

# Prevalence of American trypanosomiasis (Chagas disease) among dogs in Oklahoma

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**Objective**—To determine the prevalence of *Trypanosoma cruzi* infection among dogs in Oklahoma.

**Design**—Cross-sectional study.

**Animals**—301 owned or impounded dogs related by ownership or general geographic location to 3 dogs determined to have trypanosomiasis.

**Procedures**—Blood samples were obtained from dogs between November 1996 and September 1997. Infection status was determined by use of a radioimmunoprecipitation assay. Second blood samples were obtained from some of the seropositive dogs for study by hemoculture and polymerase chain reaction (PCR) assay. Sites where infected dogs were found were inspected for triatomine insects, and light traps were used for vector trapping.

**Results**—11 (3.6%) dogs were seropositive for *T cruzi* infection. Ten of the 11 were owned rural hunting dogs. Protozoal organisms isolated from the blood of 1 seropositive dog were identified as *T cruzi* by PCR testing. Only 1 adult *Triatoma sanguisuga* was captured in a light trap at a site near infected dogs; this insect was not infected.

**Conclusions and Clinical Relevance**—Our findings suggest that *T cruzi* is enzootic in eastern Oklahoma. Measures that would reduce the risk of dogs acquiring *T cruzi* infection are unlikely to be acceptable to their owners, and no effective drugs are available for treatment. The presence of *T cruzi*-infected dogs poses a threat of transmission to persons at risk of exposure to contaminated blood. Veterinarians who practice in the southern United States should be cognizant of this blood borne zoonosis and educate all personnel about appropriate precautions. (*J Am Vet Med Assoc* 2000;217:1853–1857)

**T***rypanosoma cruzi* is the protozoan hemoflagellate that causes American trypanosomiasis (Chagas disease). This parasite, which is found only in the

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Americas, is transmitted among its mammalian hosts by insect vectors (Family Reduviidae, Subfamily Triatominae). It can also be transmitted congenitally, through contaminated blood transfusions, or by contamination of mucous membranes or breaks in the skin with blood, insect excreta, or tissues containing infective parasites.<sup>1-3</sup> Infection with *T cruzi* is life-long, and chronic infection is characterized by detectable concentrations of specific antibodies and low concentrations of circulating parasites. In contrast to many other protozoan parasites, *T cruzi* has little host specificity, as it has been isolated from more than 100 mammalian species and dozens of insect vector species. Chagas disease is a zoonosis, and an estimated 16 to 18 million people are infected in Latin America.<sup>4</sup> It is enzootic throughout much of Latin America where raccoons, opossums, armadillos, and rodents are commonly infected, as are domestic animals such as dogs and cats. The sylvatic cycle is known to exist in the southern and southwestern United States where several cases of *T cruzi*-infected dogs have been reported.<sup>1,5-9</sup>

Our interest in studying *T cruzi* infection in dogs in Oklahoma was prompted by a veterinarian's exposure to the parasite through an accidental needle stick involving blood and lymph tissue from an infected dog. Subsequent to that event, 2 additional canine cases were identified. Given the highly infectious nature of the parasite and the potential risk of transmission to veterinarians and others who may be exposed to blood from animals infected with *T cruzi*, we conducted a serologic and parasitologic study to estimate the prevalence of *T cruzi* among domestic dogs in Oklahoma.

## Materials and Methods

**Identification of the index case**—A 4-month-old 19.5-kg (43-lb) male English Mastiff was referred to the Oklahoma State University Boren Veterinary Medical Teaching Hospital for investigation of weight loss, diarrhea, and lymphadenopathy. Numerous extracellular organisms with morphology consistent with that of *T cruzi* were seen cytologically in lymph node aspirates; the *T cruzi* antibody titer, determined by use of an indirect fluorescent assay, was high (1:512; animals with titer > 1:32 are considered seropositive); and culture of lymph node aspirates yielded *T cruzi*.

**Serologic survey**—A brief report describing the index case was published in a statewide veterinary newsletter in November 1996 to increase awareness of canine trypanosomiasis. In response to this article, 2 additional recent cases of *T cruzi* infection involving dogs were reported by veterinarians in Oklahoma. Blood samples were collected from all dogs living on the same premises as the index case and these two additional infected dogs. Between November 1996 and September 1997, a serologic survey of dogs residing in the same counties (Nowata, LeFlore, and Pittsburg counties) in eastern Oklahoma as these 3 infected dogs was conducted.

Blood samples were obtained from dogs impounded in city animal shelters, dogs known to the owners of the infected dogs, and dogs examined at participating veterinary clinics. All blood samples were collected into tubes containing EDTA (final concentration, 10 mM) to prevent coagulation.

Blood samples were tested for specific antibodies to *T cruzi*, using a radioimmunoassay (RIPA) described in an earlier report<sup>10</sup> and subsequently used to test for *T cruzi* infection in dogs.<sup>1</sup> Briefly, blood samples were centrifuged, and plasma was obtained. For each sample, 10  $\mu$ l of plasma was mixed with a volume of <sup>125</sup>I-labeled *T cruzi* (Tulahuén strain) epimastigote lysate containing 500,000 counts/min. Antigen-antibody complexes were removed from this mixture by addition of protein A-Sepharose. Samples were boiled briefly, and the immunoprecipitated <sup>125</sup>I-labeled antigens were separated by polyacrylamide gel electrophoresis and detected by autoradiography. The presence of 72- and 90-kd bands on the resulting electrophoretograms was considered a positive result.

**Isolation of *T cruzi* from blood samples**—Additional blood samples were collected from some dogs seropositive for *T cruzi* antibodies. Samples were anticoagulated with EDTA and centrifuged. The pelleted cells were washed twice in liver-digested neutralized tryptose medium containing 10% fetal calf serum, 100  $\mu$ g of penicillin/ml, and 100  $\mu$ g of streptomycin/ml (LDNT+). Cells were then suspended in a 1:1 ratio in LDNT+, and aliquots (7 ml) were placed in 25-cm<sup>2</sup> flasks for incubation at 26 C. Cultures were examined intermittently in an inverted microscope for 120 days.

**Insect collection**—Two sites where infected dogs were found were inspected for triatomine insects. Two ultraviolet light traps were operated at a Nowata county site for 1 night and at a LeFlore county site for 2 nights. In addition, 6 CO<sub>2</sub>-baited pitfall traps were operated at the LeFlore county site for 1 night. Potential triatomine habitats surrounding doghouses and pens at both of these locations were thoroughly inspected.

**Identification of protozoal isolates**—A polymerase chain reaction (PCR) assay was used to determine whether any captured triatomine insects were infected with *T cruzi* and provide positive identification of protozoal organisms isolated from infected dogs. For isolation of DNA from triatomine insects, abdominal contents were removed by dissection and mashed with an applicator stick in a microcentrifuge tube after addition of a small volume of phosphate-buffered saline solution. The DNA was extracted from a 100- $\mu$ l aliquot of this material from each insect. Five volumes of lysis buffer (10 mM Tris hydrochloride [pH 7.6], 10 mM EDTA, 0.1M NaCl, 0.5% sodium dodecyl sulfate, and 300  $\mu$ g of proteinase K/ml) were added to each sample, and the resulting mixture was incubated for 2 hours at 55 C. Samples were heated to 95 C for 10 minutes to inactivate the proteinase K and then extracted twice with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1). Nucleic acids were precipitated by addition of one-tenth volume of 3 M sodium acetate (pH 5.5), 20  $\mu$ g of glycogen, and 2 volumes of ethanol. After centrifugation in a microcentrifuge for 15 minutes, the pellet was rinsed with 70% ethanol, air dried, and suspended in 100  $\mu$ l of water. To confirm identification of protozoal organisms isolated from infected dogs, DNA was extracted from 100  $\mu$ l blood samples, using an analogous procedure.

Polymerase chain reaction assays were performed with the TCZ1 and TCZ2 primer pair, which amplify a 188-base pair nuclear repetitive sequence that is specific for *T cruzi*. The conditions for the assay were similar to those described previously.<sup>11-13</sup> Positive controls consisting of samples in which *T cruzi* DNA was used as a template were included in each run of the assay, along with samples consisting of DNA

extracted from uninfected insect excreta and dog blood, when appropriate, and standard reaction mixture negative controls. To avoid false-positive reactions attributable to contamination of reaction mixtures with 188-base pair amplicons from earlier runs, the PCR portion of the assay was performed in 1 section of the laboratory and, after amplification, tubes were opened, and electrophoresis was performed in a different area. Reagents and equipment used in the second area were never used subsequently in the first. In addition, tubes containing all reagents except template DNA were included in each assay run for the purpose of detecting contamination of reagents with amplifiable *T cruzi* DNA sequences.

To test for the specificity of the PCR assay, a second PCR assay was performed. In this second assay, oligonucleotides TCZ3 and TCZ4, which amplify a 149-nucleotide internal segment of the nuclear repetitive sequence, were used as primers, and an aliquot of the reaction mixture from the first assay that contained the 188-base pair product was used as a template.<sup>14</sup>

## Results

**Description of the index case**—The index case was a 4-month-old 19.5-kg (43-lb) male English Mastiff examined because of weight loss, diarrhea, and lymphadenopathy. In the 2 weeks prior to examination at the veterinary teaching hospital, the dog had been anorectic, defecated loose (cow-patty consistency) feces, and had lost 0.7 kg (1.5 lb). The dog had been treated with antibiotics without response and had been given prednisone (1 mg/kg [0.45 mg/lb] of body weight, PO, q 12 h, for 3 days, then 1 mg/kg, PO, q 24 h, for 3 days) by the referring veterinarian. The dog had been whelped and raised in northeastern Oklahoma. All routine vaccinations were current, and results of a fecal analysis for parasites were negative. The dog was seronegative for antibodies against *Ehrlichia canis*.

On physical examination, the dog was lethargic and thin and had mild generalized lymphadenopathy and pyrexia (rectal temperature, 39.9 C [103.8 F]). Moderate anemia (Hct, 0.27; reference range, 0.38 to 0.57), mild lymphopenia ( $0.7 \times 10^9/L$ ; reference range,  $0.8$  to  $5.6 \times 10^9/L$ ), hypoalbuminemia (19 g/L; reference range, 23 to 39 g/L), and high alkaline phosphatase activity (806 U/L; reference range, 20 to 157 U/L) were detected. Results of other serum biochemical tests were normal. Urinalysis revealed hyposthenuria (specific gravity, 1.005). On thoracic radiographs, the cardiac silhouette was normal, but the sternal lymph nodes appeared larger than normal. Hepatomegaly, inguinal lymphadenopathy, and decreased serosal detail secondary to a lack of intraabdominal fat were seen on abdominal radiographs.

Fine-needle aspirates were obtained from enlarged prescapular and popliteal lymph nodes. The smears were cellular and consisted primarily of small mature lymphocytes with scattered prolymphocytes and lymphoblasts. The numbers of plasma cells and macrophages were moderately increased. Numerous extracellular organisms morphologically consistent with *T cruzi* were also seen (Fig 1). The *T cruzi* antibody titer, determined by use of an indirect fluorescent antibody assay, was high (1:512), and *T cruzi* organisms were isolated from lymph node aspirates.

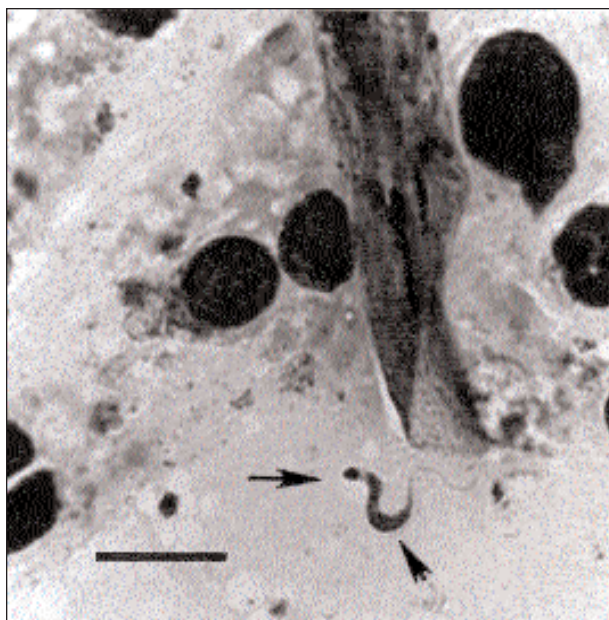


Figure 1—Photomicrograph of a lymph node aspirate from a dog with trypanosomiasis. Notice the protozoal organism in which a nucleus (short arrow) and kinetoplast (long arrow) are evident; kinetoplasts are found only in organisms belonging to the order Kinetoplastida. Giemsa stain; bar = 10  $\mu$ m. Courtesy of JH Meinkoth, Oklahoma State University Clinical Pathology Teaching Files.

Treatment with nifurtimox<sup>a</sup> (120 mg, PO, q 8 h, for 180 days) and prednisone (10 mg, PO, q 12 h) was started, and the dog was discharged to home care. Clinical improvement, as evidenced by weight gain, resolution of the lymphadenopathy, and normalization of thoracic radiographic findings, was apparent during the 6-month course of treatment. Eleven months after treatment with nifurtimox was initiated, however, echocardiography revealed mild right-sided dilated cardiomyopathy with diminished contractility and borderline function of the interventricular septum. Although no organisms were seen on lymph node aspirates, culture of blood samples obtained at that time yielded *T cruzi*.

**Serologic survey**—Blood samples were obtained from 304 dogs, including the index case and the 2 additional dogs recently identified as having been infected with *T cruzi*. One hundred eight (35.5%) of the 304 were stray (n = 99) or owned (9) dogs that lived in northeastern Oklahoma within a 1-county radius of Nowata county, where the index case resided. The remaining 196 (64.5%) dogs resided in LeFlore or Pittsburg counties in east-central Oklahoma. Of these, 82 were impounded dogs, and 114 were privately owned dogs. Overall, 161 of the 304 (53%) dogs were female. Ninety-four (30.9%) of the dogs were of mixed breeding, 39 (12.8%) were coonhounds, and 25 (8.2%) were Labrador Retrievers.

Eleven of the 304 (3.6%) dogs had clear serologic evidence of infection with *T cruzi* by RIPA (Fig 2). All but 1 of the seropositive dogs were privately owned. Four of the seropositive dogs were Mastiffs, 6 were coonhounds, and 1 was of mixed breeding. All 4 seropositive Mastiffs originated from a single breeder

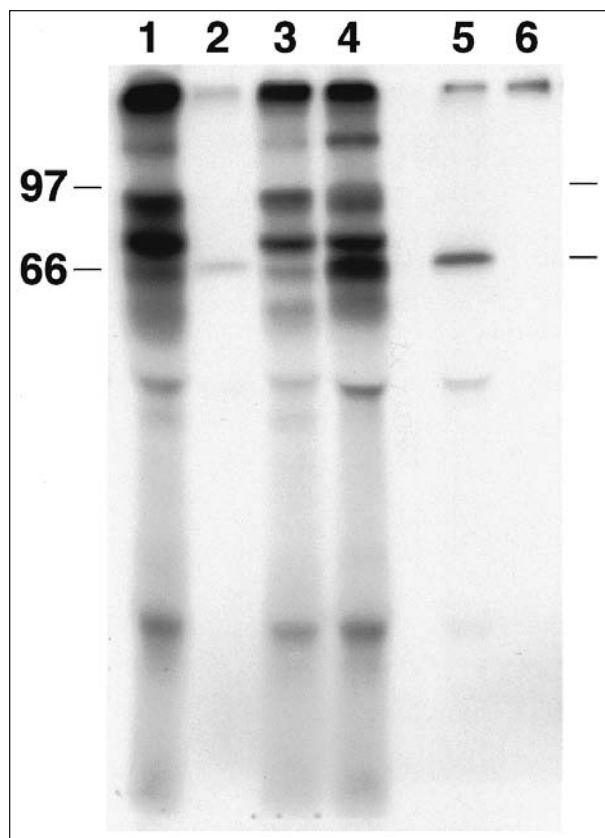


Figure 2—Electrophoretogram obtained by use of a radioimmunoprecipitation assay for antibodies that bind to <sup>125</sup>I-labeled *Trypanosoma cruzi* surface antigens in blood samples from dogs and humans. Lane 1 = Human patient from El Salvador infected with *T cruzi* (positive control). Lane 2 = Uninfected human patient (negative control). Lanes 3 and 4 = Dogs seropositive for *T cruzi* infection. Lanes 5 and 6 = Dogs seronegative for *T cruzi* infection. The 72- and 90-kilodalton bands in lanes 1, 3, and 4 are considered indicative of *T cruzi* infection. Numbers on the left indicate molecular weights.

in Nowata county. Four seropositive coonhounds lived in LeFlore county, and 2 seropositive coonhounds and the seropositive mixed-breed dog were from Pittsburg county. At least 6 of the seropositive dogs had clinical signs compatible with *T cruzi* infection.

**Isolation of *T cruzi* from blood samples**—Blood samples from 4 of the 11 seropositive dogs were submitted for protozoal culture. One was positive after 15 days, whereas the others remained negative through 120 days of observation. The protozoal organisms that were isolated had typical *T cruzi* morphology and transformed to approximately 50% culture-derived metacyclic trypomastigotes when passed into LDNT+ medium and allowed to reach stationary phase. This phenomenon is characteristic of *T cruzi*, especially *T cruzi* isolates recently obtained from natural sources. Parasites from the latter culture were inoculated into flasks containing human renal adenocarcinoma cells, and large numbers of extracellular trypomastigotes and intracellular amastigotes morphologically consistent with *T cruzi* were subsequently observed.

**Insect collection**—One adult female *Triatoma sanguisuga* was captured in a light trap operated at the



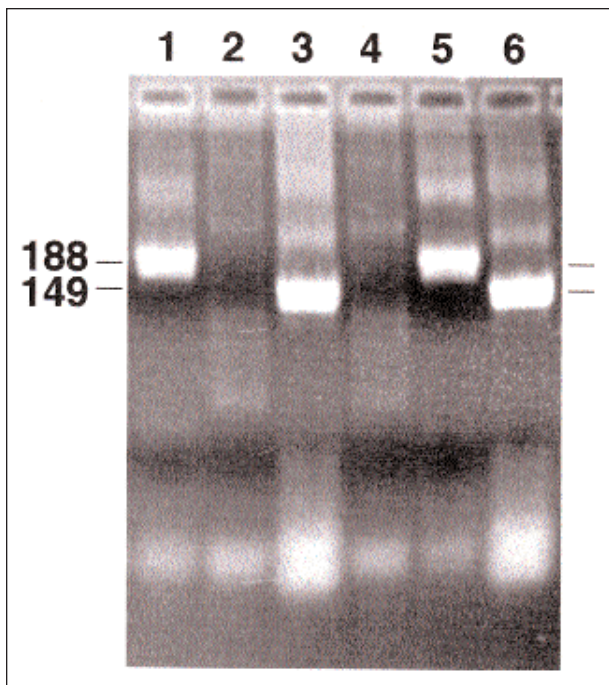


Figure 3—Electrophoretogram obtained by use of a *T. cruzi*-specific polymerase chain reaction-based diagnostic assay, using samples from dogs and humans. Lanes 1 and 3 = Human patient infected with *T. cruzi* (positive control). Lanes 2 and 4 = Uninfected human patient (negative control). Lanes 5 and 6 = Dog seropositive for *T. cruzi* infection. For lanes 1, 2, and 5, primers TCZ1 and TCZ2 were used to amplify a 188-base pair segment of the nuclear repetitive sequence of *T. cruzi*. For lanes 3, 4, and 6, primers TCZ3 and TCZ4 were used to amplify an internal 149-base pair segment of the nuclear repetitive sequence. Numbers on the left indicate base pairs.

LeFlore county site. No other triatomine vectors were collected in the light or CO<sub>2</sub> traps or found in or near runs or housing of infected dogs.

**Identification of protozoal isolates**—Results of the PCR assay indicated that the protozoal organisms isolated from blood samples from 1 infected dog were *T. cruzi* (Fig 3). The captured *T. sanguisuga* was negative for *T. cruzi* infection, as determined by the PCR assay.

## Discussion

Reports of *T. cruzi* infection in dogs in the United States have appeared sporadically, but most have been single case reports. A *T. cruzi*-infected dog in Oklahoma was described in 1986,<sup>7</sup> but broader perspectives on *T. cruzi* infection in dogs in the state have been lacking. To fill this gap, we obtained specimens from a sizable number of dogs and used a highly sensitive and specific radioimmunoassay to detect *T. cruzi* infection. Our finding of an overall prevalence of 3.6% is similar to that reported for dogs in Louisiana and Virginia.<sup>1,15</sup> However, our findings may have been biased by concentration of sample collection in areas in which 3 infected dogs were known to reside as well as by our focus on outdoor dogs with more potential exposure to sylvatic animal hosts of *T. cruzi*. In view of this, our results cannot be reasonably extrapolated to the general canine populations of the areas where the study was done nor to those of the entire state.

Nonetheless, because of the sizable number of dogs we studied, our findings extend considerably our knowledge of canine *T. cruzi* infection in Oklahoma and suggest that veterinarians include the illness in their lists of possible diagnoses when examining dogs with consistent clinical signs including fever, lymphadenopathy, hepatosplenomegaly, abnormal cardiac rhythms, and congestive heart failure.

In the present study, the prevalence of *T. cruzi* infection among coonhounds was 15.4%, raising the question of how these dogs acquired the infection. *Trypanosoma cruzi*-infected raccoons, opossums, and armadillos have been found in many areas of the southern United States.<sup>16-18</sup> It is likely that coonhounds become parasitized with *T. cruzi* by attacking and eating infected wild animals, because it is recognized that the organism is easily transmitted to mammals when parasites come into contact with the oral mucosa. The fact that wild raccoons are a reservoir of *T. cruzi* in the United States has additional public health importance in that people who hunt raccoons, which is a popular activity in Oklahoma and throughout the South, may risk acquiring *T. cruzi* infection through contact with blood while skinning infected animals.

Similarly, the role of infected triatomine insects in the transmission of *T. cruzi* to dogs in Oklahoma is unclear. *Triatoma sanguisuga* is broadly distributed in the state and tends to inhabit dens or burrows of wild mammals where blood meals can be obtained easily.<sup>19</sup> Three of the 5 littermates of the index case in the present report were available for serologic testing, and 1 was seropositive for *T. cruzi* infection. The 2 infected littermates were the last 2 puppies in the litter to be sold, suggesting a common-source exposure. The puppies were reported to have a propensity for chasing bugs, and an ultraviolet light bug zapper was hanging over the dogs' kennel. A similar type of bug trap was positioned directly adjacent to the kennel housing infected dogs in LeFlore county. Some *Triatoma* species, especially those in the southwestern United States, are attracted to light. Therefore, transmission of *T. cruzi* to these dogs may have been a result of ingestion of infected triatomine insects.

Specific treatment of *T. cruzi* infection in dogs is problematic. Nifurtimox and benznidazole are the only 2 medications that are used to treat American trypanosomiasis in humans, and they can be obtained from the Centers for Disease Control and Prevention. Their efficacy is low, as only about 70% of patients with acute Chagas disease and 20% of those with longstanding infections are cured parasitologically. Little is known about the efficacy of nifurtimox and benznidazole for treating *T. cruzi* infections in dogs, and no studies have been done to determine optimal dosing schedules and duration of treatment. Because culture of blood samples obtained from the index case 11 months after initiation of treatment still yielded *T. cruzi*, it is clear that the prolonged course of nifurtimox did not eradicate the parasites.

The question of how to prevent transmission of *T. cruzi* merits discussion in the context of our findings. The principal means of reducing a dog's risk of acquiring *T. cruzi* would be strict indoor housing that would

preclude exposure to insect vectors or mammals that may harbor the parasite. Obviously, this is not a viable option for the hunting dogs that appear to be at highest risk. Additionally, no chemo- or immunoprophylactic methods are available for preventing transmission of *T cruzi* to dogs or humans. Thus, for the foreseeable future, *T cruzi* will continue to be enzootic in dogs in the areas we studied, and this poses a risk of transmission to veterinarians and others who are exposed to dog blood. The use of sharp instruments such as needles and scalpels in the course of providing care for dogs poses the highest risk of accidental exposure. There have been a number of instances of accidental needle-stick transmission of *T cruzi* to laboratory workers and several instances of transmission following exposure to contaminated material, all of which point to the highly infectious nature of the parasite.<sup>3,20</sup> The risk of exposure to blood from dogs infected with *T cruzi* that caregivers face is underscored by the accidental puncture with a needle contaminated with blood from the index case described in the present report. A similar incident prompted an investigation of *T cruzi* infection in coon-hounds in Virginia.<sup>1</sup> Fortunately, neither the veterinarian nor 6 other persons who had direct exposure to blood from the index case described in the present report developed antibodies to *T cruzi*. Likewise, the veterinarian in Virginia did not develop clinical or serologic evidence of *T cruzi* infection.

Preventive measures are clearly warranted for veterinarians and veterinary technicians who practice in regions where *T cruzi* is enzootic. Appropriate barrier precautions against bloodborne pathogens should be used including wearing gloves while drawing blood samples, tending to intravenous catheters, and performing other invasive procedures. Proper handling and disposal of needles and other sharp instruments should be emphasized to all staff. In addition, laboratory personnel should wear gloves and protective eyewear when processing blood or tissue samples from dogs that may be infected with *T cruzi*. Dog owners should protect themselves similarly when situations in which the potential for exposure to infected blood arise. Fortunately, there is no risk of transmission of *T cruzi* from infected dogs to people through activities that do not involve exposure to blood.

<sup>a</sup>Lampit, Bayer, Buenos Aires, Argentina.

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