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RESEARCH ARTICLE

Acute Chagas disease associated with ingestion of contaminated food in Brazilian western Amazon

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Abstract

Objective: To describe clinical, epidemiological and management information on cases of acute Chagas disease (ACD) by oral transmission in the state of Amazonas in western Amazon.

Methods: Manual and electronic medical records of patients diagnosed with ACD at the *Fundação de Medicina Tropical Doutor Heitor Vieira Dourado* (FMT-HVD) were included.

Results: There were 147 cases of acute CD registered from 10 outbreaks that occurred in the state of Amazonas between 2004 and 2022. The transmission pathway was through oral route, with probable contaminated palm fruit juice (açaí and/or papatuá), and involved people from the same family, friends or neighbours. Of 147 identified cases, 87 (59%) were males; cases were aged 10 months to 82 years. The most common symptom was the febrile syndrome (123/147; 91.8%); cardiac alterations were present in 33/100 (33%), (2/147; 1.4%) had severe ACD with meningoencephalitis, and 12 (8.2%) were asymptomatic. Most cases were diagnosed through thick blood smear (132/147; 89.8%), a few (14/147; 9.5%) were diagnosed by serology and (1/147; 0.7%) by polymerase chain reaction (PCR) and blood culture. In all these outbreaks, 74.1% of the patients were analysed by PCR, and *Trypanosoma cruzi* TcIV was detected in all of them. No deaths were recorded. The incidence of these foci coincided with the fruit harvest period in the state of Amazonas.

Conclusion: The occurrence of ACD outbreaks in the Amazon affected individuals of both sexes, young adults, living in rural and peri-urban areas and related to the consumption of regional foods. Early diagnosis is an important factor in surveillance. There was a low frequency of cardiac alterations. Continuous follow-up of most patients was not carried out due to difficulty in getting to specialised centres; therefore, little is known about post-treatment.

KEYWORDS
Açaí; Chagas disease outbreaks, clinical epidemiology; oral transmission; *Trypanosoma cruzi*

Sustainable Development Goal: Good Health and Wellbeing

INTRODUCTION

Chagas disease (CD) is a neglected anthropozoonosis that remains an important health problem, even more than 100 years of its discovery. About 8 million people are *Trypanosoma cruzi* carriers in Latin America and around the world [1]. CD is caused by *T. cruzi*, a protozoan with a complex biological cycle, carried out in dozens of species of mammals and triatomine bugs insects, also known as kissing bugs. Transmission can occur via the vectorial route when faeces of infected triatomines comes in contact with broken skin, via blood transfusion and organ transplants from infected people, laboratory accidents, vertically from mother to fetus and via ingestion of contaminated food [2]. In humans, oral transmission has generated an increase in the number of acute cases of CD (ACD) and has been responsible for outbreaks in several Latin American countries [3, 4]. In Venezuela, for instance, the largest outbreak of ACD ever recorded occurred due to the consumption of guava juice [5], while in Brazil, this form was first reported in 1967, in the municipality of Teutônia, state of Rio Grande do Sul, without a defined food source [6].

In the Brazilian Amazon, the first recorded autochthonous cases of ACD occurred in Pará state, in 1969, where oral transmission was suspected [7]. Following this incident, several other outbreaks of the disease were documented, all of which shared a common source of infection/transmission, the consumption of palm fruit juices, particularly açai (*Euterpe oleraceae* and *E. precatoria*) [8]. Açai, a typical fruit of the Brazilian Amazon Region, is an economically important edible fruit and a dietary component in the region and sees increased consumption especially during the seasonal production months [9]. It should be noted that several species of palm trees are common to the vegetation around the homesteads and serve as shelter for and the establishment of colonies by some species of wild triatomines of the genus *Rhodnius*. Birds and/or small rodents that nest in these trees around the homesteads serve as source of blood meal for the vectors, thereby sustaining the insect's life cycle close to human dwellings [10].

In Amazonas state, *R. robustus* and *R. pictipes* adult forms have been found in the intra- and peri-domicile, with high rates of natural infection by *T. cruzi*, which suggested a risk in the eco-epidemiology of CD in the locations where they were discovered [10, 11]. The first record of ACD in Amazonas was in 1980 [12], but the first outbreak via oral transmission was recorded in 2004 [13]. Since then, several outbreaks have been reported in the state with people diagnosed during the acute phase after consumption of juice from regional palm fruits. In one of the outbreaks, for the first time, it was possible to prove the contamination of açaí by *T. cruzi* that was consumed by the patients [14]. Due to the relevance, this study aims to describe clinical, epidemiological and management information on cases of ACD caused by oral transmission in the Amazonas state in western Amazonia.

MATERIALS AND METHODS

Study design

Information about the cases of ACD recorded in outbreaks by oral transmission, which occurred from 2004 to 2022, in nine municipalities in the interior of the state of Amazonas, is presented in Figure 1. Primary data were obtained from patients recruited during the outbreaks, while secondary data was sourced from manual and electronic medical records of the *Fundação de Medicina Tropical Doutor Heitor Vieira Dourado* (FMT-HVD), a reference hospital for tropical and infectious diseases in Manaus, Amazonas, where the CD outpatient clinic is located. Epidemiologic analyses were carried out in all CD outbreaks by an interdisciplinary team of professionals from the FMT-HVD in collaboration with the *Fundação de Vigilância em Saúde do Amazonas Dra. Rosimary Costa Pinto* (FVS-RCP/AM).

Criteria for confirmation of the diagnosis of ACD

The following criteria were used in confirming ACD cases: (1) exposure to the probable source of contamination; (2) manifestation or absence of symptoms; (3) confirmation of laboratory diagnosis by parasitological tests: thick blood smear (TBS), hemoculture (HC), xenodiagnosis (XD) and serological tests. Serological tests were performed at the *Fundação de referência Ezequiel Dias*-FUNED, targeted at detecting anti-*T. cruzi*-IgM antibodies and considering positive values ≥1:40. Similarly, detection of anti-*T. cruzi*-IgG antibodies was performed by different methods, namely: Indirect Immunofluorescence (IFI)-IgG Chagas (Bio Manguinhos), Indirect Hemagglutination (HAI)-IgG Chagas test-HAI (Wiener Lab) and Enzyme Immunoassay (ELISA)-IgG Chagas test (Wiener Lab). For serological confirmation, a second collection was tested after a 2-week interval [2].

Identification of the *T. cruzi* strain

This aspect of the study involved the identification of the *T. cruzi* strain from the last six outbreaks that occurred in Carauari in 2011 and 2015, Uarini in 2019, Barreirinha in 2019, Ipixuna in 2021 and Amaturá in 2022. *T. cruzi* blood cultures were used for parasite DNA extraction performed according to PureLink Kit manufacturer's recommendation (Invitrogen, Life Technologies). Following that, the glucose-phosphate isomerase (*GPI*) gene was examined using the extracted DNA [15]. The polymerase chain reaction (PCR) product was purified as described by Paithankar and Prasad [16].

Sequencing was performed using the ABI 3130 DNA sequencer (Applied Biosystems, https://www.thermofisher.com), following the Big Dye Terminator v.3.1 Cycle Sequencing Kit protocol (Applied Biosystems), 10–40 ng of the

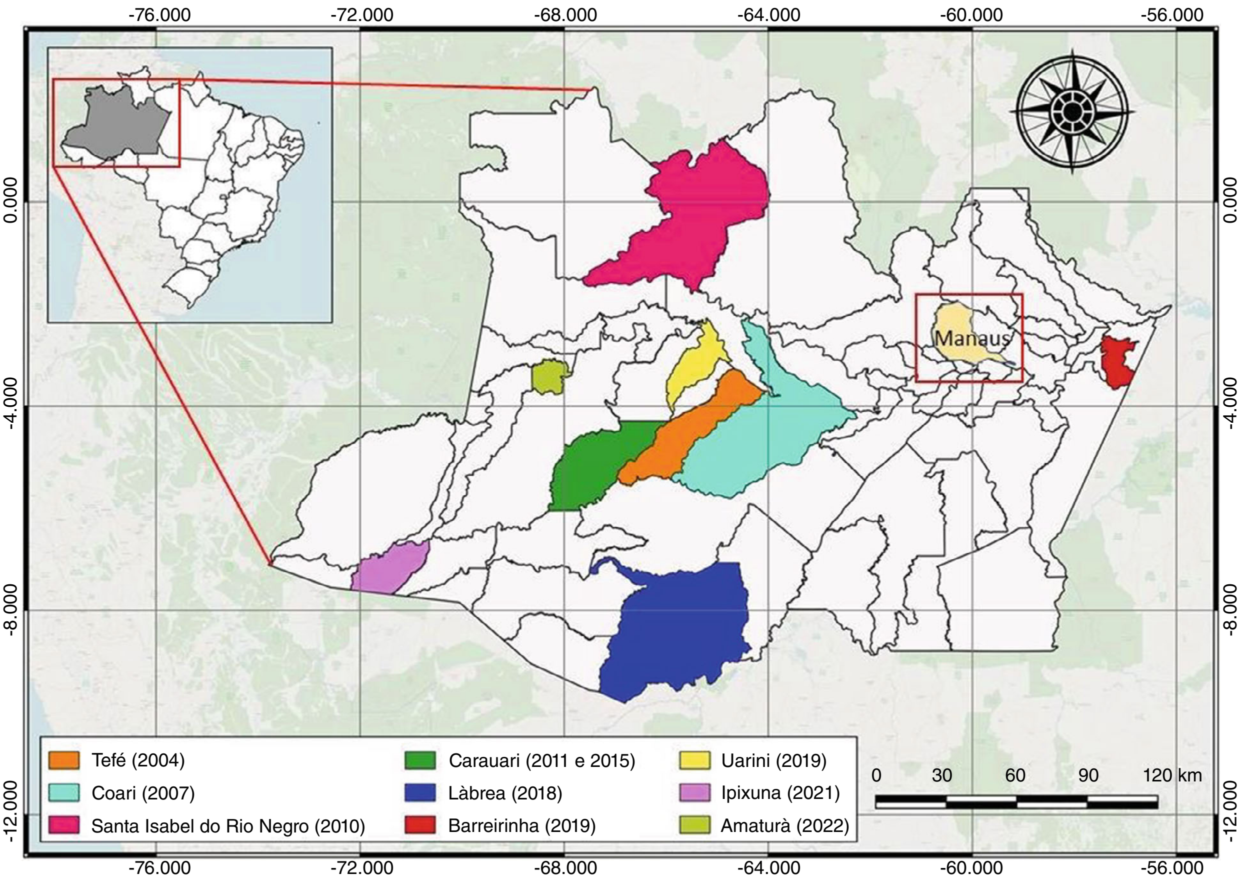

FIGURE 1 Map of Amazonas State indicating the municipalities and the year when the outbreaks occurred. *Source*: Freire R, 2022.

purified PCR product was used in the PCR reaction with the appropriate primer. The sequences obtained were deposited in Genbank (accession numbers: OP804717–OP804731) and aligned with previously published sequences and the standard strains: TcI-(Silvio10 cl1-AY540730.1), TcII-(Esmeraldo cl3, AY540728.1), TcIII-(M6241 cl6, AY484478.1), TcIV-(CANIII cl1-, AY540727.1), TcV-(Mn cl2, AY484480.1) and TcVI-(CL Brener, DQ343645.1-XM_815802.1) where the different haplotypes were identified. Phylogenetic analyses were conducted using MEGA version 11. The resultant phylogenetic tree was constructed using the Neighbour-Joining method after testing for the best-fit replacement model. Bootstrap analysis was inferred based on 1000 replicates [17].

Statistical analysis

Clinical and epidemiological data were organised using Microsoft Excel 2016 and descriptively analysed using Stata/MP 14.0. Categorical variables were described as frequencies and proportions (%). Non-parametric variables such as 'age' was described with median and interquartile range [25%–75%]. The variable 'incubation period' had a normal data distribution and was described using mean and standard deviation.

Ethical considerations

The FMT-HVD's Research Ethics Committee approved this study under the CAAE number 03164118.9.0000.0005.

RESULTS

We found 147 cases of ACD, from 10 outbreaks that occurred between 2004 and 2022, all with a history of consumption of food with probable contamination by *T. cruzi*, of which 9 outbreaks were related to the consumption of açaí juice and one of pataúá juice (*Oenocarpus bataua*). The average interval of occurrence was 2.1 years between events from 2018 to 2022; there was approximately one outbreak per year, with two outbreaks occurring in 2019 (Figure 2). In most of these events, it was found that a family member had produced and distributed the consumed palm fruit juice among their relatives and friends. There were outbreaks, though, where the açai juice was produced, packed in

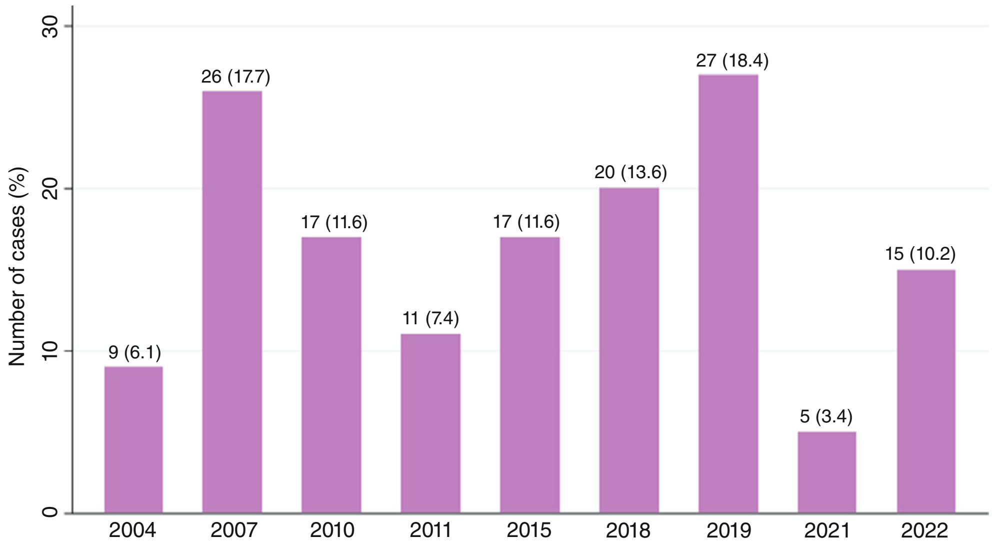

FIGURE 2 Number of acute Chagas disease cases by oral transmission between 2004 and 2022 in the state of Amazonas.

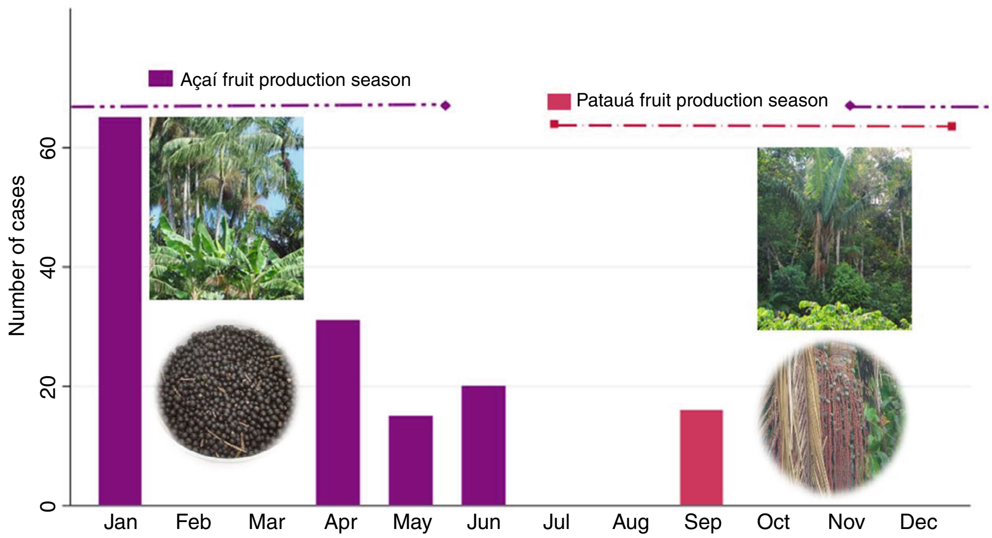

FIGURE 3 Months of Chagas disease outbreaks and the palm fruit harvest period in Amazonas. The broken line in purple represents the açaí harvest period, while the red broken line is the patauá harvest period.

another municipality, and then distributed to family and friends in the city of Manaus.

Seasonal periodicity

One of the outbreaks occurred in September, which had coincided with the patauá juice production and consumption season. Interestingly, other outbreaks were recorded in the first half of the year, whereby four outbreaks were reported in January, two each occurring in April and June and one in May, all of them associated with açaí consumption (Figure 3).

Epidemiological aspects

Of the total number of ACD cases, 134/147 (91.2%) were symptomatic; 87 (59%) were males, and the median age was 27 years [17–45], ranging from 10 months to 82 years. The recorded incubation period had a mean of 10 ± 4.8 days and clinical manifestations started between the 2nd and the 21st day after consumption of the contaminated food defined as the source of infection. Febrile syndrome was the most frequent symptom 123 (91.8%), usually accompanied by other non-specific symptoms. On the other hand, two cases (1.3%), one female (16 years) and another male (10 months), presented with meningoencephalitis (Table 1).

TABLE 1 General characteristics of the 147 patients in the Chagas disease outbreaks in Amazonas.

Variables	Number of cases (%)
Age(years), median [IQR]	27 [17–45]
Sex	
Male	87 (59)
Female	60 (41)
Area of residence	
Rural	115 (78.2)
Urban	32 (21.7)
Incubation period (days)	10 ± 4.8
Symptoms	134 (91.2)
Febrile syndrome	123 (91.8)
Asthenia	72 (53.7)
Myalgia/arthralgia	71(53)
Headache	57 (42.5)
Oedema	43 (32)
Vomiting	28 (20.9)
Epigastric pain	26 (19.4)
Rash	25 (18.6)
Palpitation	17 (12.7)
Hematuria	15 (11.2)
Dizziness	13 (9.7)
Hepatomegaly	12 (8.9)
Splenomegaly	11 (8.2)
Meningoencephalitis	2 (1.3)

TABLE 2 Parasitological and serological tests performed.

Exams	Type	Accomplished (%)	Positive (%)
Parasitological	Total	147 (100)	133 (90)
	TBS	147 (100)	132 (89.8)
	HC	110 (74.8)	100 (90.9)
	XD	79 (53.7)	70 (88.6)
	PCR	109 (74.1)	109 (100)
Serological	**Total**	**116 (78.9)**	**74 (63.8)**
	IgM	99 (85.3)	54 (54.5)
	IgG	115 (99)	49 (42.6)

Note: Data are presented in absolute and relative values.
Abbreviations: HC, hemoculture; PCR, polymerase chain reaction; TBS, thick blood smear; XD, xenodiagnosis.

Diagnostic tests

According to the evaluated records, parasitological examinations were performed on all 147 cases, with additional serological tests performed on 116 (79%) patients (Table 2). Among those who underwent parasitological tests, 132 (89.8%) tested positive by TBS. Among them was one asymptomatic child; 100/110 (91%) tested positive by HC, 70/79 (88.6%) by XD and 109/109 (100%) by PCR. The hospital records revealed that 116/147 (79%) patients underwent further serological tests, of which 74/116 (63.7%) were reactive for IgM and/or IgG anti-T. *cruzi* antibodies (Table 2).

Fourteen (9.5%) patients were diagnosed with serological tests. Of these, 12/147 (8.2%) were asymptomatic but had an epidemiological link to the outbreak loci because they had consumed the contaminated fruit juice (Table 3).

TABLE 3 Description of 14 patients diagnosed by serological tests with negative parasitological tests.

ID	Sex	Age (years)	Symptoms	IgM	IgG
1	M	15	P	NP	Reactive
2	F	30	A	R	NR
3	F	20	A	R	NR
4	F	34	A	R	NR
5	F	24	A	R	R
6	F	11	A	R	NR
7	F	17	A	R	NR
8	F	78	A	R	NR
9	F	29	A	R	NR
10	M	5	A	R	NR
11	F	23	A	R	NR
12	M	32	A	NR	R[a]
13	M	32	P	R	R
14	M	39	A	R	R

Note: P—present; A—absent; NP—not performed; R—reactive; NR—not reactive; Age in years.
[a]Individual with a reactive serology with titres of 1:320.

T. cruzi genotyping

In the 2004 ACD outbreak, the *T. cruzi* responsible was identified as zymodeme 3 (Z3; equivalent to TcIII/TcIV) using electrophoretic profile of parasite isozymes. At that time, the DTUs classification system was non-existent. In the subsequent outbreaks, the *T. cruzi* TcIV lineage was detected as a causative agent. In this study, samples from outbreaks that occurred in Carauari, Uarini, Barreirinha, Ipixuna and Amaturá were characterised as TcIV. Overall, four haplotypes were also found, which are numbered 'i to iv' (Figure 4).

Cardiological exams

Of the 147 cases, 100 (68%) underwent cardiological exams, whereby 33% (33/100) presented with cardiac alterations. Using electrocardiogram exams, the most common finding was ventricular repolarisation alteration, while echocardiogram exams revealed pericardial effusion with increased cardiac area and ventricular systolic dysfunction. Meanwhile, a 24-h Holter exam on 28 patients revealed that 10.7% (3/28)

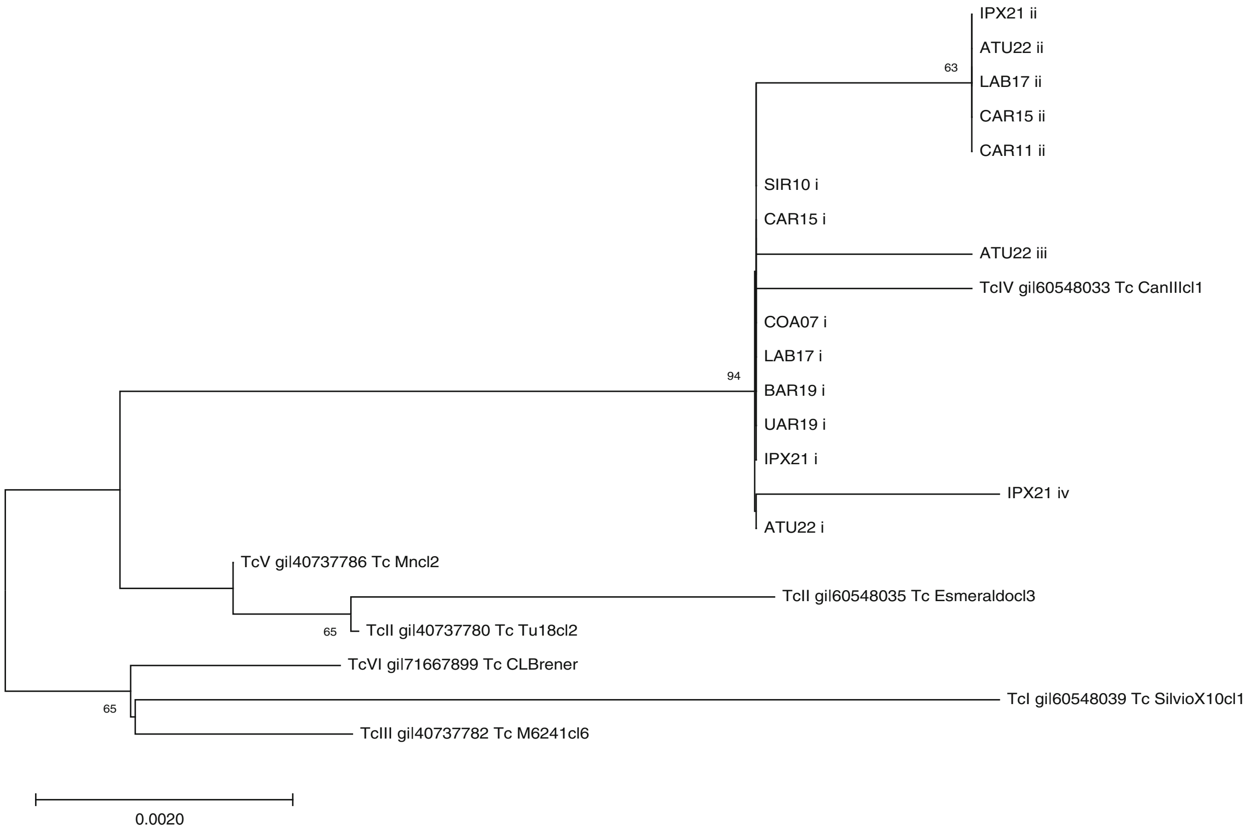

FIGURE 4 Phylogenetic position of *Trypanosoma cruzi* responsible for Chagas disease outbreaks in Amazonas based on GPI gene sequences (best-fit model: Neighbor-Joining). COA07:Coari-2007; SIR10:Santa Isabel do Rio Negro-2010 (38); CAR11:Carauari-2011; CAR15:Carauari-2015; LAB17:Lábrea-2017/2018(14); UAR19:Uarini-2019; BAR19:Barreirinha-2019; IPX21:Ipixuna-2021; ATU22:Amaturá-2022. GPI-glucose-phosphate isomerase; i-iv: different haplotypes (Sequences obtained through this and previous studies).

had paroxysmal supraventricular tachycardia and ventricular ectopias (Table 4).

Treatment and adverse effects

After a confirmed diagnosis, all patients were treated with 5–7 mg/kg/day (adults), or 10 mg/kg/day (children), orally for 60 days of Benznidazole-Rochagan®, as recommended by the Brazilian Ministry of Health and the 2015 Brazilian Consensus on Chagas Disease [2]. Unfortunately, there were no records of subsequent follow-ups and/or treatment outcome of all patients. However, from the notes in the medical records of 45/147 (30.6%) patients, 31% (14/45) presented adverse effects associated with the Benznidazole treatment. Exanthem and pruritus were the most prevalent adverse effects (Figure 5). Regarding the outcome, only one patient was known to have evolved to chronic CD with heart failure.

DISCUSSION

The transmission of *T. cruzi* through food has been proven in different experimental models [18] and in humans [8, 19]. In the Amazon, events associated with the consumption of

TABLE 4 Cardiac changes observed in the patients in the ACD outbreaks.

Variable	No. of cases (%)
Electrocardiogram	**($n = 100$)**
Normal	68 (68%)
Ventricular repolarisation alteration	14 (14%)
Right bundle branch block	5 (5%)
Antero superior divisional block	5 (5%)
Right bundle branch conduction disturbance	3 (3%)
Low QRS voltage	3 (3%)
Tachycardia	3 (3%)
Ventricular extra systoles	2 (2%)
Pre-excitation	1 (1%)
Echocardiogram	**($n = 52$)**
Normal	49 (94.2%)
Pericardial effusion + enlarged cardiac area	2 (3.8%)
Ventricular systolic dysfunction	1 (2%)
24-h Holter	**($n = 28$)**
Normal	25 (89%)
Supraventricular paroxysmal tachycardia	2 (7.4%)
Ventricular ectopias[a]	1 (3.6%)

Abbreviation: ACD, acute Chagas disease.
[a]Changed Holter equals >240 events.

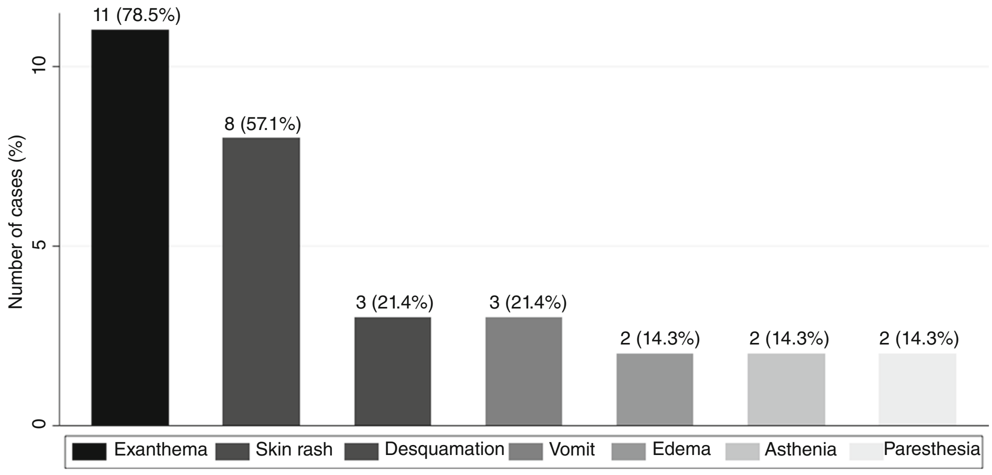

FIGURE 5 A distribution of adverse effects of benznidazole in treating ACD as reported in 14 patients. ACD, acute Chagas disease.

palm fruits, especially açaí, represent a serious problem for the population's health, due to its capacity to affect a multitude of people simultaneously [8, 20–23].

Generally, in all outbreaks, the index case was a person who sought medical attention for fever symptoms. Fever was confirmed as the most common clinical manifestation in the acute phase of CD. In the Amazon, depending on the epidemiological history, anyone seeking medical care with fever must be submitted to microscopic examination of TBS due to the region being endemic for malaria. This was the main tool used in CD surveillance in the region and the gold standard method for detection of hemiparasites [24].

Facial and lower limb oedema was observed in patients of all age groups from 8 of the 10 outbreaks that occurred. Although these symptoms are observed in low frequency in cases of ACD by oral transmission, they have also been reported both in the Amazon region [21–23], other regions in Brazil and Latin America [5, 25].

The Ministry of Health advises that people exposed during outbreaks through oral transmission, and with negative result in the TBS test, to repeat this test [26]. In Amazonas, it has been observed that it may take up to 4 days before TBS exams detect positive cases and thus it is important to do a follow-up test especially during outbreaks. We emphasise the fact that about 90% of the cases had a diagnosis confirmed by TBS, including one asymptomatic case, which demonstrates the importance of hemoscopic examination in all exposed individuals.

Immunological investigations confirmed 10% of ACD-positive cases that were clinically asymptomatic. In addition to serology, complementary parasitological exams are important in confirming or excluding *T. cruzi* infection. This is demonstrated by the case of one symptomatic patient who was TBS-negative but was diagnosed by additional PCR and HC.

In addition to tracking the probable cases, the serological investigations performed during the outbreak investigation, served as complementary diagnosis and as a useful tool in detecting existing or active but largely inconspicuous cases. From our study, one asymptomatic patient, not reactive for IgM, but reactive for IgG, with a titration of 1:320 was discovered. This patient was treated together with those involved in the outbreak in question since he had a history of exposure to the source of the outbreak and most probably had a prior infection.

It is important to highlight that during one of the outbreaks, a 10-month-old child was diagnosed with Chagas-related meningoencephalitis after a positive TBS with no history of açaí consumption during the course of the outbreak and the mother ended up also with a positive TBS. Therefore, it was suspected that the infection might have occurred during breastfeeding. Studies on the transmission of ACD through lactation in humans are old, scarce and with limitations, with some reports of contamination of milk by blood trypomastigotes from cracks in the breast [27, 28]. In this situation, it was not observed, during the outbreak period, whether there was any injury on the mother's chest; however, in cases of acute maternal infection, breastfeeding may represent a risk for the baby due to the high parasitemia in the blood during this phase. This suggests that ACD infection by oral transmission is not just limited to contaminated food/juices but can occur by way of human breast milk with infected mothers potentially infecting their infants.

Another significant fact was the absence of fatalities during these outbreaks, unlike in the outbreaks that occurred in other states, namely Pará [29], Paraíba [30], Santa Catarina [31], Rio Grande do Sul [6], Rio Grande do Norte [26], and in other Latin American countries such as Colombia [3] and Venezuela [5]. One factor that could be mitigating against ACD-related fatalities in the Amazonas is the robust surveillance system on febrile cases across the state. Early diagnosis and intervention of ACD cases has minimised risks of increased morbidity and mortality.

Cardiac alterations, evolution and genetic characterisation of *T. cruzi*

During the events, it was observed that few patients presented with cardiac alterations. This was probably because the causative *T. cruzi* strain identified comes from a sylvatic life cycle. However, this does not exempt the need for continuous monitoring. A systematic review by Chadalawada et al. [32], highlighted that patient in the acute phase of CD had a significantly higher risk (4.6%) of evolving to chagasic heart disease than patients with the indeterminate chronic form (1.9%) of CD. In addition, there has already been a record of a patient from an outbreak with evolution to chagasic heart disease even after treatment in Amazonas [33].

In the Amazon region, there are few reports pertaining to the molecular description of the *T. cruzi* lineage found in cases of CD. In this study, the TcIV lineage was identified in all the outbreaks, as well as four haplotypes of the TcIV lineage were detected. Detected in 80% of the outbreaks, haplotype 'i' was the most frequent. Our phylogenetic analysis revealed that haplotype 'ii' was the haplotype with the greatest phylogenetic difference, with 63% bootstrap and was identified in 50% of the outbreaks, all in the southern and southeastern regions of the state of Amazonas. Meanwhile, haplotypes 'iii' and 'iv' were identified in the most recent two outbreaks at Ipixuna (2021) and Amaturá (2022).

According to Monteiro et al. [34], the pathogenicity of isolates from Amazonas is low, suggesting that perhaps regions with higher lethality in outbreaks have more virulent strains with high cardiac involvement circulating. A notable virulent strain with high cardiac involvement is the TcII strain [35], a strain not yet recorded in Amazonas state, but found in samples from an outbreak outside the Amazon region and there were three deaths [8, 36]. *T. cruzi* TcI strain, on the other hand, has been related to isolated acute cases and patients with undetermined chronic CD, in vectors and reservoirs [11, 37], while the TcIV strain has been linked to acute CD outbreaks [14, 38] and isolated human cases (data not yet published). In Colombia, TcI and TcIV have also been simultaneously reported in one orally transmitted CD outbreak [39], demonstrating that TcIV, previously underreported in human isolates [40], is becoming more frequent in outbreaks of orally acquired CD.

Considerations on the epidemiological aspects and transmission routes of outbreaks in Amazonas

According to the registered cases, there is a clear association between the periodicity of the açaí harvest and the occurrence of ACD outbreaks in the Amazon, predominantly between January and June. Similar trends occurred in Pará, in the eastern Amazon, where the highest frequency of ACD outbreaks/cases between July and December corresponded to the açaí harvest in the state [20]. In Amazonas, açaí production declines in the second half of the year and juice becomes scarce, allowing residents to seek out and consume other fruit juices from palm trees, such as bacaba (*Oenocarpus bacaba*) and patauá, which are harvested from July to December [41]. In the state of Amazonas, in addition to açaí, only patauá consumption has so far been associated with an ACD outbreak.

These outbreaks occurred due to failures in hygiene practices that are vital to the production of safe products. Similar conditions occurred in the state of Pará, which had a higher frequency of outbreaks due to açaí consumption [20]. Since 2012, basic hygienic-sanitary procedures in the state have been established and applied to minimise risks and prevent foodborne illness outbreaks. These procedures have become mandatory for small producers. Juice processing requires washing with hypochlorite solution, followed by heat treatment [42].

In the homemade procedure, the beater has difficulty, especially in the heat treatment stage, where there is a lack of means to regulate the temperature. The heat treatment step is essential for the elimination of contaminating microorganisms in fruit juices and foods, considering the viability of the parasite in the foods produced. In açaí, *T. cruzi* can remain alive and infective for hours or days, depending on the temperature and humidity at which the fruit was stored. At low temperatures, it can remain viable for weeks, as observed by Santana et al. [14], who found the parasite viable in frozen açaí for more than 30 days.

Considering that infection by *T. cruzi*, particularly by oral transmission, has the potential of going undetected or misdiagnosed, good food handling practices in the region's artisanal/homemade production of palm fruit juices are vital in preventing transmission of ACD. However, we cannot overlook the importance of continued surveillance by accredited and responsible public health agencies and services over the artisanal producers. These agencies are critical in implementing health education programs as strategies for ensuring good manufacturing practices for unprocessed foods, considering that depending on the season and availability, palm fruit juices are always present in the region's alimentary habits.

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