Published in final edited form as:
J Parasitol. 2009 June ; 95(3): 775–777. doi:10.1645/GE-1734.1.

Evaluation of the Chagas Stat-Pak™ Assay for Detection of *Trypanosoma cruzi* Antibodies in Wildlife Reservoirs

Michael J. Yabsley[*,†,§], **Emily L. Brown**[*,†,§], and **Dawn M. Roellig**[†,‡,§]

Michael J. Yabsley: myabsley@uga.edu

[*]Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, Georgia 30602

[†]Southeastern Cooperative Wildlife Disease Study, Department of Population Health, Athens, Georgia

[‡]Department of Infectious Diseases, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602

Abstract

An immunochromatographic assay (Chagas Stat-Pak™) was evaluated for the detection of *Trypanosoma cruzi* antibodies in 4 species of wildlife reservoirs. Antibodies to *T. cruzi* were detected in raccoons (*Procyon lotor*) (naturally and experimentally infected) and degus (*Octodon degu*) (experimentally-infected) using the Chagas Stat-Pak. In naturally exposed wild raccoons, the Chagas Stat-Pak had a sensitivity and specificity of 66.7–80.0% and 96.3%, respectively. Compared with indirect immunofluorescent antibody assay results, serocon-version as determined by Chagas Stat-Pak was delayed for experimentally infected raccoons, but occurred sooner in experimentally infected degus. The Chagas Stat-Pak did not detect antibodies in naturally or experimentally infected Virginia opossums (*Didelphis virginiana*) or in experimentally infected short-tailed opossums (*Monodelphis domestica*). These data suggest that the Chagas Stat-Pak might be useful in field studies of raccoons and degus when samples would not be available for more-conventional serologic assays. Because this assay did not work on either species of marsupial, the applicability of the assay should be examined before it is used in other wild species.

Trypanosoma cruzi, the etiological agent of American trypanosomiasis, or Chagas' disease, is an important medical and veterinary pathogen. In some hosts, such as humans and dogs, *T. cruzi* may cause fatal myocarditis during the chronic phase of the disease. Although an estimated 10–12 million people are infected in Latin America, autochthonous human infections in the United States are rare, with a total of only 6 cases having been reported from California, Louisiana, Texas, and Tennessee since 1955 (Woody and Woody, 1955; Navin et al., 1985; Herwaldt et al., 2000; Dorn et al., 2007). However, *T. cruzi* infection in domestic dogs has been reported from Texas, Louisiana, Oklahoma, Georgia, South Carolina, and Virginia (see Meurs et al., 1998; Kjos et al., 2008).

In contrast, reports of *T. cruzi* in wildlife, e.g., raccoons and opossums, are relatively common (John and Hoppe, 1986; Yabsley et al., 2001; Brown, 2008), although disease in these reservoirs is rare. Several techniques have been utilized to determine the prevalence of *T. cruzi* in wildlife species, i.e., direct examination of blood; culture of blood or tissues, or both; polymerase chain reaction (PCR) testing of blood or tissues, or both; and serology

[§]All 3 authors contributed equally to this work.

(McKeever et al., 1958; John and Hoppe, 1986; Yabsley et al., 2001; James et al., 2002). The majority of previous studies have used hemoculture or the direct examination of blood to determine prevalence. These methods underestimate prevalence, because parasitemia decreases during the chronic stage of the infection. Serological testing is considered the most sensitive assay for determining prevalence, because antibodies to *T. cruzi* persist during the chronic phase (Yabsley et al., 2001; Yabsley and Noblet, 2002a). For example, prevalence of *T. cruzi* in raccoons (>10 raccoons tested) from the southern United States, based on culture, ranged from 15–43% (Karsten et al., 1992; Pung et al., 1995; Pietrzak and Pung, 1998; Yabsley and Noblet, 2002a; Brown, 2008), while the prevalence based on serologic testing (indirect immunofluorescent antibody test, IFA) was 33–70% (Yabsley et al., 2001; Hancock et al., 2005; Brown, 2008).

Rapid immunochromatographic assays for *T. cruzi* have been developed and validated for use in humans and dogs (Luquetti et al., 2003; Ponce et al., 2005; Cardinal et al., 2006), but have not been corroborated for use in other mammalian hosts. In general, these assays utilize a dye bound to an antibody-binding protein, e.g., protein A or G, which together will bind to all antibodies present in serum, plasma, or a blood sample. If antibodies specific to the recombinant antigens are present in a sample, then this protein-dye-antibody complex will be visualized as a colored band or a spot.

The Chagas Stat-Pak™ (Chembio Diagnostics Inc., Medford, New York) utilizes a combination of several recombinant antigens specific for *T. cruzi* (antigens described by Umezawa et al., 2003). For humans and dogs, these assays have high sensitivity and specificity and, if validated for wildlife reservoirs, would provide a field-friendly assay that is rapid, simple, and stable at room temperature, and that can be run with small quantities of blood, plasma, or serum. The objective of the current study was to evaluate the use of the commercially available Chagas Stat-Pak for use in 4 wildlife reservoirs species, 2 from North America (raccoon [*Procyon lotor*] and Virginia opossum [*Didelphis virginiana*]) and 2 from South America (degu [*Octodon degu*] and shorttailed opossum [*Monodelphis domestica*]).

Archived serum samples used in this study were collected during previous studies and stored at −20 C until testing. Samples from wild hosts were collected from raccoons (n = 57) and Virginia opossums (n = 11) from Georgia and Florida (Brown, 2008). Four wildlife reservoir species (raccoons, degus, Virginia opossums, and short-tailed opossums) were experimentally infected with *T. cruzi* to provide confirmed positive samples and to compare time to seroconversion by indirect immunofluorescent antibody (IFA) assay and Chagas Stat-Pak assays. For experimental infections, raccoons, degus, and short-tailed opossums were both captive bred and obtained from commercial sources; the Virginia opossums were from 2 litters of joeys raised in our animal facility by wild-caught nursing females (both females were IFA and culture negative for *T. cruzi*). All animals were housed indoors and shown to be IFA and culture negative for *T. cruzi* before experimental inoculation. Animals were inoculated with 1×10^6 DH82 macrophage-derived trypomastigotes (Roellig et al., 2009) by intraperitoneal (degus, short-tailed opossums, Virginia opossums) or intravenous (raccoons) routes. Negative-control animals were similarly inoculated with equivalent volumes of media. Blood samples were aseptically collected into ethylenediaminetetraacetic acid (EDTA) tubes at various days post-inoculation; plasma was collected and frozen at −20 C until testing (Roellig et al., 2009; Roellig et al., unpubl.). Numbers of animals sampled at each sampling date varied depending on the volume of plasma available for testing.

To confirm infections, culture attempts in LIT medium or DH82 canine macrophages were made on a subset of wild animals and on all experimental hosts, as described (Yabsley and Noblet, 2002b; Yabsley et al., 2004). IFA testing of all samples was performed as described

(Yabsley et al., 2001; Brown, 2008) using the following secondary antibodies, i.e., a goat anti-raccoon IgG (Kirkegaard and Perry Laboratories [KPL], Gaithersburg, Maryland) and a rabbit anti-rat IgG (KPL) for raccoons and degus, respectively. For both species of opossums, a rabbit anti-opossum IgG (Bethyl Laboratories, Montgomery, Texas) was used followed by a fluorescin-labeled anti-rabbit IgG (KPL). Slides were examined using an Olympus microscope (BH-2, Center Valley, Pennsylvania). The Chagas Stat-Pak assay was conducted per the manufacturer's instructions. Chi-square (χ^2) analysis ($P = 0.05$) was used to determine if differences existed between the serologic assay results.

The Chagas-Stat Pak detected antibodies to *T. cruzi* in both naturally and experimentally infected raccoons, as well as in experimentally infected degus (Tables I, II). No significant differences were noted in the prevalence of *T. cruzi* antibodies in 57 wild raccoons between IFA and Chagas Stat-Pak assays (42.1% vs. 33.3%, respectively, $\chi^2 = 0.934$, $P = 0.3339$). Discordant results were obtained for 9 of 57 (15.8%) samples (Table I). Using data from naturally infected raccoons, we calculated the sensitivity of the Chagas Stat-Pak in 2 ways: First, based on a comparison with IFA-positive animals only, the sensitivity of the Chagas Stat-Pak was 66.7% (16 of 24 IFA positives) (Table I); and second, based on raccoons that were both IFA and culture positive, the sensitivity of the Chagas Stat-Pak was 80% (12 of 15 positives) (Table I). The specificity of the Chagas Stat-Pak was 96.3% (based on 26 of 27 raccoons that were both IFA and culture negative). For 4 experimentally infected raccoons, the sensitivity of the Chagas Stat-Pak was lower, compared with IFA testing, during the first 3 wk of infection (Table II). In contrast, the Chagas Stat-Pak test was more sensitive for detecting early infections in some of the experimentally infected degus (Table II). Although IFA and culture provided reliably positive results, the Chagas Stat-Pak failed to detect anti-*T. cruzi* antibodies in either of the experimentally infected marsupial species we tested. Similar results were found in the naturally infected Virginia opossums (n = 7). All IFA and culture-negative opossums (4 Virginia opossums and 3 short-tailed opossums) were also negative by the Chagas Stat-Pak.

A number of different species of mammals serve as wildlife reservoir hosts for *T. cruzi*, and the availability of a rapid test would improve the ability to study the epizootiology of this important pathogen. Previously, a commercially available rapid assay (*Trypanosoma* Detect™ MRA Rapid Test; InBios International Ltd., Seattle, Washington) successfully detected *T. cruzi* antibodies in 2 IFA-positive gray foxes (*Urocyon cinereoargenteus*) from South Carolina (Rosypal et al., 2007).

In the present study, we found that the Chagas Stat-Pak successfully detected antibodies in 2 known reservoir hosts, raccoons and degus. The assay also detected *T. cruzi* antibodies in 2 experimentally infected laboratory rodents (Balb/c mice and Windsor rats) (data not shown), suggesting the assay might be useful for laboratory experiments using rodents. However, the assay failed to detect antibodies in 2 species of marsupials, which is likely because the staphylococcal and streptococcal proteins commonly used in rapid tests only variably bind with antibodies from different marsupial species (Kronvall et al., 1970; Kronvall, 1973; De Chateau et al., 1993).

The sensitivity of the Chagas Stat-Pak assay for naturally and experimentally infected raccoons was lower compared with studies on humans (93.4–98.5%) (Luquetti et al., 2003; Roddy et al., 2008) and dogs (94%) (Cardinal et al., 2006). There are several possible explanations for this finding. In the current study, we noted species differences in the sensitivity of the Chagas Stat-Pak during early *T. cruzi* infections. Experimentally infected raccoons seroconverted, as detected by IFA, between days post-infection (DPI) 4–10; however, Chagas Stat-Pak seroconversion was not detected until DPI 28, while degus seroconverted with the Chagas Stat-Pak by DPI 7. Previous studies did not evaluate the

sensitivity of the Chagas Stat-Pak during acute infections (Luquetti et al., 2003; Cardinal et al., 2006). Furthermore, previous studies of dogs and humans were conducted on banks of serum samples that were seropositive by multiple serologic assays. We only used 1 serologic assay (IFA) to determine the serostatus of the animals included in this study. Although the majority of samples are seropositive by multiple assays, discordant results between assays led to a small percentage of samples being classified as either "borderline" or equivocal (Yabsley et al., 2001; Cardinal et al., 2006). If the samples used in our study had been tested with multiple serologic assays (ELISA, HA, etc.), some borderline IFA-positive samples might have tested negative, resulting in their exclusion and a consequent increase in assay sensitivity for that species. These data suggest that the Chagas Stat-Pak might be useful in field studies of some species when samples would not be available for more conventional serologic assays, or if testing is impractical. Because this assay did not work on either species of marsupial, it must be emphasized that any commercial serologic assay or rapid test must be validated for use in wild animal species before wide-spread use in epidemiological studies.

The authors would like to thank K. McMillan and the animal care staff at the University of Georgia, College of Veterinary Medicine, for assistance with experimental animals. This study was supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases grant R15 AI067304. We also would like to thank Chembio Diagnostics Inc., Medford, New York, for donating some of the tests used in this study.

LITERATURE CITED

Brown, EL. M.S. Thesis. Athens, Georgia: University of Georgia; 2008. Seroprevalence of *Trypanosoma cruzi* in mammals of the United States; 44 p.

Cardinal MV, Reithinger R, Gürtler RE. Use of an immunochromatographic dipstick test for rapid detection of *Trypanosoma cruzi* in sera from animal reservoir hosts. Journal of Clinical Microbiology 2006;44:3005–3007. [PubMed: 16891528]

De Chateau M, Nilson BHK, Erntell M, Myhre E, Magnusson CGM, Akerstrom B, Bjorck L. On the interaction between protein L and immunoglobulins of various mammalian species. Scandinavian Journal of Immunology 1993;37:399–405. [PubMed: 8469922]

Dorn PL, Pernacario L, Yabsley MJ, Roellig DM, Balsamo G, Diaz J, Wesson D. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emerging Infectious Diseases 2007;13:605–607. [PubMed: 17553277]

Hancock K, Zajac AM, Pung OJ, Elvinger F, Rosypal AC, Lindsay DS. Prevalence of antibodies to *Trypanosoma cruzi* in raccoons (*Procyon lotor*) from an urban area of Northern Virginia. Journal of Parasitology 2005;91:470–472. [PubMed: 15986630]

Herwaldt BL, Grijalva MJ, Newsome AL, McGhee CR, Powell MR, Nemec DG, Steurer FJ, Eberhard ML. Use of polymerase chain reaction to diagnose the fifth reported U.S. case of autochthonous transmission of *Trypanosoma cruzi*—Tennessee, 1998. Journal of Infectious Diseases 2000;181:395–399. [PubMed: 10608796]

James MJ, Yabsley MJ, Pung OJ, Grijalva M. Amplification of *Trypanosoma cruzi*-specific DNA sequences in formalin-fixed raccoon tissues using polymerase chain reaction. Journal of Parasitology 2002;88:989–993. [PubMed: 12435142]

John DT, Hoppe KL. *Trypanosoma cruzi* from wild raccoons in Oklahoma. American Journal of Veterinary Research 1986;47:1056–1059. [PubMed: 3087247]

Karsten V, Davis C, Kuhn R. *Trypanosoma cruzi* in wild raccoons and opossums from North Carolina. Journal of Parasitology 1992;78:547–549. [PubMed: 1597808]

Kjos SA, Snowden KF, Craig TM, Lewis B, Ronald N, Olson JK. Distribution and characterization of canine Chagas' disease in Texas. Veterinary Parasitology 2008;152:249–256. [PubMed: 18255233]

Kronvall G. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin G. Journal of Immunology 1973;111:1401–1406.

Kronvall G, Seal US, Finstad J, Williams RC Jr. Phylogenetic insight into evolution of mammalian Fc fragment of γG globulin using staphylococcal protein A. Journal of Immunology 1970;104:140–147.

Luquetti AO, Ponce C, Ponce E, Esfandiari J, Schijman A, Revollo S, Añez N, Zingales B, Ramgel-Aldao R, Gonzalez A, et al. Chagas' disease diagnosis: A multicentric evaluation of Chagas Stat-Pak™, a rapid immunochromatographic assay with recombinant proteins of *Trypanosoma cruzi*. Diagnostic Microbiology and Infectious Disease 2003;46:265–271. [PubMed: 12944018]

McKeever SG, Gorman GW, Norman L. Occurrence of *Trypanosoma cruzi*-like organisms in some mammals from southwestern Georgia and northwestern Florida. Journal of Parasitology 1958;44:583–587. [PubMed: 13621315]

Meurs KM, Anthony MA, Slater M, Miller MW. Chronic *Trypanosoma cruzi* infection in dogs: 11 cases (1987–1996). Journal of the American Veterinary Medical Association 1998;213:497–500. [PubMed: 9713531]

Navin TR, Roberto RR, Juranek DD, Limpakarnjanarat K, Mortenson EW, Clover JR, Yescott RE, Taclindo C, Steurer F, Allain D. Human and sylvatic *Trypanosoma cruzi* infection in California. American Journal of Public Health 1985;75:366–369. [PubMed: 3919598]

Pietrzak SM, Pung OJ. Trypanosomiasis in raccoons from Georgia. Journal of Wildlife Diseases 1998;34:132–136. [PubMed: 9476234]

Ponce C, Ponce E, Vinelli E, Montoya A, De Aguilar V, Gonzalez A, Zingales B, Rangel-Aldao R, Levin MJ, Esfandiari J, et al. Validation of a rapid and reliable test for diagnosis of Chagas' disease by detection of *Trypanosoma cruzi*-specific antibodies in blood of donors and patients in Central America. Journal of Clinical Microbiology 2005;43:5065–5068. [PubMed: 16207963]

Pung OJ, Banks CW, Jones DN, Krissinger MW. *Trypanosoma cruzi* in raccoons, opossums, and triatomine bugs in southeast Georgia, U.S.A. Journal of Parasitology 1995;81:324–326. [PubMed: 7707220]

Roddy P, Goiri J, Flevaud L, Palma PP, Morote S, Lima N, Villa L, Torrico F, Albajar-Viñas P. Field evaluation of a rapid immunochromatographic assay for *Trypanosoma cruzi* infection using whole blood in Sucre, Bolivia. Journal of Clinical Microbiology 2008;46:2022–2027. [PubMed: 18400910]

Roellig DM, Ellis AE, Yabsley MJ. Oral transmission of *Trypanosoma cruzi* with opposing evidence for the theory of carnivory. Journal of Parasitology 2009;95:360–364. [PubMed: 18763853]

Rosypal AC, Tidwell RR, Lindsay DS. Prevalence of antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in wild canids from South Carolina. Journal of Parasitology 2007;93:955–957. [PubMed: 17918387]

Umezawa ES, Bastos SF, Coura JR, Levin MJ, Gonzalez A, Rangel-Aldao R, Zingales B, Luquetti AO, Da Silveira J Franco. An improved serodiagnostic test for Chagas' disease employing a mixture of *Trypanosoma cruzi* recombinant antigens. Transfusion 2003;43:91–97. [PubMed: 12519436]

Woody NC, Woody HB. American trypanosomiasis (Chagas' disease): First indigenous case in the United States. Journal of the American Medical Association 1955;159:676–677. [PubMed: 13263111]

Yabsley MJ, Noblet GP. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. Journal of Wildlife Diseases 2002a;38:75–83. [PubMed: 11838232]

Yabsley MJ, Noblet GP. Biological and molecular characterization of a raccoon isolate of *Trypanosoma cruzi* from South Carolina. Journal of Parasitology 2002b;88:1273–1276. [PubMed: 12537130]

Yabsley MJ, Noblet GP, Pung OJ. Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). Journal of Parasitology 2001;87:1155–1159. [PubMed: 11695383]

Yabsley MJ, Norton TM, Powell MR, Davidson WR. Molecular and serologic evidence of tick-borne ehrlichiae in three species of lemurs from St. Catherine's Island, Georgia, USA. Journal of Zoological and Wildlife Medicine 2004;35:503–509.

TABLE I

Results of hemoculture, indirect immunofluorescent antibody (IFA), and Chagas Stat-Pak™ assay testing of 57 wild raccoons from Georgia and Florida.

Chagas Stat-Pak	Hemoculture positive		Hemoculture negative	
	IFA positive	IFA negative	IFA positive	IFA negative
Positive	12	2	4	1
Negative	3	4	5	26

TABLE II

Indirect immunofluorescent antibody (IFA) and Chagas Stat-Pak™ testing results for raccoons (*Procyon lotor*) and degus (*Octodon degu*) experimentally-infected with *Trypanosoma cruzi*.

Raccoons												Degus											
DPI* 3		DPI 10		DPI 28		DPI 42		DPI 112‡		Negative controls		DPI 7		DPI 14		DPI 21		DPI 56		DPI 112‡		Negative controls	
IFA	Stat†	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat	IFA	Stat
2/4	0/4	3/4	0/4	3/3	2/3	2/2	2/2	3/3	3/3	0/2	0/2	0/5	2/5	6/6	3/6	4/4	3/4	2/2	2/2	3/3	1/3	0/1	0/1

*DPI, days post-inoculation.

†Stat, Chagas Stat-Pak™ results.

‡All animals were hemoculture positive on DPI 112, which confirmed infection in each animal.