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DIAGNOSIS AND TREATMENT OF A NATURAL INFECTION WITH *TRYPANOSOMA CRUZI* (CHAGAS DISEASE) IN A SYMPTOMATIC DE BRAZZA'S MONKEY (*CERCOPITHECUS NEGLECTUS*) IN ALABAMA

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Abstract: *Trypanosoma cruzi*, the causative agent of Chagas disease, is a zoonotic, vector-borne, protozoan hemoflagellate with a wide host range. An 11-yr-old, captive-bred male De Brazza's monkey (*Cercopithecus neglecus*) presented with weight loss despite normal appetite. Examination revealed hypoglycemia, nonregenerative anemia, and many trypanosomes on a blood smear. A whole blood sample was PCR-positive for *T. cruzi* discrete typing unit TcIV and the monkey seroconverted using two different methods. The monkey was treated with the standard human dose of benznidazole twice daily for 60 d; however, blood obtained over the next 1.5 yr posttreatment remained PCR-positive for *T. cruzi*. A second course of benznidazole at a higher dose but lower frequency for 26 wk was required for the monkey to convert to sustained PCR-negative status. The monkey recovered with no apparent lasting effects.

INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas disease, or American trypanosomiasis, is a zoonotic, vector-borne, protozoan hemoflagellate with a wide host range. It is transmitted via triatomine insects ('kissing bugs') and is endemic across Central/South America, Mexico, and the southern United States. Parasite reservoirs in the United States include wildlife hosts such as raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), and Virginia opossums (*Didelphis virginiana*.)[2,3,11,15] The infectious stage of the organism is transmitted via the insect's feces through mucous membranes or breaks in the skin, or ingestion of infected triatomines or food, which are important routes in animals.[2,11] During the acute phase, the parasites can often be found in the blood, and patients may be asymptomatic or exhibit fever, lethargy, anorexia, weight loss, or lymphadenopathy. Data from infected humans suggest that 30%–40% of infected patients develop cardiomegaly, heart failure, arrhythmia, megaesophagus, or megacolon.[12] Diagnosis is made through PCR, serology, or identification of parasites in blood smears or growth of parasites from blood. While natural infections have been documented in nonhuman primates, this monkey required a modified treatment protocol to clear parasitemia.[6,7,9,13]

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CLINICAL BRIEF

An 11-yr captive-bred male De Brazza's monkey (*Cercopithecus neglectus*) presented with 10% weight loss over 3 mo, despite normal appetite, in August 2018. The monkey was born in New York State and arrived in Alabama in 2012 with no significant medical history. The monkey allowed voluntary hand injection with ketamine (Ketathesia, Henry Schein Animal Health, Dublin, OH 43017, USA; 40 mg IM) and dexmedetomidine (Dexmedesed, Dechra Veterinary Products, Overland Park, KS 66211, USA; 0.2 mg IM). Physical examination findings included pale mucous membranes and thin body condition. Thoracic and abdominal radiographs showed gas-distended intestines which appeared hypermotile on ultrasound. A CBC revealed a normal leukogram and anemia (PCV = 17%) and a biochemistry panel revealed severe hypoglycemia (26 mg/dl; Table 1).[17] Urinalysis was unremarkable. The monkey was given two boluses of 25% dextrose (Nova-Tech, Inc, Grand Island, NE 68801, USA; 2 ml IV), dexamethasone sodium phosphate (Dexaject SP, Bimeda-MTC Animal Health, Inc., Cambridge, Ontario

Table 1. Clinicopathologic results of a De Brazza's monkey (*Cercopithecus neglectus*) naturally infected with *Trypanosoma cruzi*.

Date	PCV (%)	Trypanosome presence on blood smear	Glucose (serum or whole blood; mg/dl)	qPCR Ct (whole blood)	IgG ELISA (OD)[a]	IgM ELISA (OD)	Stat-Pak band score (whole blood)	Cardiac troponin I (serum; ng/L)
12 Mar 2017	NT[a]	NT	NT	NT	NT	NT	NT	65
21 Aug 2018[b]	17	+	26	15.77	+ (0.294)	− (0.211)	−	43
29 Aug 2018	23	NT	NT	NT	NT	NT	+	NT
31 Aug 2018[c]	24	+	56	NT	NT	NT	NT	NT
04 Sep 2018	32	NT	90	NT	NT	NT	NT	NT
11 Sep 2018	34	−	45	NT	NT	NT	NT	NT
18 Sep 2018	32	+	59	NT	+ (0.588)	− (0.242)	NT	NT
25 Sep 2018	36	+	52	25.97	NT	NT	+	NT
16 Oct 2018	35	+	54	NT	NT	NT	NT	NT
30 Oct 2018[d]	NT	−	83	31.41	+ (0.627)	− (0.089)	+	102
26 Dec 2018	36	+	54	NT	NT	NT	+	NT
24 Apr 2019	NT	−	NT	NT	+ (1.115)	− (0.025)	NT	48
14 Jun 2019	33	−	91	26.34	+ (1.832)	− (0.116)	+	NT
10 Sep 2019	NT	−	NT	NT	+ (1.571)	− (0.022)	NT	NT
24 Sep 2019	34	−	NT	NT	NT	NT	NT	NT
12 Feb 2020	37	NT	49	NT	NT	NT	+	367
18 Mar 2020[e]	NT	NT	39	NT	NT	NT	NT	NT
14 May 2020	37	−	41	NT	NT	NT	NT	NT
04 Jun 2020	37	−	40	NT	NT	NT	NT	NT
02 Jul 2020	37	NT	60	33.79	+ (0.555)	− (0.076)	+	20
06 Aug 2020	34	NT	50	NT	NT	NT	NT	NT
11 Sep 2020[f]	35	NT	55	−	NT	NT	+	NT
25 Sep 2020	33	NT	47	NT	+ (0.467)	− (0.092)	NT	NT
30 Oct 2020	34	NT	51	−	NT	NT	+	NT
12 Nov 2020	36	NT	38	NT	NT	NT	NT	40
17 Dec 2020	41	NT	59	NT	NT	NT	NT	NT
22 Jan 2021	42	NT	NT	−	NT	NT	+	NT
11 Feb 2021	39	NT	58	NT	NT	NT	NT	NT
05 Mar 2021	40	NT	54	−	NT	NT	+	NT
26 Mar 2021	40	NT	63	NT	+ (0.516)	− (0.076)	NT	NT

[a] OD, optical density; NT, not tested.
[b] Date of diagnosis.
[c] First treatment protocol initiated 1 d prior.
[d] First treatment protocol concluded 2 d prior.
[e] Second treatment protocol initiated 4 d prior.
[f] Second treatment protocol concluded.

N3C 2W4, Canada; 3.6 mg SC), iron dextran (Hematinic, MWI, Boise, ID 83705, USA; 70 mg IM), ceftiofur crystalline free acid (Excede, Zoetis Inc., Kalamazoo, MI 49007, USA; 140 mg SC) and vitamin B complex (Sparhawk Laboratories, Lenexa, KS 66215, USA; 0.2 ml IM). Dexmedetomidine was antagonized with atipamezole (Revertidine, Modern Veterinary Therapeutics, Miami, FL 33186, USA; 0.5 mg IM). After recovery, the monkey was started on enrofloxacin (Taylor's Pharmacy, Winter Park, FL 32789, USA; 5 mg/kg PO q12h), and staff offered small, frequent meals and ad libitum browse. Additional diagnostics, including a fecal Gram stain, fecal culture, and serum insulin (<7.5 μU/ml; Michigan State University Diagnostic Laboratory, Lansing, MI 48910, USA) were all unremarkable.

Blood smear microscopy revealed many trypanosomes, which were also visible in the buffy coat of a spun hematocrit tube under 40× magnification. Serum was seropositive and PCR-positive for *Trypanosoma cruzi* (VRL, San Antonio, TX 78229, USA), indicating that this monkey was in the acute phase of Chagas disease when treatment can be most effective.[12] Treatment with benznidazole (Exeltis USA, Inc, Florham Park, NJ 07932, USA; 3.5 mg/kg PO q12h × 60 d),

dissolved in water and administered via syringe, was initiated 9 d after diagnosis. Benznidazole is a nitroimidazole derivative approved for use in children 2–12 yr, which is used off-label for patients outside of that age range. No side effects were appreciated in this monkey, which in humans can include rash, headache, anorexia, peripheral neuropathy, and leukopenia.[12] The monkey remained PCR-positive for Chagas disease at the end of this treatment (Table 1).

Failure of benznidazole treatment using a daily dosing regimen has recently been associated with the capacity of *T. cruzi* intracellular amastigotes to enter a dormant state that is resistant to benznidazole[16] but which can be eliminated by pulse treatment with higher doses in mice.[4] Accordingly, the monkey was treated with benznidazole again, using a dose of 35 mg/kg (fivefold the normal daily dose) once weekly. The monkey remained PCR-positive after 11 wk on this regimen, so the frequency was increased to twice a week (Wednesday and Saturday) for the remaining 15 wk of treatment. The monkey was PCR-negative on the last day of treatment and remained negative during the following 6 mo.

Blood was sent to a research laboratory (College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA) for further characterization of the parasite and an antibody rapid test on whole blood. Following DNA extraction from 500 µl of whole blood (Omega Bio-Tek, Norcross, GA 30071, USA), a qPCR was used to amplify the repetitive satellite DNA of the parasite,[8] after which a multiplex qPCR amplifying the spliced leader intergenic region (SL-IR) was used to determine *T. cruzi* discrete typing unit (DTU),[5] resulting in the detection of DTU TcIV in this monkey. There are seven major discrete typing units of *T. cruzi*, Tc I-VI plut TcBat.[15] In the southern United States, TcIV is most commonly seen in raccoons and striped skunks, whereas TcI is most commonly seen in Virginia opossums.[2,11,15] All of these wildlife reservoirs are found around the zoo. Additionally, a rapid immunochromatographic assay (Chagas Stat-Pak; Chembio Diagnostic Systems, Inc, Medford, NY 11788, USA) using 10 µl of whole blood showed a negative result, indicating a lack of antibody detection at that time using this test, with later positive results (Table 1).

Natural infections with *T. cruzi* have been documented in wild and captive nonhuman primates including macaques (*Macaca* spp.), lemurs (*Lemur catta*, *Eulemur macaco flavifrons*, *Varecia variegata variegata*), squirrel monkeys (*Saimuri sciureus*), golden lion tamarins (*Leontopithecus rosalia*), a baboon (*Papio cynocephalus*), a chimpanzee (*Pan troglodytes*), and a gibbon (*Hylobates pileatus*) in the Americas; however, reports of treatment are limited.[6,7,9,11,13] Clinical signs in nonhuman primates are variable and range from asymptomatic infection to heart failure.[10] In this monkey, levels of cardiac troponin (cTnI), a measure of cardiac damage, increased, reaching a peak of 367 ng/L at 18 mo (Animal Health Diagnostic Center, Cornell University, Ithaca, NY 14852, USA), but then decreased during the second treatment protocol (i-STAT cardiac troponin I, Zoetis Inc). Increases can be seen with the cardiac form of *T. cruzi* infection in humans.[1] A limited voluntary echocardiogram was performed at 3 mo postdiagnosis and appeared unremarkable. Complete echocardiogram and electrocardiogram performed under anesthesia during routine examinations at 10 mo and 2 yr were unremarkable, suggesting no long-term cardiac damage.

The ability to collect blood from this monkey routinely during voluntary training session was invaluable in monitoring this case. The severe anemia noted at diagnosis resolved 5 d after starting the initial treatment. Mild hypoglycemia has persisted intermittently in this individual and has been reported in mice experimentally infected with *T. cruzi* as a result of pancreatic inflammation and defective hepatic gluconeogenesis.[14] Serial qPCR screening showed the highest load of parasite in the blood at the time of diagnosis, with an increase in CT (lowered parasite burden in blood) during the first treatment, followed by an increase in parasite again. After the revised treatment protocol was initiated, there was an increase in CT (decrease in parasite load) followed by four sequential negative results. *Trypanosoma cruzi* IgG increased initially and then decreased, and the StatPak rapid test showed seroconversion from an initial negative result to positive results throughout the remainder of the monitoring period.

Failure of benznidazole treatment regimens is well documented in humans and other hosts.[16] High-dose pulse administration of benznidazole has achieved parasitological cure in mice[4] as well as in dogs and nonhuman primates (Bustamante, unpubl. data). In this monkey, once-weekly dosing with five times the typical daily dosage of benznidazole for 11 wk failed to resolve the infection, but increasing the frequency to twice weekly yielded a presumptive

cure based on consistently PCR-negative blood samples over 6 mo and declining IgG in serum. While some hosts do occasionally spontaneously resolve the infection, that is unlikely to be the case with this monkey because it was consistently PCR-positive for 2 yr prior to the final course of treatment and then consistently negative thereafter.

No other animals have been diagnosed with Chagas disease at the zoo, despite blood smear reviews being standard practice on all animals that have a CBC performed. This monkey lived with two offspring at the time of diagnosis and neither developed Chagas disease. While triatomines were not found at the zoo, the relatively large, meshed-in habitat of these monkeys could make detection difficult.

The relatively high parasite load in the blood of this subject provided an opportunity to detect treatment failure during and after the various treatment regimens. Fortunately, a failed initial treatment did not appear to select for resistance to benznidazole, as evidenced by apparent resolution after the second treatment utilizing a higher dosage and increased frequency. Benznidazole appeared to be safe in this monkey, which recovered from the acute disease with no observed lasting effects. *Trypanosoma cruzi* infection should be considered in zoo animals in endemic areas, and blood film evaluation may aid in rapid diagnosis.

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