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Chagas disease in a naturally-infected dog from Northeast Brazil: a case report

Vicente Toscano de Araújo-Neto[1], Andressa Noronha Barbosa-Silva[1], Lucas Abrantes Batista[2], Rodrigo de Souza Mendes[3], Christiane Carlos Araújo de Negreiros[1], Barbara de Araújo Fidellis[4], George Harisson Felinto Sampaio[2], Yannara Barbosa Nogueira Freitas[5], Carlos Ramon do Nascimento Brito[4,6], Paulo Marcos da Matta Guedes[4], Lúcia Maria da Cunha Galvão[1,2] and Antonia Claudia Jácome da Câmara[1,4,6*]

Abstract

Background Dogs are the main domestic reservoir host of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, and they are considered sentinel animals for the detection of new cases of human infection. *Canis familiaris* is also a well-established experimental *T. cruzi* infection model, but little is known about the progression of Chagas disease in naturally-infected dogs, especially during the acute phase in these hosts. Triatomine species infected with discrete typing units (DTUs) I, II and III of *T. cruzi* have been previously found in many of the municipalities of the state of Rio Grande do Norte. The current study describes the clinical, hematological, biochemical, cardiological and parasitological characteristics of a single dog during the acute phase of its naturally-acquired *T. cruzi* infection, and characterizes the isolate obtained from this individual host using biological, molecular and phylogenetic methods.

Results A juvenile dog exhibiting discomfort during defecation, itchy skin, and enlarged popliteal lymph nodes showed a prolonged period of patent parasitemia, with normocytic and hypochromic anemia. In addition, cardiac damage was suggested by high concentrations of the biomarkers cardiac troponin I and NT-ProBNP, as well as Doppler echocardiography, which showed qualitative segmental hypokinesia. The parasites isolated from this individual canine were genotyped, using three molecular markers and phylogenetic analysis, as the DTU TcIII. First detected in sylvatic environments, our current observations also demonstrate the presence of this DTU in domestic/ peridomestic locations.

Conclusions The cardiac alterations that we observed in a naturally-*T. cruzi*-infected dog contribute to expanding our knowledge of both Chagas disease in *Canis familiaris*, and the epidemiological scenario in locations where *Triatoma brasiliensis* is the main triatomine vector of *T. cruzi*.

Keywords Biomarkers, Dog, DTU III, Heart changes, Phylogenetic analysis, Peridomicile, Transmission cycle, *Trypanosoma cruzi*

*Correspondence:
Antonia Claudia Jácome da Câmara
antonia.claudia.camara@ufrn.br
Full list of author information is available at the end of the article

Background

Chagas disease is an anthropozoonosis caused by the parasite *Trypanosoma cruzi*, which is a neglected tropical disease that affects the most vulnerable populations in endemic countries [1, 2]. The parasite is mainly transmitted between different vertebrate hosts in enzootic cycles by different species of triatomines, insects of the Triatominae subfamily (Hemiptera: Reduviidae), and can infect over 150 species of domestic and wild mammals [3].

Trypanosoma cruzi was initially identified using morphological characteristics [1, 4, 5], and later further characterized using several different molecular markers [6–11]. *Trypanosoma cruzi* is currently divided into seven distinct subgroups according to its genetic diversity, known as "discrete typing units" (DTUs): TcI to TcVI, and TcBat [12–14]. TcI and TcII are the predominant DTUs in Brazil, with TcI mostly associated with sylvatic transmission cycles between wild mammalian hosts, while TcII is mostly associated with domestic transmission cycles, involving humans and their associated domesticated mammals. Consequently, TcII is the DTU most detected in humans [15–17]. In the state of Rio Grande do Norte, in the Northeast Brazil, the three DTUs TcI, TcII and TcIII have all been described in both humans and triatomines [16–19], while TcII and TcIII have also been reported in various non-human mammals [17, 20].

Domesticated dogs (*Canis familiaris*) are the main domestic reservoir hosts of *T. cruzi* [21]. These animals are, therefore, considered useful sentinels for new cases of human Chagas disease, as they are likely to participate in the domestic, peridomestic and sylvatic transmission cycles of the parasite, acting as "bridge hosts" between the former and the latter transmission cycles, and, thereby, both introducing and/or maintaining *T. cruzi* infection in domestic and peridomestic settings [22]. As such, epidemiologically, domesticated dogs often become infected before humans [21], have a persistent and elevated parasitemia [23], and are estimated to infect up to 100-times more triatomines than adult humans under experimental conditions [21, 23–26]. In addition to their epidemiological importance as reservoir hosts in the field, dogs are an important for the study of *T. cruzi* infection in the laboratory, because the progression of the acute and chronic phases of this parasite infection in them is similar to that observed in humans, and their use as an experimental model is essential for understanding the transmission dynamics of the parasite, vertebrate host immune responses, and drug development [27–29].

Previously, *Trypanosoma cruzi* infection in domesticated dogs in the state of Rio Grande do Norte has been demonstrated using serological and molecular tests [30, 31]. Recent studies have also shown that these non-human hosts co-occur in the same locations as the triatomine vector species *Triatoma brasiliensis* Neiva, 1911, in rural areas of the state of Rio Grande do Norte [30], and dogs have already been detected as a food source for *T. brasiliensis* infected with *T. cruzi* [19]. Here, we describe the clinical, hematological, biochemical and parasitological aspects of a single dog found naturally-infected with *T. cruzi*, which was in the acute phase of parasite infection. Furthermore, we report the characterization of the parasite isolate obtained from this dog by biological, molecular and phylogenetic methods.

Methods

Case

A 72-day-old female crossbred dog, coded for the purposes of this study as individual "C681", from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, Brazil, was acquired by its owner at an adoption fair in Natal, the capital of the state. This municipality is 71 km from Natal and is located in the semi-arid zone of the state [32] and is considered at medium risk for *T. cruzi* transmission [33]. The dog was taken to a veterinary service because it exhibited discomfort during defecation, and apparently had itchy skin. The initial clinical examination was otherwise normal except for enlargement of popliteal lymph nodes. Another clinical examination was carried out 11 days after the initial examination, and no changes were observed. At this latter time, the dog had normal coloration of the mucous membrane, a capillary perfusion time of three seconds, normal skin turgor, and normal body temperature. Multiple blood collections were performed at the Yannara Freitas, on each occasion without anesthesia using a mechanical restraint. The timeline of blood collections is shown in Table 1. The first blood collection was taken at the onset of symptoms (D0), followed by the next 11 days later (D11), the third on day 19 (D19), and subsequent collections carried-out on days 26, 40, and 62, with the last collection 137 after symptom onset. As well as blood counts, other complementary tests such as blood culture, biochemical tests, Doppler echocardiogram and serology were also performed on D11. The Doppler echocardiogram examination was performed using an EsaoteMyLab™ Gamma, SP2730 2.3 – 6.1 MHz sectoral transducer (Esaote, Genova, ITALY). On 9th March 2022, the blood of the dog was sent to the *Laboratório de Biologia de Parasitos e Doença de Chagas* (LABIOPAR) of the *Universidade Federal do Rio Grande do Norte* (UFRN), where parasite identification was conducted. For the blood collection performed on D11, three aliquots were separated for: (i) direct examination, (ii) blood culture, and (iii) DNA extraction. For the final aliquot used

Table 1 Follow-up of the blood count parameters for the dog "C681" naturally-infected with *Trypanosoma cruzi* from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, Brazil

	Time (days after first clinical examination)							
	D0	**D11**	**D19**	**D26**	**D40**	**D62**	**D137**	**Reference ranges**
Red blood cells	3,200	4,400	4,700	5,300	5,500	5,740	7,700	3,500–6,000/mm^3
Hemoglobin	5.6	7.4	8.7	9.9	9.6	11.4	17	8.5–13 g/dl
Hematocrit	22	25	28	34	33	37	49	26—39%
MCV	68	57	59	64	66	64	62	69–83 fL
MCH	12	17	18	18	19	20	22	22–25 pg
MCHC	15	30	31	29	29	30	35	31–33%
RDW	17	14	13	13	16	13	12	12—15
White blood cell	6,700	6,900	6,500	8,800	8,700	8,300	12,400	8,000–16,000/mm^3
Band neutrophils	0	0	0	0	0	0	0	0—1
Neutrophil	57	53	56	68	50	54	61	46—68
Eosinophil	0	1	0	0	2	1	4	01—05
Basophil	0	0	0	0	0	0	0	0—1
Lymphocytes	36	38	40	29	43	42	28	30—48
Monocytes	7	8	4	3	5	3	7	01—10
Platelets	96,000	308,000	288,000	265,000	450,000	342,000	308,000	200,000–500,000/mm^3
Trypomastigotes	absence	presence	presence	absence	presence	absence	absence	
Comments	Microcytic hypochromic anemia	Microcytic hypochromic anemia	Microcytic hypochromic anemia	Microcytic hypochromic anemia	Microcytic hypochromic anemia	Without changes	Polycythemia	
	Leukopenia	Leukopenia	Leukopenia					

D0 day zero, *D11* day 11, *D19* day 19, *D26* day 26, *D40* day 40, *D62* day 62, *D137* day 137, *MVC* Mean Cell Volume, *MCH* Mean Corpuscular Hemoglobin, *MCHC* Mean Corpuscular Hemoglobin Concentration, *RDW* Red Cell Distribution Width

for DNA extraction, 6 M Guanidine-HCl/0.2 M EDTA (Sigma Chemical Company, USA) at pH 8.0 [34], was added to the blood in a ratio of 1:2. Biochemical evaluations were performed at the Yannara Freitas using commercial kits according to the recommendations of the manufacturer, in order to measure canine cardiac troponin-I, N-terminal prohormone B-type natriuretic peptide (NT-ProPNB), creatinine, alkaline phosphatase, alanine aminotransferase (ALT), total proteins, albumin and globulin.

Direct microscopic examination, blood culture and parasite isolation

Approximately 2 mL of venous blood from the dog was collected at D11 in a vacuum tube containing EDTA and transported under refrigeration to LABIOPAR. A drop of this blood was then placed between slide and coverslip for direct light microscopic examination. The remaining blood was used for blood culture. The red blood cells from the dog were separated from the plasma, added to Liver Infusion Tryptose (LIT) medium supplemented with 10% bovine serum, and placed in an incubator at 26 °C (± 1ºC). The culture was examined microscopically between a slide and a coverslip on days 15 and 30 after culture initiation using an optical microscope at 400× magnification [35]. In order to obtain parasites for the molecular PCR assay, positive cultures were amplified on a logarithmic scale to obtain a concentration of 10^8 parasites/mL, according to the protocol of Camara et al. [16].

DNA extraction

DNA extraction from the whole blood of the dog preserved using guanidine-HCl/EDTA (pH 8.0), as well as the parasites mass isolated from blood culture, was performed using the phenol–chloroform method, following one of two protocols, respectively: the blood extraction method by Gomes et al. [36], and the parasite isolation protocol by Macedo et al. [37]. The extracted DNA was then used as the template for the PCR assays.

Diagnostic PCR for the detection of *T. cruzi*

The amplification of the constant regions of the minicircles of the kinetoplast DNA of *T. cruzi* was performed as described by Gomes et al. [36], using the primers *121* (5'-AAATAATGTACGGG(G/T)GAGATGCATGA-3') and *122* (5'-GGTTCGATTGGGGTTGGTGTAATATA-3'). For the PCRs, DNA extracted from both the blood of the dog and the parasite cultures was used. DNA from *T. cruzi* CL-Brener clone was used as positive control, and a sample from an uninfected human as a negative control. The PCR products were analyzed by electrophoresis using 6.0% polyacrylamide gels, and the DNA fragments visualized by silver staining.

Genetic characterization of *T. cruzi*

The genotypic characterization of the *T. cruzi* parasites isolated from the infected dog sample (i.e., parasite culture "C681") was performed according to the protocol by D'Ávila et al. [38], using as markers the: (i) mitochondrial *cytochrome* c *oxidase II* (*coii*) gene [11], (ii) the D7 divergent domain of the *24Sα rRNA* gene [39] and the intergenic region *SL-Irac* [40]. PCR reactions were performed using a Mastercycler® Nexus Gradient thermal cycler (Eppendorf®, Hamburg, Germany). The following reference clones/strains of *T. cruzi* were used as positive controls: the TcI Col1.7G2 clone/Colombia, the TcII Y strain/São Paulo/Brazil, the TcIII CBS56Tb isolate/Rio Grande do Norte/Brazil, the TcIV AM64 strain/Amazonas/Brazil, the TcV 3253 strain/Minas Gerais/Brazil, and the TcVI CL-Brener clone/Rio Grande do Sul/Brazil.

Nested PCR, purification, sequencing and phylogenetic analysis

Nested amplification of the *18S SSU rRNA* gene region was carried out according to Noyes et al. [41] using two sets of primers: the *TRY927F* (5'-GAAACAAGAAAC ACGGGAG-3') and *TRY927R* (5'-CTACTGGGCAGC TTGGA-3') outer primer pair were used in the first round of PCR, and the *SSU561F* (5'-TGGGATAACAAA GGAGCA-3') and *SSU561R* (5' -CTGAGACTGTAA CCTCAAAGC-3') inner primer pair were used in the second round of PCR [42]. The PCR products amplified and detected on a polyacrylamide gel were quantified and adjusted to a concentration of 20 ng/µL DNA.

Purification of the PCR products was performed using ExoSAP-IT™ PCR Product Cleanup (Thermo Fisher Scientific) according to the manufacturer's instructions. The BigDye® Terminator v3.1 Cycle Sequencing Kit (Applera, Applied Biosystems, CA, USA) was used for the sequencing reactions. A final concentration of 20 ng of purified DNA, 1 µL of either the forward and reverse primers (5 pmol), 1.75 µL of Save Money buffer (200 mM Tris–HCl, pH 9; 5 mM $MgCl_2$), 0.5 µL of BigDye® and sterile deionized water up to 10 µL were used for this reaction. The sequencing runs were performed on an automatic sequencer (ABI 3500 DNA Analyzer, ThermoFisher). The electropherograms were analyzed, and consensus sequences created using the software program MEGA X v. 10.2.2 [43], and then aligned using the MAFFT online v. 7 multiple alignment program for amino acid or nucleotide sequences [44]. Additional in-group, as well as the out-group, sequences were obtained from the NCBI/GenBank database using the BLAST tool (National Center for Biotechnology Information – https://www.ncbi.nlm.nih.gov/BLAST/). The BLAST search was optimized for highly similar sequences (megablast), as well as selection of sequences previously reported from the state of Rio Grande do Norte. The nucleotide alignments were manually inspected and corrected as necessary using MEGA Xnucleotide substitution models, selected using the ModelFinder plugin of the software PhyloSuite v. 1.2.2 [45], were used for the phylogenetic analysis: (i) MrBayes v. 3.2.6 [46] was used for phylogenetic analysis by Bayesian inference with a GTR+F model, in which two parallel runs and 10,000 sampling frequency were performed; and (ii) IQ-TREE [47] under the TIM3+F model for 10,000 standard bootstraps. In addition, the approximate likelihood ratio test similar to Shimodaira-Hasegawa [48] was performed. The resulting phylogenetic trees were plotted using Figtree v. 1.4.4 v. 1.0.1.

Results

Microscopic diagnosis for hemoparasites, whole blood counts, biochemical and echocardiogram parameters of a naturally-infected dog

In the dog C681, trypomastigotes suggestive of *T. cruzi* were easily visualized at D11 (Fig. 1a) with up to two parasites observed in each microscopic field during direct blood examination and/or in blood smears. This patent parasitemia persisted for approximately 29 days, with trypomastigotes observed in the blood smears until D40 inclusive (Table 1). Blood count changes in this period were discreet, with hypochromic microcytic anemia and leukopenia between days D0 and D40 inclusive (Table 1).

On D11, the biochemical parameters, shown in Table 2, revealed important elevations from the normal reference ranges in cardiac troponin I (0.50 ng/mL) and NT-ProBNP (3,701 pmol/L), while the Doppler echocardiogram performed on the same day showed a pronounced qualitative segmental hypokinesia in the posterior wall P1, P2 and P3, with alternating radial kinesia ranging from 26 to 41%. In addition, a reduction in the hypokinesia of the longitudinal myocardial fibers, with a normalized left ventricle < 1.7 (LVEDD / Weight 0.294), no evidence of volume overload or predictive indicators of congestion (E/A, E/IVRT and E/E´),

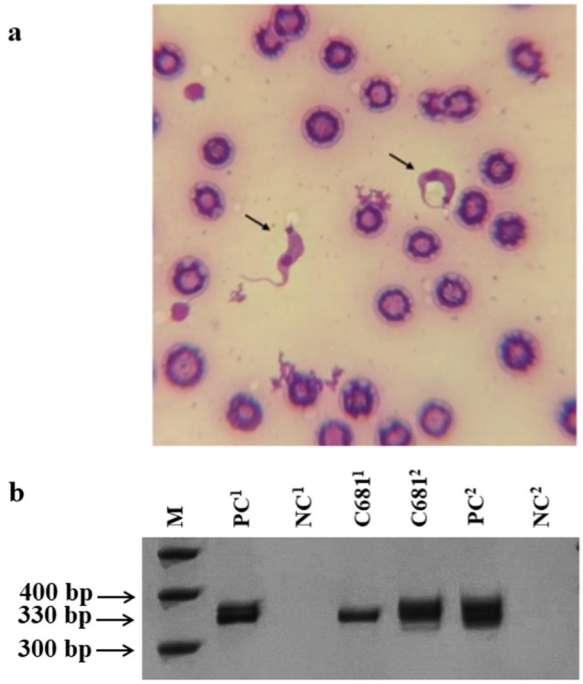

Fig. 1 Microscopic and molecular diagnosis of *Trypanosoma cruzi* parasites in the naturally-infected dog "C681" from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, Brazil. **a** Blood smear showing panoptic-stained trypomastigotes in the whole peripheral blood of during the acute phase of infection. Light microscopy at 1000× magnification. **b** Polyacrylamide gel showing specific amplification of 330 bp kDNA fragments of the minicircle of *T. cruzi* from the blood ($C681^1$) and in vitro cultured parasites isolated ($C681^2$) from the dog C681. NC^1: uninfected human blood. PC^1: positive control for the DNA extraction, which used blood from the dog from an endemic area, also naturally-infected with *T. cruzi*. PC^2: positive control for the PCR using DNA extracted from the *T. cruzi* clone CL-Brener. $NC.^2$: negative control for the PCR reaction (H_2O). M: molecular size marker (100-bp ladder; Life Technologies, Gaithersburg, MD)

and maintained cardiac output (VTI x π (radius)^2) of the aortic artery were observed. At this time, D11, a serological indirect immunofluorescence test for IgG antibodies against *T. cruzi* was also performed, which was specifically-reactive at a 1/40 dilution, without the presence of detectable antibody against *Trypanosoma evansi* (results not shown).

Trypanosoma cruzi kDNA PCR and genetic characterization of DTU

Conventional standard PCR targeting the trypanosome kDNA was used both on samples of whole blood taken from the dog "C681" and the parasite culture derived from the blood taken from this dog (C681). Both samples gave an approximately 330 base pair (bp) band consistent with specific amplification of the constant region of the minicircle of *T. cruzi* kDNA (Fig. 1b). After this

Table 2 Biochemical parameters on the 11th day of follow-up in the dog "C681" naturally-infected with *Trypanosoma cruzi* from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, Brazil

Biochemical parameters	D11	Reference ranges
Cardiac troponin I	0.50	0.00 to 0.07 ng/mL
NT-ProBNP	3,701	< 900 pmol/L
Creatinine	0.8	0.5–1.5 mg/dL
Alkaline phosphatase	53	9.4–249 U/L
ALT/GPT	20	21–74 U/L
Urea	20	15–40 mg/dL
Total protein	6.1	5.8–7.7 g/dL
Albumin (A)	2	2.6–3.3 d/dL
Globulin (G)	4.1	2.7–4.4 g/dL
Ratio A/G	0.4	0.45–11 g/dL

Abbreviations: NT-ProBNP N-terminal prohormone B-type natriuretic peptide, *ALT* alanine aminotransferase, *GPT* glutamic-pyruvic transferase

PCR-based kDNA identification, genotyping of the in vitro cultured C681 isolate was performed using the three genetic markers used for *T. cruzi* DTU-typing: *coii*, *24Sα rRNA* and *SL-Irac. COII* had haplotype B, *24Sα rRNA* amplified the 110 bp fragment, and for the *SL-Irac* gene two fragments of 150 and 200 bp were visualized. The bands observed for the C681 isolate were similar to those for the reference isolate CBS56Tb, confirming that the isolate C681 belongs to DTU TcIII (Fig. 2a, b, c).

Phylogenetic analysis

PCR of the *18S SSU rRNA* gene from the in vitro cultured C681 isolate amplified a 650 bp fragment. DNA sequencing showed a high-quality electropherogram. The consensus sequence was submitted to the BLASTn tool for alignment with other sequences in the NCBI/GenBank database. This analysis identified other previously deposited *T. cruzi* DTU TcIII *18S SSU rRNA* sequences, which had similarities between 99.81% and 100% with our new C681 sequence, and a query coverage of between 67 to 100% with the other sequences which it hit, and *E*-values of zero. In both the Bayesian inference and maximum likelihood phylogenetic analyses, the sequence from C681 clustered in the "TcIII" clade, together with the sequence from the RN02 isolate previously obtained from *T. brasiliensis* collected in the state of Rio Grande do Norte (Fig. 3). The basal node of the clade for the TcIII DTU genotype had very high support values for both the Bayesian (~ 1.0 posterior probability) and maximum likelihood (~ 100% bootstrap) analyses, consistent with the DTU genetic characterization performed and described above using the other three loci. The outgroup,

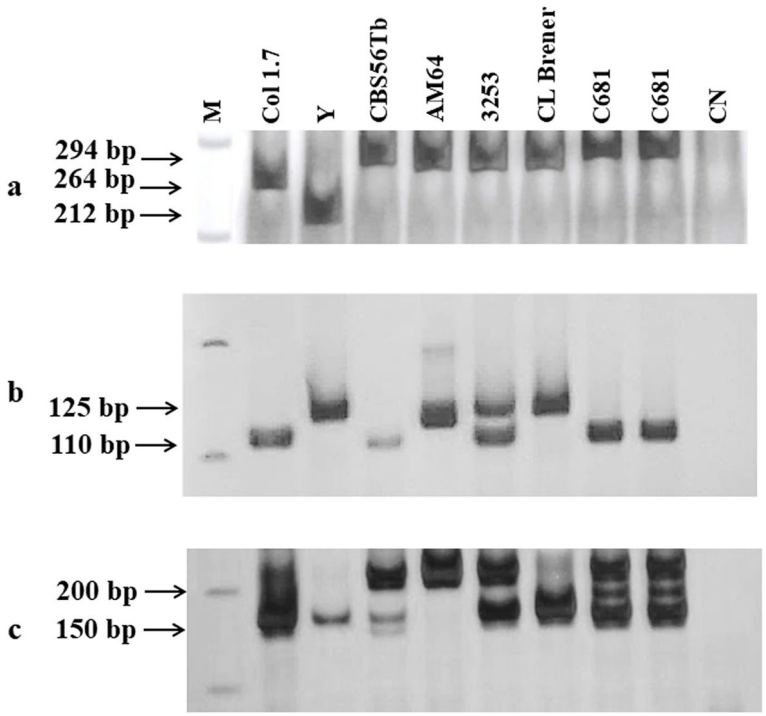

Fig. 2 Polyacrylamide gels showing the molecular characterization of the in vitro cultured *Trypanosoma cruzi* C681 isolate derived from the naturally-infected dog "C681" from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, Brazil. Each of the three gels shows amplification of one of the three genes used for *T. cruzi* DTU-typing for the C681 isolate, and various other known reference clones and strains of *T. cruzi*. In all three gels, the products have been loaded in the same order for the isolate and the clones/strains. **a** Amplification of the mitochondrial *coii* gene. Lane 2: the Col1.7G2 clone, showing amplified restriction fragments of 264 bp, characteristic of "haplotype A". Lane 3: the Y strain with the 212 bp fragment of "haplotype C". Lane 4: the CBS56Tb isolate. Lane 5: the AM64 strain. Lane 6: the strain 3253. Lane 7: the CL-Brener clone. Lanes 8 and 9: duplicate reactions for the C681 isolate, showing a 294 bp band indicative of haplotype B. Lane 10: negative control (H_2O). **b** Amplification of the *24Sα rRNA* gene. Lane 2: the clone Col1.7G2 (110 bp). Lane 3: the Y strain (125 bp). Lane 4: the CBS56Tb isolate (110 bp). Lane 5: the AM64 strain (117/119 bp). Lane 6: the 3253 strain (110 and 125 bp). Lane 7: the CL-Brener clone (125 bp). Lanes 8 and 9: duplicate reactions for the C681 isolate, which amplified a fragment of 110 bp. **c** Amplification of the *SL-IRac* gene. Lanes 2, 3, 6 and 7: the Col1.7G2 clone, the Y strain, the 3253 strain, and the CL-Brener clone respectively, both with 150/157 fragments bp. Lanes 4, 5, 8 and 9: the CBS56Tb isolate, the AM64 strain, and the C681 isolate duplicate, respectively. In all three gels shown, Lane 1: "M" = molecular size marker (100-bp ladder, Life Technologies, Gaithersburg, MD)

represented by *Trypanosoma minasense* (MF141847) formed an external clade (Fig. 3).

Discussion

A dog in the acute phase of natural infection with the *T. cruzi* DTU TcIII, exhibiting patent parasitemia for almost a month, was evaluated using clinical, hematological, biochemical and parasitological methods. In general, the natural history of *T. cruzi* infection begins with the interaction of the parasite with the vertebrate host, followed by a prepatent period of variable duration when few parasites are present and their detection is limited. Then, the acute phase starts, with exponential reproduction of the parasite at the cellular level, resulting in a gradual increase in the number of trypomastigotes in the blood, which can now be observed using fresh blood [1, 49]. Therefore, when there is patent parasitemia, *T. cruzi* infection is in its acute phase, the laboratory criterion for which is the identification of trypomastigotes in peripheral blood through direct examination, as suggested by Laranja et al. [50] and Dias et al. [51].

Our analyses showed changes in the whole blood count, such as hypochromic microcytic anemia and leukopenia during the patent parasitemia, corroborating with previously described experimental infection of Beagle dogs with different *T. cruzi* strains, which showed that reductions in blood cell counts were closely correlated with parasitemia levels during the acute phase of infection [52]. In this study, we also observed along with the presence of parasite forms in the blood, low red blood cell counts and low hemoglobin levels. Other authors have also correlated higher parasitemia in vertebrate hosts infected with the Y strain of *T. cruzi*, with anemia, leukopenia, and clinical signs, such as lymph node enlargement, lethargy, loss of appetite and weight loss [53]. Studies on experimental infection in dogs using

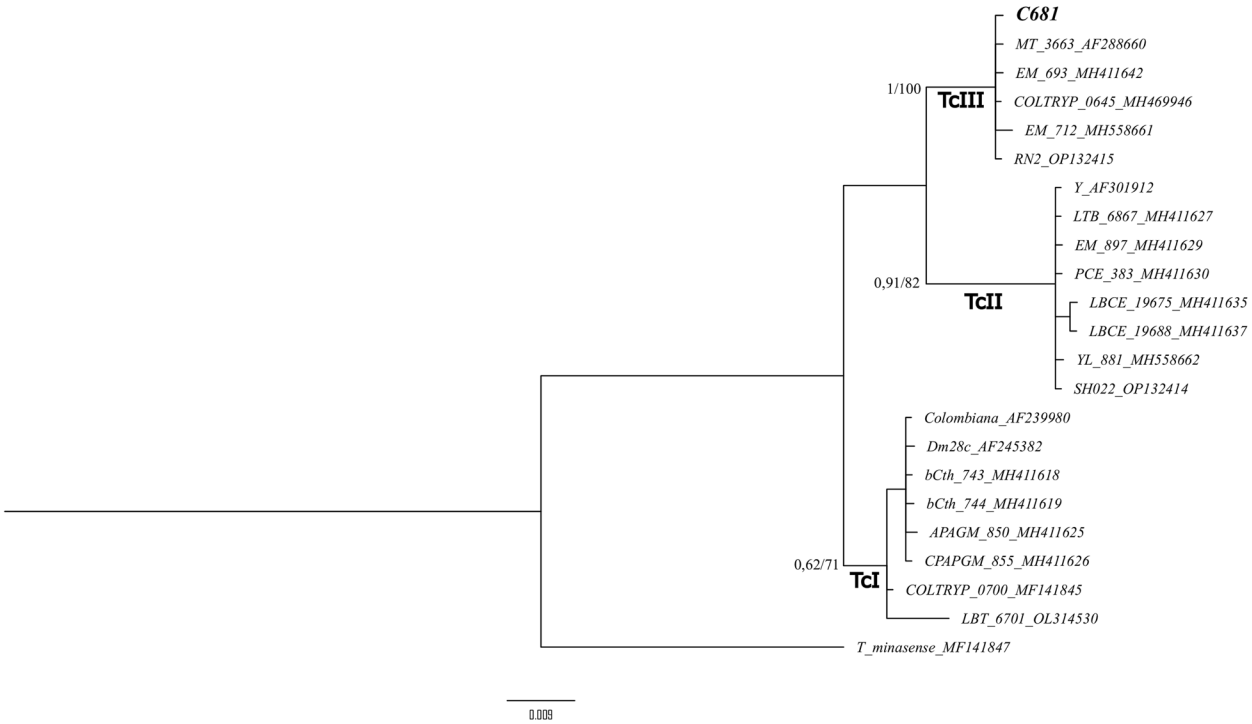

Fig. 3 Phylogenetic relationships of the *18S SSU rRNA* gene for sequences derived from various isolates and strains of *Trypanosoma cruzi*. A Bayesian phylogenetic tree was constructed using *18S SSU rRNA* sequences derived from the *T. cruzi* C681 isolate reported in this study and various representative sequences for the TcI, TcII and TcIII DTUs obtained from GenBank, a sequence *T. minasense* being used as an outgroup. The numbers on the nodes are the support values, with the posterior probabilities from the Bayesian inference given first, and the bootstrap values from IQ-TREE shown after

different strains of *T. cruzi* have also shown that high parasitemia in acute phase is related to more severe cardiomyopathy [27, 28, 54].

Cardiac troponin I and NT-ProBNP have been described as biochemical markers of cardiac damage in dogs [55, 56]. The findings of this case report demonstrated alterations in these cardiac biomarkers – both cardiac troponin I and NT-ProBNP – in addition to segmental hypokinesia in the posterior heart wall suggesting important evidence of myocardial kinetic alteration. These changes have previously been reported in dogs chronically-infected with *T. cruzi* [57]. Myocarditis is a disease caused by numerous etiological factors and characterized by a non-specific course [58], although post-mortem histopathological examination of cardiac muscle specimens can enable precise characterization of the inflammatory changes. In the current context, this latter examination was not possible to perform on the dog, as it remains alive and is clinically well. It is important to note that alterations in these cardiac function markers can also occur in other situations, such as NT-ProBNP being a relevant biochemical marker for predicting mortality in older dogs with degenerative mitral valve disease (DMVD) [59], as well as elevated cardiac troponin I levels in dogs with systemic inflammatory response syndrome (SIRS) [60]. In felines, the presence of cardiac troponin I is associated with inflammatory cardiac injuries [61]. Our study describes a naturally-infected dog, and it is known that there is great variability in the clinical symptoms of infection with *T. cruzi*. The dog investigated in this study came from the municipality of São Paulo do Potengi, in the state of Rio Grande do Norte, an area considered to be at medium risk for *T. cruzi* transmission, with the triatomine vector species *T. brasiliensis*, *Triatoma pseudomaculata* Corrêa and Spínola, 1964, *Panstrongylus lutzi* Neiva and Pinto, 1923 and *Rhodnius nasutus* Stal, 1859, widely distributed in the semi-arid region, which includes São Paulo do Potengi [33]. It is noteworthy that *T. brasiliensis* is the species most frequently found in anthropogenically-altered environments, and infected dogs have been identified in places where this triatomine species is also present [30].

Parasite infection of dog C681 was confirmed by morphological analysis of trypomastigotes identified by direct examination, and blood smears, and by amplification of the constant kDNA minicircle region using specific primers for *T. cruzi*. Dogs naturally-infected with *T. cruzi* have been previously described in the different

states of Brazil, including Rio Grande do Norte [30, 31], as well as Paraíba [62, 63], Bahia [64] and Mato Grosso do Sul [65]. Naturally-infected domestic dogs have also been described in other countries, such as Argentina [66, 67] and the United States of America [68, 69].

Molecular characterization of the *T. cruzi* isolate C681 – using the *coii*, *24Sα rRNA* and *SL-Irac* genetic markers, and phylogenetic analysis of the *18S SSU rRNA* region – identified it as belonging to the DTU TcIII. Previously, in the state of Rio Grande do Norte, TcIII has been reported in the triatomine species *P. lutzi*, *T. brasiliensis*, as well as in wild mammals and human [16, 17, 19]. Overall, these observations suggest an overlap between the sylvatic and domestic/peridomestic transmission cycles of the TcIII DTU [16–19], with domestic dogs acting as bridge hosts that facilitate the movement of *T. cruzi* between different transmission cycles, as previously suggested in the Chaco region of Argentina by Enriquez et al. [66].

Conclusion

In summary, our case report demonstrates the occurrence of a dog with a natural infection with the *T. cruzi* DTU III, in the acute phase of Chagas disease and its associated cardiac damage. These findings suggest that dogs could be important indicators and sources of domestic *T. cruzi* infection of humans.

Abbreviations

NT-ProPNB	N-Terminal Prohormone B-Type Natriuretic Peptide
ALT	Alkaline phosphatase, alanine aminotransferase
LIT	Liver Infusion Tryptose medium
E	Peak velocity of early diastolic transmitral flow
E´	Peak velocity of early diastolic mitral annular motion measured at the lateral mitral annulus
A	Peak velocity of late diastolic transmitral flow
IVRT	Isovolumic relaxation time
VTI	Velocity time integral
LVEDD	Left ventricular diameter in diastole
DPI	Day post-infection
kDNA	Kinetoplast DNA
MAFFT	Multiple alignment program for amino acid or nucleotide sequence online
BLAST	Basic Local Alignment Search Tool
iq-Tree	Software for maximum likelihood analysis
PPP	Prepatent period
PP	Patent period

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04486-5.

Supplementary Material 1.

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Authors' contributions

Conceptualization: V.T.AN., A.N.BS., P.M.M.G., L.M.C.G., A.C.J.C. Funding: A.C.J.C. Dog`s examination: V.T.AN. T. cruzi isolation and genotyping: A.N.BS., L.A.B., B.A.F., G.H.F.S., C.C.A.N., A.C.J.C. Ecodopplercardiogram: R.S.M. whole blood counts and biochemical parameters: Y.B.N.F.; Sequencing and phylogenetic analysis: V.T.AN., A.N.BS., A.C.J.C.; Original draft writing: V.T.AN., A.N.BS., R.S.M., C.R.N.B., A.C.J.C.. Reviewed and corrected the manuscript: A.C.J.C., L.M.C.G., C.R.N.B., A.N.BS., P.M.M.G. All authors reviewed the manuscript.

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Data availability

The sequence was submitted to GenBank under the following access numbers: OR394654. The isolate C681 and blood sample are available in the LABI-OPAR collection at Department of Clinical and Toxicological Analyses/UFRN.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee on the Use of Animals/UFRN, protocol no.134.062/2018 and no.138.066/2018. The dog owner was informed about the research and gave written informed consent before samples were collected.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Graduate Program in Pharmaceutical Sciences, Federal University of Rio Grande Do Norte, Natal, Brazil. [2]Graduate Program in Health Sciences, Federal University of Rio Grande Do Norte, Natal, Brazil. [3]Veterinary Cardiology and Pulmonology Service, Rio Grande Do Norte, Natal, Brazil. [4]Graduate Program in Parasitic Biology, Federal University of Rio Grande Do Norte, Natal, Brazil. [5]Veterinary Service Laboratorial, Natal, Rio Grande Do Norte, Brazil. [6]Department of Clinical and Toxicological Analyses, Health Sciences Center, Federal University of Rio Grande Do Norte, Avenida Gal. Gustavo Cordeiro de Farias, N/N 2nd floor, Petrópolis, Natal 59012-570, Rio Grande Do Norte, Brazil.

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