The intracellular amastigote forms can multiply within a variety of different cell types. Reticuloendothelial, myocardial, adipose, and neuroglial cells are preferred sites for replication. Amastigote binary fission fills and destroys infected cells releasing the amastigote and trypanomastigote forms. The latter are spindle shaped and approximately 20 μ m long, and characteristically assume a U shape in stained blood smears. Trypanomastigotes are ingested by the reduviid bug as it obtains a blood meal. Protozoa multiply in the posterior portion of the insect midgut. In 8 to 10 days trypanomastigotes are passed in the feces to infect humans. Acute-stage symptoms occur only in about 1% of patients and are usually observed in younger children. Patients may die in a few weeks or months, may recover, or may enter a chronic stage of infection. Chronic Chagas' disease may become apparent years or decades after infection. Here the most frequent clinical sign is cardiomyopathy characterized by cardiomegaly and conduction changes. In endemic areas, the chronic form of Chagas' disease is diagnosed more commonly than the acute disease (Garcia, 2001).

Chagas' disease is a significant cause of disease in Central and South America. Within the United States, human health issues affiliated with this parasite are most often related to potential infection among immigrants

from endemic areas (Kirchhoff, 1993). However, during the last 20 years studies have documented the occurrence of *T. cruzi* in a variety of mammals and in triatomine vectors within the borders of the United States (reviewed in Yabsley et al., 2001). Typically these reports are from southern states with *Triatoma sanguisuga* likely serving as the most important triatomine vector in the southeastern states (Beard et al., 2003).

The first report of *T. cruzi* in a triatomine from Tennessee occurred in 2000 (Herwaldt et al., 2000). A single *T. sanguisuga* was captured in the crib of an infant residing in a well constructed home located in a rural area (Rutherford County) near Nashville, Tennessee. Black spots were observed on the crib sheets and upon dissection in our laboratory the *T. sanguisuga* was found to be engorged with blood. Motile trypanosomes were observed in the intestinal contents and identified as *T. cruzi*. Whole blood from the infant sleeping in the crib tested positive for *T. cruzi* by PCR and DNA hybridization. The family dog had an anti-*T. cruzi* titer of 1:1024 and 2 of 3 raccoons trapped in the vicinity had positive hemoculture for *T. cruzi*.

In the present report we describe the identification of additional *T. sanguisuga* that were infected with *T. cruzi*. The known range of *T. cruzi* in Tennessee is extended to include Cannon and Davidson counties. It is apparent also that infected insects can occur in both urban and rural environments.

MATERIALS AND METHODS

In the summer of 2001 a *T. sanguisuga* (Fig. 1) was brought to this laboratory after its recovery adjacent to a well-constructed apartment complex in metropolitan (Davidson County) Nashville, Tennessee. The presence of highly motile

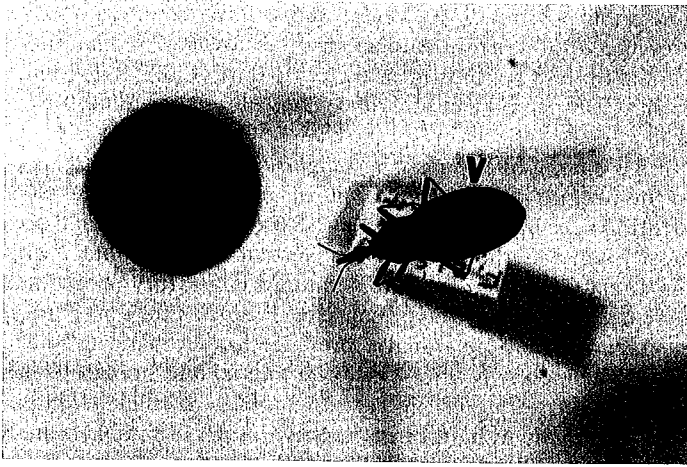


FIG. 1. A *Triatoma sanguisuga* from middle Tennessee. Adults may be 1.0 to 2.5 cm long (note one-cent coin in photograph for size comparison). The body is flattened and dark brown to black in color. On each side of the abdomen are reddish-orange marks (arrow).



FIG. 2. Appearance of *Trypanosoma cruzi* from infected *Triatoma sanguisuga* using phase microscopy. Bar = 10 μ m.

trypanosomes and Giemsa stained preparations of intestinal contents were identified as *T. cruzi*. This was subsequently confirmed at the Centers for Disease Control and Prevention based on morphological features, antibody reactivity, and polymerase chain reaction analysis.

During the fall of 2005 a *T. sanguisuga* was captured from within a home in a rural area of Cannon County, Tennessee, located approximately 60 km from metropolitan Nashville. Intestinal contents likewise contained trypanosomes (based on phase microscopy and Giemsa staining of intestinal contents) later confirmed as *T. cruzi* by the Centers for Disease Control and Prevention.

In each of these instances, for microscopic identification in our laboratory, the abdomen of the triatomine was cut lengthwise with a razor blade and the intestinal contents placed in a 1 ml microfuge tube containing 100 μ L of RPMI-1640 (Gibco, Grand Island, New York) media held at room temperature. For examination of motile trypanosomes, 15 μ L aliquots were pipetted onto standard laboratory slides (25 by 75 by 1 mm), covered with cover glass (22 by 50) and viewed at 200x and 400x using phase contrast microscopy (Olympus BH-60 microscope). In all the infected *T. sanguisuga* many actively motile protozoa were quickly apparent and easily identified using phase microscopy (Fig. 2). For Giemsa staining, 10 to 20 μ L aliquots were spread onto slides, allowed to air dry, and immediately fixed in methyl alcohol for 1 to 2 min. Slides were then Giemsa stained within 48 h. For this, 1:20 dilutions of stain (Sigma-Aldrich, St. Louis, MO.) and 1:50 dilutions of stain (Harleco, EM Diagnostic Systems, Gibbstown, New Jersey) were used. Slides were stained 50 min, rinsed in tap water, allowed to dry overnight, cover glassed using Cytoseal 60 (Stevens Scientific, Kalamazoo, Michigan) and viewed under oil immersion at 630x and 1000x magnification. Stained specimens were often characterized by trypomastigotes and epimastigotes with a large oval kinetoplast and a nucleus in the center of the body. Some trypomastigotes assumed a characteristic C or U shape that is a feature of *T. cruzi* (Fig. 3).

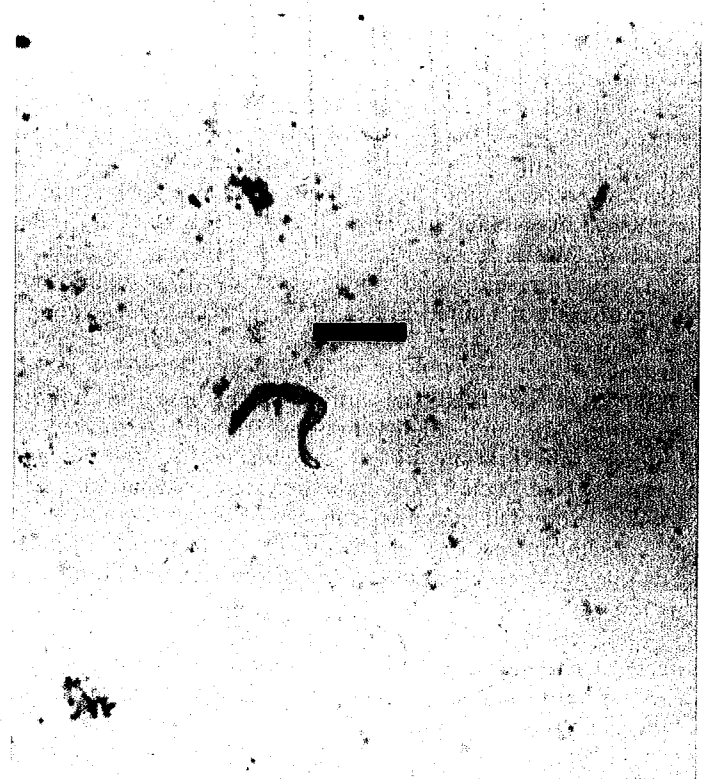


FIG. 3. Appearance of *Trypanosoma cruzi* from infected *Triatoma sanguisuga* following Giemsa staining. Note prominent kinetoplast and U shape. Bar = 10 μ m.

RESULTS AND DISCUSSION

Not all *T. sanguisuga* brought to our laboratory and viewed by light microscopy were positive for *T. cruzi*. Two other *T. sanguisuga* (one taken from the basement and one captured on the front porch) from the original report site were negative. In addition, 1 adult was found in a light fixture from a residence of one of the coauthors of this report (CRM) residing within a well constructed home in a rural area of Rutherford County, Tennessee. This specimen was highly desiccated and the intestinal contents were unsuitable for visual examination for *T. cruzi*.

Sixty years ago, Tennessee was included in a generalized distribution for the genus *Triatoma* (Usinger, 1944). The report noted that the common species within the southeastern United States were *T. sanguisuga* and *Triatoma lecticularis*. Twenty-two years later, Tennessee was considered within the known range of *T. sanguisuga* (Kagan et al., 1966). In states adjacent to Tennessee (Alabama, Georgia, and North Carolina) *T. cruzi* infections have been reported in *T. sanguisuga* or various mammal species (Olsen et al., 1964; Karsten et al., 1992; Pung et al., 1995; Pietrzak, et al. 1998). These reports suggested potential animal and human health issues that would be associated with the presence of *T. sanguisuga* infected with *T. cruzi*. In more recent years, a pattern of endemic *T. cruzi* within the southern United States has become apparent. It is clear infected triatome vectors, wild animals, and in several reports domestic animals such as canines are subject to infection (Tomlinson et al., 1981; John et al., 1986; Barr et al., 1995; Meurs et al., 1998; Bradley et al., 2000; Beard et al., 2003). Throughout this reporting period, there was an absence of reports of endemic or autochthonous human infection. Even a more recent serological survey failed to note a significant presence of *T. cruzi* antibodies in endemic populations in the southeastern United States (Barrett et al., 1997). The lack of documented human infection has been attributed to circumstances such as relatively good housing standards, delay in defecation until 20 to 30 minutes after feeding in North America triatomes, and the possibility virulence for humans is diminished in endemic strains of *T. cruzi*. (Kagan et al., 1966; Kirchhoff, 1993).

Despite present good housing standards in regions of the southeastern United States, it is apparent *T. sanguisuga* can be recovered from within and in proximity to well constructed housing (Olsen et al., 1964; Herwaldt et al., 2000). This has been the authors experience in middle Tennessee. Although quality housing would be expected to diminish inhabitation by *T. sanguisuga*, it should not necessarily be expected to exclude the vector. Human presence certainly promoted increased chances of visual identification and subsequent recovery of triatomes, and it played a significant role in the recovery of triatomes from occupied homes in this region.

Since *T. sanguisuga* is an obligate blood feeder, the infected triatomes identified by our laboratory must have fed on *T. cruzi* infected animals. Because insects were recovered in or near houses, the source of infection is likely related to animals adapted to living in close association to human housing. This does not exclude mammals such as rats, raccoons or opossums which can often be found in residential settings. It is also possible domestic canines harbor this parasite. To date there have been no studies in Tennessee to

access this possibility. However, studies have demonstrated the presence of *T. cruzi* in canines in other areas of the southern United States (Tomlinson et al., 1981; Barr et al., 1995; Meurs et al., 1998; Bradley et al., 2000; Herwaldt et al., 2000; Beard et al., 2003).

To date, 3 of 5 mature *T. sanguisuga* found in or adjacent to homes in a 3 county area of middle Tennessee were identified as harboring *T. cruzi*. Recovery of the triatomes most often was the result of inadvertent human contact with the insect. Following capture, standard laboratory procedures were used to identify the presence of *T. cruzi*. This observation is suggestive of a meaningful presence for the parasite in the middle Tennessee region. The exact animal and human health significance of *T. cruzi* in Tennessee merits further investigation. Especially in light of the reported autochthonous human infection, a pet dog infection, and the fact that *T. cruzi* infection is not ordinarily considered when addressing health issues in both humans and domestic animals in Tennessee.

Addendum—Shortly after review of this manuscript, two additional *T. sanguisuga* were captured inside the domestic residence in Cannon County, Tennessee. Based on light microscopy and examination of intestinal contents, these two triatomes were infected with *T. cruzi*.

ACKNOWLEDGEMENTS

Appreciation is expressed to J. Lawrence, Division of Parasitic Diseases, Center for Disease Control and Prevention, Atlanta, Georgia, for confirmation of suspected *T. cruzi* in the triatomes brought to our laboratory. The authors wish to express their appreciation to J. M. Gragg for recognition and collection of triatomes from Cannon County, Tennessee.

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