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Case Report

Transmission of Chagas Disease via Blood Transfusions in 2 Immunosuppressed Pigtailed Macaques (*Macaca nemestrina*)

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A 2.25-y-old male pigtailed macaque (*Macaca nemestrina*) was experimentally irradiated and received a bone marrow transplant. After transplantation and engraftment, the macaque had unexpected recurring pancytopenia and dependent edema of the prepuce, scrotum, and legs. The diagnostic work-up included a blood smear, which revealed a trypomastigote consistent with *Trypanosoma cruzi*, the causative agent of Chagas disease (CD). We initially hypothesized that the macaque had acquired the infection when it lived in Georgia. However, because the animal had received multiple blood transfusions, all blood donors were screened for CD. One male pigtailed macaque blood donor, which was previously housed in Louisiana, was positive for *T. cruzi* antibodies via serology. Due to the low prevalence of infection in Georgia, the blood transfusion was hypothesized to be the source of *T. cruzi* infection. The transfusion was confirmed as the mechanism of transmission when screening of archived serum revealed seroconversion after blood transfusion from the seropositive blood donor. The macaque made a full clinical recovery, and further follow-up including thoracic radiography, echocardiography, and gross necropsy did not show any abnormalities associated with CD. Other animals that received blood transfusions from the positive blood donor were tested, and one additional pigtailed macaque on the same research protocol was positive for *T. cruzi*. Although CD has been reported to occur in many nonhuman primate species, especially pigtailed macaques, the transmission of CD via blood transfusion in nonhuman primates has not been reported previously.

Abbreviations: CD, Chagas disease; NHP, nonhuman primates; TNPRC, Tulane National Primate Research Center; WaNPRC, Washington National Primate Research Center.

Trypanosoma cruzi, the causative agent of Chagas disease (CD), is a protozoan parasite that is transmitted by bloodsucking triatomines, a type of reduviid bug. Also known as kissing bugs, triatomines transmit *T. cruzi* through infected feces that enter the host via a bite wound or mucous membranes. In humans, other mechanisms of transmission include transplacental, blood transfusion, organ transplantation, and ingestion of contaminated food products.[9,10,12,15,21,22,29,34] CD is endemic in Latin America but also is present in the southern United States, and its geographic range has continued to expand.[3,13] CD is estimated to affect approximately 8 million people in Latin America and can be found in more than 150 species of domestic and wild animals.[30]

Typically CD is a lifelong infection, with acute and chronic stages. Acute CD is generally asymptomatic, but clinical signs can include fever, malaise, hepatomegaly, splenomegaly, enlarged lymph nodes, subcutaneous edema, and chagoma—an inflammatory nodule that forms at the site of entry. ECG changes can include sinus tachycardia, first-degree atrioventricular block, low QRS voltage, and T-wave changes.[30] Radiographs can reveal cardiomegaly. In chronic CD, approximately 10% to 15% of patients develop megaesophagus, megacolon, or both. The more common and life-threatening presentation of chronic CD is cardiomyopathy, which occurs in 20% to 40% of infected persons.[24] The most common cardiac symptoms of chronic CD include right bundle-branch block, left anterior fascicular block, and ventricular premature contractions.[30] Clinical disease due to chronic CD can include sudden death, heart failure, and thromboembolism.

Natural Chagas disease has been diagnosed in numerous nonhuman primates (NHP) in the United States, including pigtailed macaques, cynomolgus macaques, baboons, chimpanzees, golden-lion tamarins, capuchins, and squirrel monkeys.[2,5,8,13,17,25,32,35-38] Clinical signs and disease progression are similar to those described in humans. Although these animals were diagnosed with CD across the country, it is believed that all were infected while they were housed in the southern United States. *T. cruzi* has been found in numerous reservoir species in the southern United States, with the highest prevalence in raccoons and opossums.[7] A recent survey at the Tulane National Primate Research Center (TNPRC; Covington, LA) reported the prevalence of CD as 1.6% in more than 2000 NHP, with a higher prevalence of 3.4% in pigtailed macaques.[13] NHP are believed to acquire CD primarily via ingestion of the reduviid bug in addition to inoculation via a bite

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wound or mucous membranes. NHP have also been experimentally infected with CD.[6,8] Although congenital transmission has not been documented, CD has been associated with a stillbirth in an NHP.[17] Here we describe the first reported cases of transmission of *T. cruzi* via blood transfusions in NHP.

Case Report

A 2.25-y-old, 3-kg male pigtailed macaque (animal A) born at the Yerkes National Primate Research Center (Atlanta, GA) was transferred to the Washington National Primate Research Center (WaNPRC; Seattle, WA) when he was approximately 1 y old. At both AAALAC-accredited facilities, the animal was on an IACUC-approved protocol and housed and managed in accordance with the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act.[1,20] At the WaNPRC, he was assigned to a stem cell gene transfer project. Prior to the experimental protocol, the macaque was clinically normal on physical exam and blood work. Blood work consisted of a CBC and chemistry screen. He was negative for *Macacine herpesvirus* 1, simian foamy virus, SIV, simian retrovirus 2, simian T-cell leukemia virus, and simian varicella virus.

Animal A had bone marrow aspirates performed on multiple limbs and was irradiated with a fractionated treatment of 255 Gy 4 times over 2 d (days –1 and 0). A femoral catheter was surgically placed, and a tether system was used for continuous intravenous access. The macaque received a bone marrow transplant of 115×10^6 $CD34^+$ cells that were transduced with a vector that included GFP and methylguanine methyltransferase, which potentially would confer increased drug resistance to chemotherapy. The macaque was placed on the protocol's standard treatment for irradiated animals which included ceftazidime, vancomycin, and gentamicin until day 15, fluconazole until day 50, and acyclovir until day 150. He also received enrofloxacin, granulocyte colony-stimulating factor, and transfusions of fresh whole blood and platelet rich plasma as needed (Table 1). In light of the CBC results and 5% GFP markers in blood, he was considered to be engrafted on day 22. However, on day 40, the macaque began experiencing recurrent pancytopenia. He received blood transfusions once or twice each month to treat severe anemia or thrombocytopenia (Table 1), granulocyte colony-stimulating factor to stimulate neutrophil production, and enrofloxacin to prophylactically prevent infection. Because of the unexpected pancytopenia, the bone marrow was biopsied. The biopsy revealed moderately hypocellular bone marrow, with a modest relative decrease in neutrophilic progenitors and normal megakaryocytes.

On day 110, animal A was sedated for a physical exam, and semiannual tuberculosis testing was performed. The animal was pyrexic (103.3 °F) and had dependent edema of the prepuce, scrotum, and legs (Figure 1). Because of the severe edema of the prepuce, the macaque was monitored closely and observed to urinate without difficulty. To reduce the edema, the macaque was started on 2 mg/kg oral furosemide twice daily. In addition, the animal was placed on acyclovir, fluconazole, enrofloxacin, and amoxicillin–clavulanic acid because of the persistent pancytopenia. The dependent edema decreased in the scrotum and legs but persisted in the prepuce 5 d after furosemide was started. Therefore, the furosemide dose was increased to 4 mg/kg BID and the route was switched to intramuscular to ensure that the macaque received the full dose. After 5 d of intramuscular furosemide, the edema had resolved completely.

Table 1. Recurrent cycle of pancytopenia treated with blood transfusions after the macaque was fractionally irradiated on days –1 and day 0 and received a bone marrow transplant on day 1.

	PCV (%)	WBC (x 10^3/μL)	Platelets (x 10^3/μL)	Transfusion (mL)
Reference range	34–46	2.2–13.9	236–693	NA
Day				
–7	39	5.5	512	—
1	25	0.2	61	—
2	22	0.0	25	—
3	19	0.0	15	60
4	33	0.0	70	—
6	29	0.1	15	—
8	26	0.2	3	60
9	29	0.4	28	
11	26	0.5	14	**60**
12	35	0.7	64	
14	32	1.2	4	60
17	38	5.9	6	60
20	43	4.5	15	30[a]
22	42	6.9	71	—
25	34	8.4	6	80
43	22	1.5	18	60
50	24	2.7	49	—
70	25	3.2	55	**60**
106	18	0.9	20	—
107	—	—	—	60
110[b]	16	1.0	21	60
127	18	3.4	57	—
128	—	—	—	60
133	21	3.3	39	—
136[c]	—	—	—	**60**

NA, not applicable.
All transfusions were performed with irradiated whole blood unless otherwise noted; bold type indicates transfusions from animal B, the source of CD.
[a]Platelet-rich plasma provided.
[b]Approximate date of diagnosis of CD in animal A by blood smear.
[c]Approximate date when animal A was found to be antibody-positive for CD.

Because of the continued CBC abnormalities, additional diagnostics were performed, including thick and thin blood smears which were sent to the University of Washington Hospital Clinical Laboratory. On the blood smears, a trypomastigote consistent with *T. cruzi* was observed (Figure 2). Because this macaque had lived outdoors for 1 y in Georgia, where CD is present, the infection was presumed to have been acquired there. In light of the diagnosis of CD, a cardiac work-up was performed. ECG, indirect blood pressure, oxygen saturation, thoracic radiography, and echocardiography were all normal.

Although observation of the trypomastigote on blood smear is specific for the diagnosis of CD, the animal also was found to be antibody-positive to *T. cruzi* (Trypanosoma Detect test strips, InBios International, Seattle, WA). In addition, the strips were tested with 3 known positive *T. cruzi* archived plasma samples as positive controls.[32] All 3 archived samples were positive for

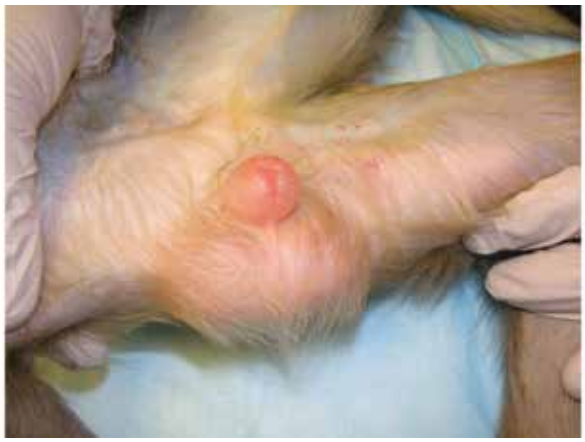

Figure 1. Animal A had dependent edema of the scrotum, prepuce, and legs.

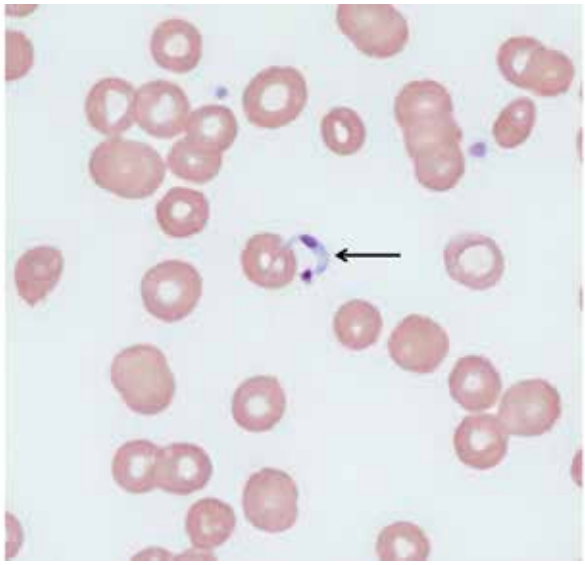

Figure 2. Trypanosome trypomastigote in a thin blood smear of animal A.

antibodies. Animal A's archived samples from 10 mo prior, 1 d prior, and 1 mo after the diagnosis of *T. cruzi* were tested also (Table 2). Only the sample collected 1 mo after diagnosis was positive for trypanosomal antibodies. The macaque had been in Seattle for more than 1 y, and the 2 archived plasma samples were negative, implying that the infection was recently acquired in Seattle rather than in Georgia. Therefore, all colony blood donors were screened for *T. cruzi* via the test strips.

Seven unique macaques had donated blood to animal A during his 12 total transfusions. Of the 7 blood donors tested, 1 pigtailed macaque (animal B) was found to be positive for *T. cruzi*. Animal B donated blood 3 times for transfusions to animal A and had lived outdoors in Louisiana for approximately its first 6 y and indoors at the WaNPRC for 3 y. Because triatomine bugs are not found in the state of Washington, animal B acquired CD while living in Louisiana in an outdoor enclosure with exposure to the bugs. Animal B was removed from the blood donor pool and received a full cardiac work-up, which revealed peaked T-waves on ECG and a mildly enlarged cardiac silhouette. Echocardiography revealed an increased thickness of the right ventricular free wall

Table 2. Results from Trypanosoma Detect test strips for antibodies to *Trypanosoma cruzi*.

Animal	2009	August 2010	October 2010	December 2010
A (index case)	—	—	+	ND
B (blood donor)	ND	+	ND	ND
C (2nd case)	ND	—	—	+

—, negative; ND, not done; +, positive
For animal B, the archived sample from August 2010 was tested in October 2010.

(0.59 cm; normal, 0.23–0.43 cm) and a decreased ejection fraction (0.34; normal, 0.36–0.94). Animal B exhibited no other clinical signs or abnormalities associated with CD. He was euthanized shortly thereafter, and on gross necropsy and histology had no signs of CD. The mild cardiac abnormalities observed on echocardiography were not evident at necropsy or histology.

Animal A, the newly infected experimental animal, was monitored regularly via blood work and thoracic radiographs. Blood work revealed hyperproteinemia (10.1 g/dL; normal range, 5.4 to 7.9 g/dL) due to hyperglobulinemia (8.3 g/dL; normal range, 2.4 to 4.7 g/dL). By day 140, blood work had stabilized, with normal RBC, WBC, and platelet counts, and globulin levels had decreased to 6.0 g/dL. By day 180, CBC counts remained stable, and globulins had returned to a normal level of 4.1 g/dL. The pigtailed macaque also had annual radiography and echocardiography examinations, which showed no abnormalities, and was euthanized 2.5 y after the initial clinical presentation. On necropsy, no gross lesions were apparent, but histology revealed a single small focus of mixed myocarditis within the left ventricle, with histiocytes, lymphocytes, and rare neutrophils mildly displacing myofibers. Separate, scattered areas of mild myofibrillar disarray without evidence of significant inflammation were present, and there was moderate subvalvular perivascular fibrosis.

Other pigtailed macaques on the same research protocol and who had received blood transfusions from animal B were screened for CD. One other macaque (animal C) was antibody-positive for *T. cruzi*, although no parasites were visualized on blood smear (Table 2). Animal C had received a blood transfusion from animal B at 10 d after irradiation. Animal C did not demonstrate any clinical signs or abnormalities related to CD. Full cardiac work-up and annual follow-up radiography and echocardiography revealed no significant abnormalities. Animal C was euthanized approximately 2 y after diagnosis, and no gross or histologic changes associated with CD were noted on necropsy.

Discussion

The transmission of CD via blood transfusion had not been documented previously to occur in NHP. This absence likely reflects the limited number of blood transfusions, lack of screening for CD, and low prevalence of CD in NHP. Although numerous cases of CD in NHP have been reported,[2,5,6,8,17,25,28,32,35-38] reference 13 reports the first large-scale screening of NHP for CD, in which pigtailed macaques at the studied institution had a higher prevalence of CD (3.4%) than did baboons (1.7%) and rhesus macaques (1.1%). Therefore, a randomly selected pigtailed macaque blood donor would more likely be positive for *T. cruzi* than would another NHP. Why pigtailed macaques have a higher prevalence of CD than other macaques is not understood. *T. cruzi* organisms

are found in the blood, and transmission of CD via blood transfusion has been documented in humans; it is therefore unsurprising that transfusion-associated CD would occur in NHP.[12,15,22,34] In humans, it is estimated that there is a less than 10% to 20% chance of acquisition of CD from a single unit of blood (approximately 450 mL) from an infected donor.[4] Animal B donated 60 mL of blood 3 times to animal A, for a cumulative total of 180 mL. On the basis of blood volume donated and the assumption that the percentage of acquisition is proportional with blood volume, the likelihood of transmission of *T. cruzi* to this macaque was less than 4% to 8%. However, the probability of transmission could be increased by the multiple blood transfusions and differing parasitemia dynamics between humans and NHP. Prior to transfusion in animal A, the donor blood was irradiated with 4000 rads of γ radiation for 10 min to eliminate potential pathogens. However, *T. cruzi* reportedly can remain viable after X-ray or γ radiation doses from 10,000 to 100,000 rads.[14,16] Therefore, irradiation of donated blood with 4000 rads likely would not kill *T. cruzi*.

The index macaque described here lacked clinical signs of CD, probably due to the relatively short time of infection and temporary, nonspecific clinical signs of acute CD. In NHP, only a low percentage of animals with acute and chronic CD develop any type of clinical signs or cardiac changes.[6,8,32,36,38] Nonspecific clinical signs of acute CD include leukocytosis, general malaise, lymphadenopathy, fever, myocarditis, and organomegaly. The only specific clinical sign of the acute phase of CD is the chagoma, which is a nodule that can develop at the site of infection. Because the animals we report were infected via blood transfusion, there was not a parenteral site of entry and a chagoma would not be observed. In acute CD, symptoms can last 4 to 8 wk and typically resolve spontaneously. The more visible and detrimental clinical signs of CD, such as cardiomyopathy and heart failure, are associated with the chronic stage of infection. Even in chronic CD, only a maximum of 40% of humans ever become symptomatic. Only animal B, the blood donor, was potentially in the chronic stage of infection. Animal A did exhibit pancytopenia, dependent edema, and hyperglobulinemia. None of these signs are typical of acute CD. However, because this macaque was significantly immunosuppressed, the pathogenesis and clinical signs of CD may have been altered.

Although not well-documented, bone marrow hypoplasia with concurrent pancytopenia has been reported to occur with acute CD.[23,33] Because macaque A had had recurrent pancytopenia prior to the transfusion of *T. cruzi*-contaminated blood, pancytopenia likely was related to the experiment and not to CD. The dependent edema could have been due to a thromboembolism that decreased venous return to the heart and that eventually spontaneously resolved via fibrinolysis. In light of the normal cardiac exam results and minimal cardiac involvement seen histologically, the edema was not likely associated with the heart failure that can be observed during chronic CD. Hyperglobulinemia can be due to an increase in α, β, and γ globulins, which can be distinguished via protein electrophoresis but was not performed. However, both β and γ globulins can be increased due to inflammation, which could have been caused by acute CD. Gross necropsy and histology showed only changes potentially associated with CD in animal A. The myocardial fibrosis observed in animal A has been reported in other NHP with CD.[2,5,35] It is possible that the focus of myocardial fibrosis was attributable to CD and that, given additional time, animals B and C would have developed chronic CD. However, the myocardial fibrosis could have been an incidental finding. As a result of these cases, all future blood donors and animals that will be immunosuppressed are screened for CD by using the test strips. Because of the possibility of congenital transmission, breeder animals that had previously been housed in the southern United States were screened for CD and were all negative.

The presence of *T. cruzi* trypomastigotes in a blood smear allows for a definitive diagnosis; no other 'gold standard' for the diagnosis of CD is available.[11] Animal A was diagnosed via observation of a trypomastigote in the blood smear, but macaques B and C lacked trypomastigotes on multiple blood smears. However, blood smears have a low sensitivity for the diagnosis of CD, especially after the acute stage.[28] Animals B and C were diagnosed as having CD by using an immunochromatographic dipstick. In humans, this test has had high reported sensitivities of 84.8% and 99.2% and specificities of 97.9% and 99.1%.[19,31] All of the 4 known *T. cruzi*-positive samples from NHP at the WaNPRC were positive via the test strips. In addition, this dipstick test has been used to screen NHP at the TNPRC and yielded positive results for 6 of 7 positive controls and negative results for all 10 negative controls. Although both sample sizes were small, the sensitivity and specificity of the dipstick test in NHP are expected to be similar to those in humans.

In humans, treatment options for CD include the antiprotozoal drugs benznidazole and nifurtimax, which are only available from the Center for Disease Control. These drugs have considerable side effects, including seizures and peripheral neuropathies, which often cause treatment interruptions.[18,26,27] Ketoconazole has been investigated as a treatment in NHP but was found to be ineffective.[32] Because of the limited availability and significant side effects of the agents, treatment options were not pursued for these animals. In a large breeding colony of NHP, animals positive for CD typically are culled.

Here we describe the first reported cases of transmission of CD via blood transfusion in NHP. Bloodborne pathogens can negatively affect the health of recipient animals and are a significant experimental variable. These cases emphasize the need for pathogen screening of blood donors, depending on the nature of the research, the species involved, and the risk of exposure. Examples of other potential transfusion-transmitted pathogens include simian T-cell leukemia virus, simian retrovirus, malaria, and cytomegalovirus. In light of these cases, we recommend CD screening for all NHP that have lived outdoors in the southern United States, especially if they will be immunosuppressed or used in cardiac research.

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