Case Report

Chagas Disease in 2 Geriatric Rhesus Macaques (Macaca mulatta) Housed in the Pacific Northwest

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Chagas disease (American trypanosomiasis) is caused by the protozoan parasite Trypanosoma cruzi. It is endemic in Latin America but also is found in the southern United States, particularly Texas and along the Gulf Coast. Typical clinical manifestations of Chagas disease are not well-characterized in rhesus macaques, but conduction abnormalities, myocarditis, and encephalitis and megaesophagus have been described. Here we report 2 cases of Chagas disease in rhesus macaques housed in the northwestern United States. The first case involved a geriatric male macaque with cardiomegaly, diagnosed as dilated cardiomyopathy on ultrasonographic examination. Postmortem findings included myocarditis as well as ganglioneuritis in the esophagus, stomach, and colon. The second case affected a geriatric female macaque experimentally infected with SIV. She was euthanized for a protocol-related time point. Microscopic examination revealed chronic myocarditis with amastigotes present in the cardiomyocytes, ganglioneuritis, and opportunistic infections attributed to her immunocompromised status. Banked serum samples from both macaques had positive titers for T. cruzi. T. cruzi DNA was amplified by conventional PCR from multiple tissues from both animals. Review of their histories revealed that both animals had been obtained from facilities in South Texas more than 12 y earlier. Given the long period of clinical latency, Chagas disease may be more prevalent in rhesus macaques than typically has been reported. T. cruzi infection should be considered for animals with unexplained cardiac or gastrointestinal pathology and that originated from areas known to have a high risk for disease transmission.

Abbreviations: DCM, dilated cardiomyopathy; CMV, cytomegalovirus; NHP, nonhuman primate.

Chagas disease is caused by the hemoflagellate protozoan parasite Trypanosoma cruzi. The disease is endemic in many regions of South and Central America, and its range extends to the southern United States. In the United States, there is evidence that the parasite has established a domestic transmission cycle with canine reservoirs,19 and there are numerous wildlife reservoirs, most importantly armadillos, raccoons, rodents, and opossums.6 The main mode of transmission is via arthropod vectors, primarily triatomine species (kissing bugs or cone-nosed bugs), which serve as intermediate hosts. Vector species are present in the southern half of the United States.2 Infection has been reported sporadically in domestic nonhuman primate (NHP) colonies.12 Autochthonous insect vector-mediated transmission in humans in the United States has been reported rarely.13 Transmission of T. cruzi to NHP is thought to occur mainly through insect vectors, specifically by contamination of the oral mucous membranes with parasite-containing feces during consumption of the bug. The infection may remain subclinical for years and, similar to that in people, affects the nervous system, digestive system, and heart. Clinical findings in NHP are infrequent but can include subcutaneous edema, fever, anorexia, lethargy, heart failure, and sudden death.4,5 As in humans, the disease in NHP consists of an acute phase, with a paucity of clinical manifestations, and a chronic phase, characterized by irreversible cardiomyopathy leading to cardiac dysfunction and death. Chronically infected NHP in the indeterminate form of the chronic phase can exhibit subclinical conduction and echocardiographic abnormalities.8 T. cruzi infections have been reported in a number of NHP species housed in Texas, Louisiana, and Georgia. Species affected include rhesus macaques (Macaca mulatta),15,17 cynomolgus macaques (M. fascicularis),29,41 yellow baboons (Papio cynocephalus), olive baboons (P. anubis),12,14,41 pig-tailed macaques (M. nemestrina),12,38 squirrel monkeys (Saimiri sciureus),13 ring-tailed lemurs (Lemur catta),19,30 black-eyed lemurs (Eulemur macaco flavifrons), black and white ruffed lemurs (Varecia variegata variegata),15 pileated gibbons (Hylobates pileatus),26 a lion-tailed macaque (M. silenus),30 a Celebes crested black macaque (M. nigra),27 and a chimpanzee (Pan troglodytes).4 Here we report on 2 cases of Chagas disease in rhesus macaques housed in the northwestern United States but that originated from South Texas.

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The first case involves a 25-y-old male rhesus macaque assigned to a primate aging protocol. The second case affected a 19-y-old female rhesus macaque assigned to an AIDS vaccine...
study. Both animals were captive-reared Indian-origin rhesus macaques obtained from a primate breeding facility in Alice, TX, in 1998 and 1997, respectively. Both animals had previously been tested for B virus (Macacine herpesvirus 1), simian T-lymphotropic virus, and simian retrovirus type D and were serologically negative. These animals were singly housed at an AAALAC-accredited facility, and the research protocols were approved by the IACUC. All animals at the facility are maintained and used in accordance with the Guide for the Care and Use of Laboratory Animals.

Routine husbandry parameters include 12:12-h light-dark cycles; controlled temperature, humidity, and ventilation; commercial primate chow (Purina 5000, Purina Mills, St Louis, MO); water ad libitum; and daily supplementation with a variety of fresh fruits and vegetables.

Clinical findings. In our first case, cardiomegaly was noted on protocol-related MRI in January 2009. The macaque was clinically asymptomatic and had no history of a cardiac murmur or arrhythmia. On physical examination under ketamine sedation (10 mg/kg IM; Ketasetria, Butler Schein Animal Health, Dublin, OH), there was no evidence of clinical heart disease and the CBC and serum chemistry results were unremarkable. Thoracic radiographs and ECG results were supportive of generalized cardiomegaly. Echocardiographic features including 4-chamber dilation, ventricular-wall thinning, and hypokinesis confirmed a diagnosis of dilated cardiomyopathy (DCM). In July 2009, angiotensin-converting enzyme inhibitor therapy with enalapril maleate (0.5 mg/kg PO twice daily; Butler Schein Animal Health) was started, and the patient was monitored for azotemia. Approximately 1 mo later, α and β blockade was initiated with carvedilol (0.03 mg/kg once daily; Diamondback Drugs, Scottsdale, AZ). The patient was followed with biannual physical exams and monthly Doppler systolic blood-pressure measurements. The carvedilol dose was titrated to a goal dose of 0.4 mg/kg over 10 mo, according to reported experience with the drug in a chimpanzee. A cardiac evaluation was repeated in December 2010 by using tiletamine hydrochloride–zolazepam hydrochloride sedation (3 mg/kg IM; Telazol, Fort Dodge Animal Health, Fort Dodge, IA). No overt signs of congestive heart failure were noted and no changes from the previous evaluation were noted on echocardiography. However, the patient was hypoxic under sedation, with peripheral oxygen saturation of 88% to 90%. Supplemental oxygen support was provided via a nasal cannula, and oxygen saturation immediately improved to 100%. Due to this patient’s poor long-term prognosis and the subsequent limitations for his projected research use, he was euthanized.

The second case presented with a 1-day history of lying down in her cage and reluctance to sit up. Four months previously, she had been infected with SIVmao239 as part of a research protocol. Her previous history and physical exam findings were otherwise unremarkable. She received tramadol HCl (25 mg PO every 12 h; Butler Schein Animal Health) for supportive care. The investigative staff reported that she had reached the protocol endpoints of low CD4+ lymphocyte counts and elevated plasma viral load, and she was euthanized the following day.

Gross necropsy findings. Necropsies were performed on both animals at the time of euthanasia; both were in overweight to obese nutritional condition. In the first case, the heart was generally enlarged, flabby, and dilated (Figure 1 A). Both the left and right ventricular free walls were thinned and the chambers expanded. There were several 0.5- to 1-cm areas of pallor within the ventricular free wall that were visible from the epicardial surface. On cut section of both ventricular free walls and the interventricular septum, there were foci of pallor and fibrosis within the myocardium. The mitral valve leaflets were nodular and thickened. There were areas of pallor on the endocardial surface of the left ventricle (Figure 1 B). The papillary muscles within the left ventricle were predominately pale and fibrotic. The aortic arch was dilated to approximately 2 times normal diameter. There were multiple fibrofatty streaks and plaques within the thoracic and abdominal aortic intima; the abdominal aorta was more severely affected than was the thoracic. Multiple 3- to 5-mm air-filled subpleural bullae were scattered throughout the lung lobes, consistent with prior lung mite infection.

In the second case, gross findings included a small, rounded spleen with no lymphoid tissue visible on cut section. The dorsal aspect of the left caudal lung lobe contained mild, multifocal pulmonary hemorrhages and a small number of subpleural bullae scattered throughout the lung lobes. A parovarian cyst was noted adjacent to the left ovary, and there were multiple diverticula in the ascending, transverse, and descending colons. Gross examination of the heart was unremarkable.

Histopathologic findings. Microscopic examination of the heart from the first macaque revealed multifocal interstitial fibrosis with moderate to marked chronic lymphoplasmacytic and eosinophilic myocarditis with fibrosis and cardiomyocyte degeneration (Figure 1 C). The second case had mild chronic lymphohistiocytic myocarditis and rare intracellular protozoal pseudocysts containing numerous amastigotes (Figure 1 D). In addition, in the first case there was a moderate chronic lymphoplasmacytic ganglioneuritis and leiomysitis in the esophagus, stomach, and colon (Figure 1 E). The second macaque exhibited mild chronic ganglioneuritis and leiomysitis in the esophagus, rectum, and gall bladder (Figure 1 F) as well as findings associated with SIV infection, including lymphoid depletion, trichomonad gastritis, neutrophilic neuritis and ganglionitis, as well as pneumonia and lymphohistiocytic meningomencephalitis attributed to SIV giant cell disease. Both macaques had atherosclerosis and bronchiolar dilatation, consistent with a previous lung mite infection.

Serology. Stored serum from initial quarantine (29 June 1998) and necropsy samples (21 December 2010) from the first case were submitted to the Division of Bacteriology and Parasitology (Tulane National Primate Research Center, Covington, LA) and analyzed by using a commercial kit (Trypanosoma Detect Rapid Test; Inbios International, Seattle, WA), with positive results on both samples. Serum obtained at necropsy (23 February 2012) from the second case was tested at VRL Laboratories (San Antonio, TX) and was positive for IgG antibodies against T. cruzi.

Immunohistochemistry for rhesus cytomegalovirus (CMV). Sections of stomach and sciatic nerve exhibiting neutrophilic neuritis and ganglionitis from the macaque in case 2 were evaluated for CMV antigen by using a monoclonal antibody specific for the immediate-early protein of rhesus CMV.

PCR analysis for T. cruzi. DNA extraction was performed on formalin-fixed, paraffin-embedded samples. Sections (n = 5; width, 10 µm) were cut from paraffin blocks and placed in microcentrifuge tubes. DNA was extracted by using the DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer’s specifica-
Figure 1. Chagas disease in rhesus macaques. (A) Heart from case 1 shows generalized enlargement and globoid outline. Aorta is markedly dilated (arrow). Scale bar, 1 cm. (B) Opened left ventricle of heart from case 1 shows enlargement of left ventricle and pale papillary muscles (arrow). Scale bar, 1 cm. (C) Photomicrograph of heart from case 1 exhibits extensive chronic lymphoplasmacytic inflammation with fibrosis and myocyte degeneration (arrow). Hematoxylin and eosin stain; bar, 100 µm. (D) Photomicrograph of heart from case 2 shows multiple T. cruzi amastigotes (arrow) within a myocyte fiber. There is mild lymphohistiocytic inflammation (asterisk). Hematoxylin and eosin stain; bar, 50 µm. (E) Photomicrograph of muscular wall of the esophagus of case 1 exhibits marked chronic lymphocytic ganglioneuritis (asterisk). Hematoxylin and eosin stain; bar, 100 µm. (F) Photomicrograph of muscular wall of the esophagus of case 2 exhibits mild chronic lymphocytic ganglioneuritis (asterisk). Hematoxylin and eosin stain; bar, 50 µm.
tions and quantified by a spectrophotometer (NanoDrop Technologies, Wilmington, DE). Standard techniques of conventional PCR analysis were used to test for the presence of parasite and host DNA from both animals. Reference DNA for T. cruzi (Tulahuen strain) was purchased from American Type Culture Collection (Manassas, VA) and was used as the positive control.

A 330-bp kinetoplast minicircle DNA sequence specific to T. cruzi amplified by using primers S35 (5′-AAA TAA TGT ACG GAG GAG ATG CAT GA 3′) and S36 (5′-GGG TTC GAT TGG GTT TGG TGT 3′) and Platinum Taq DNA polymerase (Life Technologies, Carlsbad CA) under ‘touchdown’ conditions. Amplification reactions were performed in a final volume of 25 µL containing 0.5 U Platinum Taq DNA Polymerase High Fidelity (Life Technologies), 0.2 mM of each of the dNTPs, 2 mM MgSO4, 0.4 µM of each primer, 1× high-fidelity buffer, and 1 µL (40 to 113 ng/µL) of template DNA. The thermal cycling conditions were as follows: after initial denaturation at 94 °C for 2 min, the annealing temperature was set to 59 °C for 2 cycles, and thereafter decreased by 1 °C for every 2 cycles until the temperature reached 52 °C. At those conditions, the samples were run for an additional 20 cycles. The PCR product was loaded on 1% agarose gels, and electrophoresis was performed. The gels then were stained with ethidium bromide, visualized under UV illumination, and photographed. In separate reactions, a 244-bp sequence of the 16S rRNA gene was amplified from the samples as a control for failure to obtain good-quality DNA. It was done using the primer set L2513 (5′-GCC TGT TTA CCA AAA ACA TCA C 3′) and H2714 (5′-CTC CAT AGG GTC TTC TCG TCT T 3′).

Discussion

This report describes T. cruzi infection in 2 rhesus macaques from South Texas that had been housed in a nonendemic area for more than a decade. The first case was diagnosed by serology, the second by direct observation of amastigotes. There is limited information on the current prevalence of T. cruzi infection, screening measures for NHP in colonies in the southern United States (particularly Texas), and the effects of infection on animal models for biomedical research. One South Texas institution reported an infection rate of 8.5% in an outdoor colony of rhesus macaques in 1977. Another 2009 report estimated a seroprevalence of 2% to 3% in a colony of baboons. Most recently, a 2013 study established a prevalence of 8.5% in an outdoor colony of cynomolgus macaque in South Texas. In addition, a 2013 study reported a prevalence of infection (1.6%) in a Louisiana NHP colony. Currently in Texas, dogs, armadillos, coyotes, raccoons, opossums, and woodrats have been identified as reservoir hosts of T. cruzi. The vector is widely distributed throughout the state, with at least 7 triatomine species found in over 40% of counties. The T. cruzi infection rate of the vector in Texas has been reported to be greater than 50%. South Texas represents a high-risk area for disease transmission based on abundance of the vector, suitable habitat for the vector, and incidence of parasites.

In people, diagnosis of T. cruzi infection is based on the detection of parasites in the blood during the acute phase and through detection of circulating antibodies in the chronic phase. Serology can be highly sensitive, but antibody levels vary due to oscillating parasitemia, and the tests have variable specificity. PCR, hemoculture, and xenodiagnosis are highly specific but have low sensitivity, particularly in the chronic phase. It is suggested that 2 parallel tests be performed, one with high sensitivity and one with high specificity. Immunochromatographic dipstick tests have been developed for a range of tropical diseases, recently including Chagas disease, in an effort to provide an easy to use, inexpensive, and accurate diagnostic tool. The lateral flow test has been determined to have high specificity and sensitivity for detecting T. cruzi antigens in canine and human sera and was recently validated for NHP.

Several years after the chronic phase has started, 20% to 40% of people develop clinical signs of Chagas disease. Chagas cardiomyopathy is the most important presentation of Chagas disease because of its characteristics, frequency, and severity and is the main cause of death in people infected with T. cruzi. The pathogenesis of the cardiomyopathy is not completely understood but is thought to be dependent on parasite persistence; with either direct damage to the myocardium or via parasite-driven immunopathology with subsequent myocardial damage and fibrosis. Clinical manifestations in people include arrhythmias and other ECG abnormalities as well as heart failure, thromboembolism, stroke, and sudden death. Due to the parasite’s tendency to concentrate in muscle and ganglion cells, chronically infected patients may develop an enteric neuropathy and Chagas digestive megasymphyses, such as megasphagous. Clinical signs of chronic Chagas disease appear to be less common in NHP; however, both cardiac and gastrointestinal disease have been reported.

On the basis of clinical and pathologic features, the rhesus macaque in the first case was diagnosed with DCM secondary to chronic Chagas disease. DCM has been seen secondary to infectious processes, such as SIV, and as a sequela to angiomaticous pheochromocytomas in rhesus macaques. Prior reports of chronically infected rhesus macaques describe cardiac dysfunction characterized by conduction disturbances and less commonly, echocardiographic changes. DCM secondary to Chagas disease has not been reported in the rhesus macaque.

History and pathology findings in the second case suggest reactivation of Chagas disease after infection with SIV. Reactivation, characterized by circulating parasitemia in chronically infected hosts, has been reported in immuno compromised patients with HIV–AIDS since the early 1990s. The clinical manifestations of reactivated Chagas in HIV patients differ from those immunosuppressed from other causes, with approximately 79% experiencing central nervous system disease. Similarly, Chagas encephalitis developed in an immunosuppressed Celebes macaque positive for simian type D retrovirus and treated with corticosteroids. One case of T. cruzi reactivation subsequent to SIV infection has been reported in a rhesus macaque. This animal originated from a T. cruzi-endemic area and had a subclinical infection until infection with SIV. The clinical findings in the reported animal were consistent with SIV infection, and at 60 wk postinfection, at a stage of severe immunosuppression, flagellates were detected on blood smears and in coculture with peripheral blood mononuclear cells. Postmortem findings included an interstitial myocarditis and identification of a pseudocyst with amastigotes in the myocardium, similar to findings from our second case.
Ganglioneuritis and leiomyositis of the gastrointestinal tract were observed in both of our macaques. We were able to use PCR analysis to confirm the presence of T cruzi DNA in the esophagus and stomach of the first case and the esophagus of the second case. Interpretation of the histologic findings of ganglioneuritis was somewhat complicated in the second case by the concurrent presence of CMV-induced ganglioneuritis in several tissues. However, cytomegalovirus infection in immunosuppressed macaques causes a characteristic neutrophilic response in tissues, including peripheral nerves. In the second case, necrotizing and neutrophilic inflammation was present in nerves and ganglia associated with the gastric mesentery and within the sciatic nerve; CMV antigen was present within mesenchymal and endothelial cells in these lesions. Although animals coinfected with T cruzi and CMV could exhibit lesions of both diseases in the same tissues, differentiation of etiology can be attempted through the use of microscopic evaluation and ancillary diagnostic tools, as we did in this case. Similar to that in humans with Chagas disease, inflammation of the enteric plexuses in NHP appears to be more common than the development of megaesophagus and megacolon.

Specific treatment for Chagas disease was not attempted in either of our cases. Benznidazole and nifurtimox are the only drugs shown to have efficacy against Chagas disease in humans, and both have drawbacks, including toxicity and drug resistance. The acute phase of Chagas disease is responsive to antiparasitic treatments, with parasitological cure thought to occur in 60% to 85% of patients and 90% of congenitally affected infants treated in the first year of life. However, the benefits of these therapies are unclear in the chronic form of the disease. A multicenter, randomized, double-blinded, placebo-controlled trial of benznidazole for patients with mild to moderate Chagas cardiomyopathy is currently under way. Traditional therapy for heart failure, such as angiotensin-converting enzyme inhibitors and β-blockers, is needed. Infection should be considered in animals originating from this region, even if many years have elapsed since acquisition. The disease can have long-term health consequences, particularly for NHP entering longitudinal or immunosuppressive protocols, and screening may be warranted.

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