Orally Transmitted Chagas Disease: Biology, Epidemiology, and Clinical Aspects of a Foodborne Infection

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Abstract Chagas disease has been one of the great neglected infectious pathologies, affecting not only developing countries but also the most vulnerable sectors of the population with greater intensity. In the spectrum of its mechanisms of infection, the most forgotten, unknown, and underestimated is the oral infection. Despite being the usual form of infection among mammals and the increasing frequency of oral *Trypanosoma cruzi*-transmitted outbreaks since 1967, many aspects about the physiopathology, the histotropism, clinical evolution, evasion mechanisms of *Trypanosoma cruzi*, the consequent host immune response, and the therapeutic efficacy are still unknown. However, nowadays we already know, and are discussed in this chapter, the risk factors, the clinical aspects, the incriminated vectors, the ideal diagnostic methods, and the therapeutic approach primarily applicable to the acute phase.

1 Introduction

American trypanosomiasis is one of the most important zoonoses of the New World since its causative agent *Trypanosoma cruzi* infects all mammals that are exposed to this protozoan. Its original mechanism of transmission between the wild and, more recently, domestic reservoirs has been fundamentally the oral route, since the fur

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and the thickness of the skin are a physical barrier almost insurmountable for its penetration. Hence, it is considered that the oral route is the usual way of infection in different species. Today we know the intrinsic mechanisms of the cellular biology of this parasite, by its more efficient penetration through the gastric mucosa [1]. Perhaps the exception to this route of penetration is represented by marsupials which seem to be the ancestral reservoirs, among which *Didelphis marsupialis*, which has the capacity to house vertebrate and vectors cycles, the latter in their odoriferous glands. Under situations of stress, they are able to vaporize their secretions containing metacyclic trypomastigotes that can infect remotely other animals through mucous membranes such as the conjunctiva [2].

The participation of humans in the epidemiology of this hemoparasite is related to the arrival of Asian migrants that populated America, and their infection has been demonstrated by molecular biology techniques in mummies of different Amerindian ethnic groups, at least for 7000 years [3]. Unlike wild mammals, humans and in particular Amerindian ethnic groups have a skin devoid of mechanical barriers such as fur, which allow the easy penetration of metacyclic trypanosomes through small fissures and even at the stinging point of their vectors, which eliminated trypanosomes through the excreta. For these reasons, humans are the only species in which the cutaneous route is considered a natural via of infection. *Trypanosoma cruzi*, the causal agent of this infection, multiplies intracellularly in the vertebrate host causing damage especially in the heart, the target organ, causing Chagas disease (ChD).

When Carlos Chagas published the first report of a new disease in 1908 [4], it was recognized that this parasitic tropical disease was acquired through the skin [5]. Typical entry-site symptoms and clinical signs (Romaña sign, cutaneous indurations known as "chagomas") were described by this outstanding Brazilian physician and researcher. Other entry ports have been recognized (blood transfusions, vertical transmission, organ transplantation, laboratory accidents), and since 1960 the oral route has been recognized as one of the causes of foodborne diseases [6]. This fact is one of the reasons why this mode of infection has been neglected and minimized by health authorities, as rare accidents of secondary importance. However, the increased number of oral ChD (OChD) reports especially originating from the Amazon region and in four other countries (Venezuela, Bolivia, Colombia, and French Guyana) [7] has elicited and increased interest in this particular form of infection. In Venezuela, 14 outbreaks have been reported—ten are recorded in Alarcón de Noya et al. [8] and the other four in Ruiz-Guevara et al. [9]—being the first outbreak occurred in 2007, the largest registered in Latin America [10]. In September 19, 2017, the Colombia Health Ministry (Ministerio de Salud y Protección Social) warned about the situation of a family of Venezuelans who had three children died with acute ChD, one of 9 months in Táriba (Táchira State), Venezuela, and the other two children of 5 and 9 years old died in the North of Santander, Colombia. The two grandparents had the same symptoms. All belonged to the same family and came from the same locality (https://www.minsalud.gov.co/Paginas/Intensificadas-medidas-de-salud-p%C3%BAblica-por-muertes-de-venezolanos-por-Chagas.aspx). Due to the simultaneity in related persons of the

same family, and the identification of acute ChD, this episode could be identified as the 15th outbreak of oral transmission of *T. cruzi* in Venezuela.

2 Particularities of the Orally Transmitted Chagas Disease

Although transcutaneous transmission has been considered the most important transmission mechanism until now, in Venezuela in the last 11 years, only a total of ten patients have been reported [11], while in that interim, there have been 15 OChD outbreaks affecting 273 people. Furthermore, among these ten cases, four were members of the same family, and possibly they were infected by the oral route [8, 11]. This decline in prevalence coincides with that observed in the rest of Latin America, as a result of the integration of the Chagas disease control programs of the Latin American countries, promoted by PAHO (Central America, Andean, and Southern Cone initiatives) [12]. However, in contrast to the decreased transmission shown in these figures, an increase in acute cases of orally acquired infection has been observed in the last two decades [13, 14].

There are multiple reasons that could explain this change in the transmission patterns in the endemic region of Latin America. Among them, the following are worth highlighting:

2.1 Bioecological Factors

1. Human migrations: great demographic changes have occurred in this continent such as the reversal in the percentages of rural vs. urban population, which decreased from 70.5% in 1950 to 21% in 2013 [15, 16]. This allowed the migration of infected populations to the periphery of the cities, which today make up the belts of misery and that, consequently, correspond to recently invaded and deforested areas, now absent from the natural wild reservoirs that were the original source of food for the triatomines. These demographic changes in some way forced changes in the ethology of vectors, which were now forced to feed on domestic reservoirs (rodents, dogs, and cats) and humans. In short, a rural pathology now becomes a pathology of urban predominance in some countries, as is the case of Venezuela [17].
2. Triatomine vectors: domiciliation is one of the major bioecological changes that have occurred in this disease. It has been reported by a series of species that are not necessarily good vectors through the cutaneous route (*Triatoma dimidiata*, *Panstrongylus rufotuberculatus*, *Rhodnius stali*, *Eratyrus mucronatus*, and *Panstrongylus geniculatus*). It is the case of *Panstrongylus geniculatus*, which nevertheless has revealed to be a species of less importance for the transmission through the skin, since the speed factor in the defecation reflex that is essential for skin transmission, is irrelevant for the contamination of food and therefore

Fig. 1 Homemade or artisan fresh (unpasteurized) juices are the main source of infection with metacyclic trypomastigotes from completely blended triatomines or their excretions. *Panstrongylus geniculatus* is the species most frequently incriminated as the source of infection (drawing by Alberto Monteagudo)

for the oral route [18, 19]. Both nymphal stages and adults can be the source of infection, either through their depositions on food or by the fact of being blended during the processing, particularly of fruits, in the form of artisanal juices (guava, mango, and juice of sugarcane) [17, 20] (Fig. 1).

3. Reservoirs: a total of 180 species of 25 families of infected mammals have already been described as potential hosts for *T. cruzi* (marsupials, monkeys, bats, horses, dogs, cats, rats, raccoons, skunks, coyotes, etc.) [21, 22]. Among them, *Didelphis marsupialis* is the most important synanthropic reservoir of the continent due to its wide geographical distribution, high levels of natural infection with *T. cruzi*, and the presence of infective metacyclic trypomastigotes in the perianal glands similar to triatomines and also able to infect through oral, nasal, ocular, and skin routes of infection [2]. The rat, *Rattus rattus*, deserves special attention as three basic conditions have enabled it to become a very efficient urban reservoir for *T. cruzi*. Firstly, rats are very susceptible to *T. cruzi* infection maintaining the parasite for a long period of time; secondly, they have a high urban population density, tending to increase with the garbage in the cities, and thirdly, they are a preferred food source of *Panstrongylus geniculatus* [23, 24].

2.2 Cultural Patterns

This is a fundamental aspect to understand some of the OChD outbreaks, in which the habits of nutrition and customs of the population in the different social strata have conditioned the expansion of outbreaks of this serious pathology.

The consumption of homemade, street, and school-canteen fruit juices is frequent in Latin America, from which these outbreaks have been generated. Fruits such as guava, açai, mango, pineapple, and sugarcane have been incriminated as vehicles of the outbreaks. These juices either came from palm trees where triatomines are present, which were then processed without checking or without pasteurizing the juice, or the contamination occurred in the kitchens where adults and nymphs wander at night from any of the triatomine species that can contaminate with their defecations with metacyclic trypomastigotes or they are blended in the preparation process.

Unlike skin contamination in which triatomines excrete a few microliters of urine containing between 3000 and 4000 metacyclic trypomastigotes per microliter, in the case of the contamination with an entire triatomine, it has been estimated that a *Triatoma infestans* can contain 684,000 trypomastigotes in the intestine [25]. It explains the high parasitic load that a patient can receive, having a mechanism of penetration more efficient than the skin; in consequence the morbidity and mortality are much higher.

2.3 Other Environmental Factors: Vegetation, Housing, Brightness, and Climate

Perhaps the deforestation in the periphery of the cities is the main component for the bioecological changes, because it introduces very important changes on the ecology of the vectors and their habits and in particular the domiciliation of vectors of previously wild behavior. The parasites move from a wide variety of species of wild reservoirs to only four urban species (dogs, cats, rodents, and humans), limiting the number of parasitic variants in a sort of screening or funnel decreases the genetic variability of the variants that affect the domestic and peridomestic species as well as humans.

These other factors also have an important role in the epidemiological changes that are occurring in this disease. In relation to vegetation, it has already been mentioned how deforestation or the simple fact of living near it increases the risk as a factor of its transmission. This is what is called “border phenomenon,” which has been one of the important factors in the acute outbreaks of the Caracas area, since all of the oral outbreaks occurred near the forest.

The houses most likely to be infested by triatomines are mostly inhabited by marginalized populations located in slums (“favelas,” “barrios,” “villas miseria,” “ranchos,” etc.) in which most of the walls are not plastered with numerous crevices in which vectors can enter, hide, and multiply [17].

The periods of drought (March–May) and the increase of the luminosity in the communities have been evidenced as risk factors additional to those already men-

tioned, as the invasions of the vectors increased. In the latter case, the streetlights and houses are powerful attractors of the triatomines, in particular of *Panstrongylus geniculatus* [18].

What characterizes oral transmission and what differentiates it from cutaneous transmission

- Usually seen in a form of outbreaks.
- Can be transmitted by any triatomine species and also by bedbugs.
- Metacyclic and sanguineous trypomastigotes are likely infectious.
- The entire triatomine with very high parasite load could be the infectious source.
- Infection source: complete triatomines or their feces and *D. marsupialis* anal excretions (food contamination).
- Occurs in rural as well as urban areas.
- There is no previous history of cutaneous manifestations.
- It affects all social status.
- Patients often deny having seen triatomines.
- It is the most common infection route in animal species other than humans.

All the previously described items should make clinicians to suspect that they are facing an OChD outbreak, which should be managed as an emergency of clinical severity and high mortality.

3 Clinical Aspects of the Acute Cases in Orally Transmitted Chagas Disease

The suspicion of oral transmission of ChD can almost only be made during the acute phase of the disease or when a chronically case is found to be related to people who suffered the acute form of the infection.

The acute condition of oral infection with *T. cruzi* is different from the clinical manifestations of other acute cases due to other mechanisms of infection. In the case of blood transfusion, organ transplantation, and congenital transmission, the immediate antecedent is essential for raising the suspicion of a possible acute infection by *T. cruzi.* In the transcutaneous vector transmission, there is usually a previous history of contact with triatomines, and even the patient usually brings the insect full of blood found in the bed or in the house.

The epidemiological antecedent most frequently described by patients is their association with a meal or a drink shared with friends and family, who also agree with a similar symptomatology. It can take days or weeks to establish the network of people exposed simultaneously and who should be evaluated as soon as possible to avoid clinical severity and mortality [23, 26, 27]. Among the antecedents, there is no recognition of contact with triatomines nor the entrance door of the parasite (Romaña and/or chagoma signs) as it can appear in acute cases due to transcutaneous vectorial infection [28]. The incubation period is 3–22 days in oral transmission [10, 29]. In Chacao, the maximum incubation period was determined in 11 days, as

one of the infected teachers joined their work just 11 days before the onset of symptoms [20].

1. Clinical presentation of acute cases: In the review of several outbreaks of OChD (two in Colombia, a compilation from Brazil, and two from Venezuela) [20] and the most numerous and recent outbreaks reported in Colombia [29] which in total add up to 434 acute cases, the predominant symptomatology has been fever, headache, facial and lower limb edema (Fig. 2), abdominal pain, diarrhea, myalgias, arthralgias, and asthenia (Table 1).

   The clinical picture of the acute phase of oral transmission is that of a systemic infection with cardiac involvement as the heart is the target organ. Frequent cardiological manifestations result in chest pain, palpitations, and dyspnea, which are reasons for hospitalization. Cardiomegaly (Fig. 2) and heart failure can occur as a result of severe arrhythmia or cardiac tamponade due to pericardial effusion leading the patient to a fatal outcome [33]. The most frequent findings on the EKG and ECHO can be seen in Table 2.

   Regarding the laboratory findings observed in hematology and blood chemistry, there are no particular findings. During the first microepidemic episode, troponin was evaluated only in hospitalized patients, and it was elevated in 8/11 (73%) of patients; the globular sedimentation rate was altered in 57%, the C-reactive protein in 87.5%, the lactate dehydrogenase in 88.8%, and leukocytosis in seven patients [36]. During the second large outbreak, laboratory exams were made to 43 out of 88 infected persons. They showed anemia (78%), elevated troponin (50.6%), leukocytosis (>10,000) (69%), and elevated transaminases 26% AST and 51.6% ALT, and 2.3% of the patients showed elevated creatinine [33].
2. Severity and mortality. Oral infection with *T. cruzi* has a faster and more severe course than the cutaneous transmission, probably due to the size of the inoculum to which the patient's immune system faces and also better efficacy of parasites to penetrate the gastric mucosa and accessing to systemic circulation. The severity of symptoms and mortality depend on time between the onset of symptoms, the confirmed diagnosis, and treatment delivery. Mild and severe cases were more frequent as much as 87.2% in 227 persons orally infected from ten outbreaks registered in Venezuela from which only 12.7% of the confirmed cases

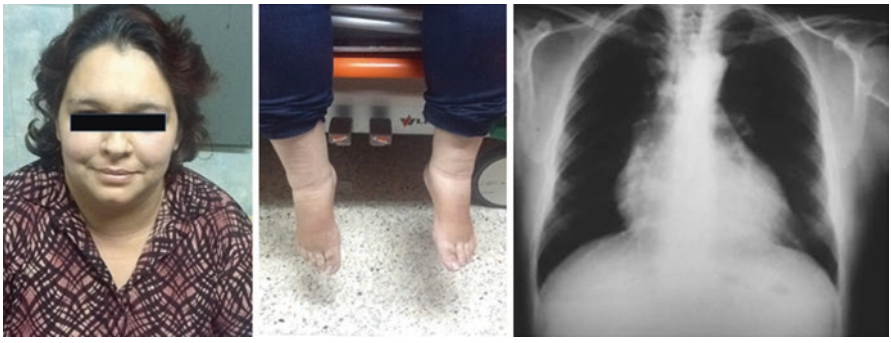

Fig. 2 Facial, lower limb edemas and pericardial effusion in patients with oral Chagas disease

Table 1 Predominant symptomatology in 434 acute oral cases of Chagas disease registered in Brazil, Colombia, and Venezuela

Symptomatology	Frequency (%)
Fever	80–100
Headache	25–93
Facial edema	28–70
Edema in lower limbs	4–57
Abdominal pain	22–50
Diarrhea	2–30
Myalgias, arthralgias	11–85
Asthenia	10–73

n = Brazil 181 [13]; Venezuela 192: 103 [10] and 89 [30]; Colombia 61: 11 [31], 10 [32], and 40 [29].

Table 2 Frequency (%) of EKG and ECHO alterations in acute oral *Trypanosoma cruzi*-infected patients from four outbreaks

	Brazil [34]	Colombia [32]	Venezuela [30, 35]
ST-T changes	100		37.8[a]
Right branch blockage	25	40	1.9[a]
Atrial or ventricular fibrillation	8.3	20	3[a]
Left ventricular hypertrophy			17.5[b]
Pericardial effusion	36.4		81.3[b]

[a]Marques et al. [35]
[b]Alarcón de Noya [30]

were asymptomatic [8]. In relation to mortality, it varies from 0% to 100% being the highest frequency in two outbreaks in Colombia where death occurred in regions of Magdalena and Santander with 5/13 (38.5%) and 3/3 (100%) in 2003 and 2008, respectively [33]. From ten cited outbreaks in Venezuela, ten persons died from 249 infected persons, with 4% mortality, being predominantly children, pregnant woman and her stillborn, and a woman that had given birth 2 months before and was still breastfeeding.

3. Vertical transmission. One of the most feared consequences in oral infection is the possibility of congenital transmission. Generally, congenital cases of mothers in the chronic phase of *T. cruzi* infection are asymptomatic, and if the mother is not screened for infection, the congenital case may go unnoticed into the chronic phase. Different is the case of acute infection when it affects the pregnant woman. Vertical transmission in acute patients with oral transmission is manifested in any of its forms as spontaneous abortion [34, 37], death of the fetus in the uterus [38], and the birth of an infected newborn [39]. The dissemination of the parasite reaches all the organs of the mother, including the placenta, infiltrating it from where it reaches the fetus to spread and affect all organs as evidenced by the presence of the parasite by direct microscopic examination of

the tissues or by PCR [38]. The treatment of the pregnant woman with Chagas disease is contraindicated [40]; however each acute case in pregnant women should be evaluated because not giving treatment puts the mother at risk [37].

4 Laboratory Diagnosis

Once there is epidemiological and clinical suspicion, confirmation of the probable case of acute infection by oral transmission of *T. cruzi* must be done. In isolated cases that are usually the index cases, one of the frequent diagnostic errors is the confusion with malaria, although it has a clinic and a different pattern of fever. Nonetheless, when performing the thick and thin blood smears, it is possible to discriminate these two hemoparasites and apply the appropriate treatment [26]. The parasitological diagnosis should be made in all patients with epidemiological and clinical suspicion, but cases present unexpectedly, and sometimes the disease can affect large groups making it difficult to comply with the entire diagnostic protocol.

1. Immunological diagnosis: ELISA tests should be applied to the entire study population, searching simultaneously for IgG and IgM antibodies. Following the PAHO/WHO guidelines, two serological diagnostic tests of different bases [41] should be tested. Tests such as indirect hemagglutination, direct agglutination, and immunofluorescence have been used for this purpose. An example of this approach is the flowchart used in our two large oral outbreaks, in which diagnosis of the cases was initiated by the ELISA test and thereafter indirect hemagglutination or Western blot were used as confirmatory methods [30, 42].
2. Parasitological diagnosis: To confirm that we are facing an acute outbreak of oral transmission ChD, it is necessary to identify *T. cruzi* in at least one case of all clinically and epidemiologically related cases. Thick and thin blood smears are used as traditional techniques, although fresh examination of a drop of blood under a coverslip is very useful because it allows for immediate visualization of mobile trypomastigotes. Nevertheless, the best and rapid diagnostic test for the acute phase is microhematocrit [43], due to its parasite concentrating effect, which can be observed at the level of the buffy coat after centrifugation of the capillary tube (Fig. 3). In reference laboratories, it is important to obtain the parasitic isolate to allow for subsequent molecular studies and to know the circulating genotype [44, 45], so that inoculation in mice and blood culture could be performed in all possible patients.
3. Molecular diagnosis: Upon suspicion of acute cases of ChD, all blood samples should be taken from the first contact, even without previous serological diagnosis, since in some occasions there is no new opportunity to take a second sample from the patient, or by the time the serology result arrives, the patient is already receiving treatment. The first blood sample should be of enough volume to allow pretreatment testing with polymerase chain reaction (PCR) and animal culture in order to obtain *T. cruzi* DNA for molecular studies. The follow-up studies of outbreaks carried out by the Tropical Medicine Institute in Caracas tested for the

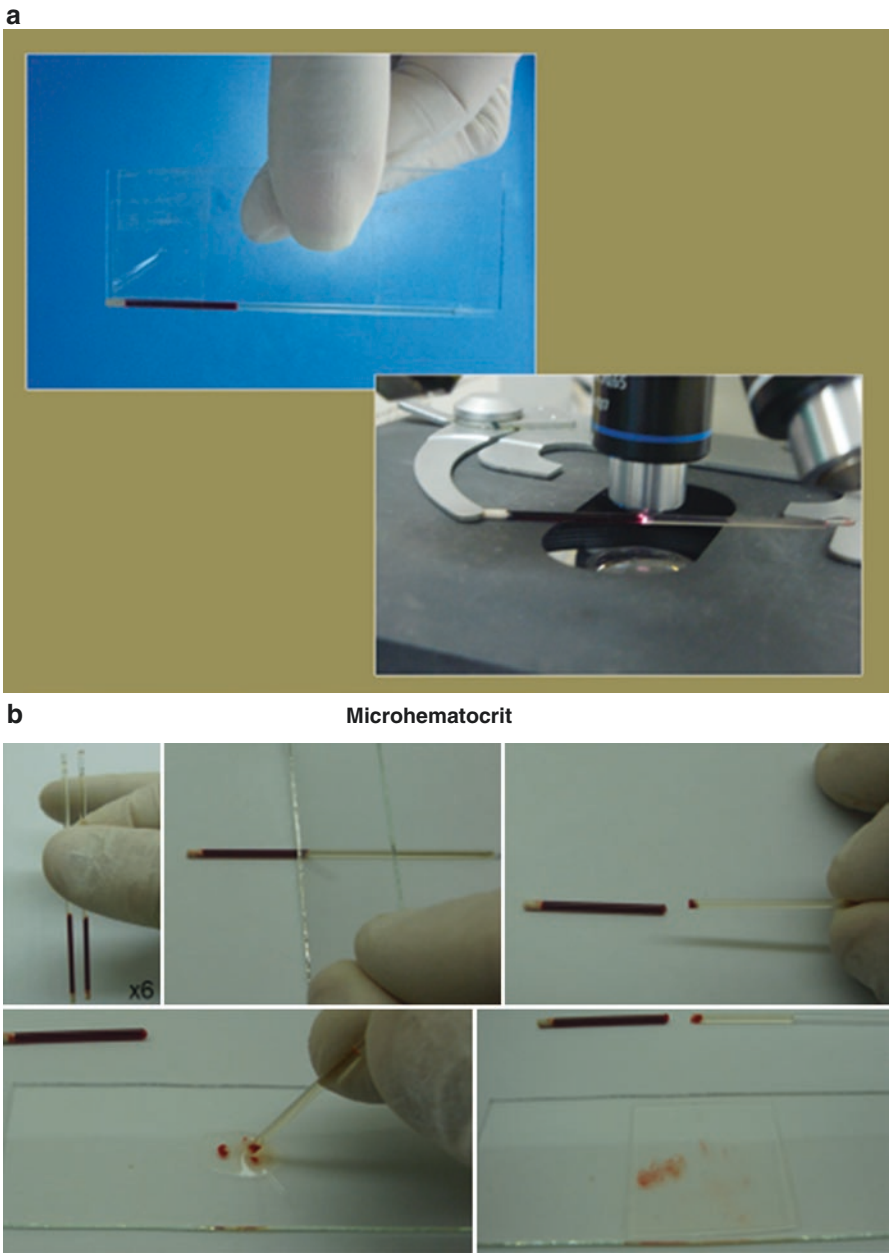

Fig. 3 (**a**) Microhematocrit technique, for the rapid demonstration of blood trypomastigotes in acute infected patients. Mobile parasites are concentrated on the top of the buffy coat. (**b**) Blood sample obtained with the microhematocrit capillary could be examined under the coverslip to observe motile trypomastigotes and, after fixation, must be stained with Giemsa

presence of parasitic DNA [30, 42]. Oral ChD acute cases have shown the highest parasite load by PCR when compared to vectorial acute and chronic cases [46, 47]. PCR can be considered more as an indicator of treatment failure rather than a marker of cure [48]. Molecular studies also allow to determine the genotype. Studies by Diaz et al. analyzing parasite DTUs from six Colombian OChD outbreaks found that these molecular biomarkers were not strictly correlated with clinical forms of the disease [49].

4. Diagnostic strategies depend on the size of outbreak. The first urban ChD outbreak in Venezuela allowed to compare the performance of different diagnostic methods used under medical emergency conditions and their contribution to accurately confirm the acute infected persons [42]. A complete parasitological and immunological evaluation was performed in the initial small group of patients, confirming the etiology of the outbreak in a non-endemic area for ChD. Thereafter, only conventional serology methods, including ELISA (IgM/IgG) and IHA, were applied in all individuals at risk. The selection of these methods was based on the speed of implementation, sensitivity, and specificity. The use of fresh blood smears, Giemsa stains, and PCR assays was restricted to a very limited number of cases because these techniques are considered cumbersome and time-consuming in an urgent situation. The parasitological outcomes were different between the severely ill group and the subset of mildly symptomatic individuals. The parasitemia was lower in the second group, probably due the previous use of different antibiotics that may have inhibited the parasite's growth in culture. In severely symptomatic patients, fresh blood smears were more sensitive for detecting parasites than Giemsa-stained smears, suggesting that the former technique should be routinely applied in people with long-lasting fever of unknown origin. When *T. cruzi* trypomastigotes are detected in peripheral blood by any technique during an outbreak, it is necessary to investigate all symptomatic and asymptomatic cases suspected of sharing the same epidemiological risk in order to prevent increased morbidity and mortality. The importance of simultaneous screening for specific IgM and IgG antibodies by ELISA can confirm the acute condition of the infection. The detection of IgM was very specific in that study, as there were no false positives detected when comparing with the results obtained by ELISA IgG test. In spite of the application of all these serologic techniques, the diagnosis of ChD was inconclusive in 16 individuals who were positive in only one of the immunological tests, not complying with the international criteria of identification of a Chagas case. Due to the epidemiological link, these individuals were treated with nifurtimox. Subsequently, seven of them tested positive for PCR.

These experiences reinforced the widespread use of conventional serology methods detecting IgM and the use of all available diagnostic techniques to confirm infection. For the second largest outbreak in Venezuela, blood culture, serology, and PCR were carried out during the first contact to the probable infected persons. In this study more parasite isolates and PCR were recorded [30]. In conclusion, all available parasitological, immunological, and molecular procedures are essential for identifying the causal agent of the T. cruzi oral transmission.

5 Treatment and Follow-Up

1. Routine treatment: In ChD, the same drugs are used for both the acute and chronic phases: nifurtimox (NFX) (Lampit®, Bayer), a nitrofuran, and benznidazole (BNZ) (Rochagan®, Roche), a nitroimidazole [50]. Due to better tolerance, BNZ is considered the first drug of choice; however, individual tolerance varies widely, and NFX represents an alternative. The choice depends mostly on the international availability [51]. For the acute phase, treatment is recommended for all patients, regardless of the mode of transmission and age of the patient [40]. BNZ is indicated at 6 mg/kg/day during 60 days in three daily doses and NFX, at 8 mg/kg/day during 90 days divided into two daily doses. When necessary, antiallergic, antineuritic, or gastric protectors are prescribed. During the follow-up of the treatment, laboratory tests that include complete hematology, urea and creatinine, aminotransferases, and bilirubin are recommended [52].
2. Adverse events of treatment during the acute phase in patients infected by the oral route. The undesirable effects of the two unique available compounds to treat ChD are usually caused by oxidative and reductive damage on host tissues that limit considerably their use [50]. A cross-sectional analytical study was carried out in Venezuela on 122 patients who received 176 courses of treatment with any of the two anti-*T. cruzi* drugs (113 with NFX and 63 with BNZ). Treating symptomatic persons and taking into account the drug side effects, it was important to know how much of the adverse effects were caused by the disease and how much by the medication. When the occurrence of side effects was compared among individuals with confirmed acute ChD and suspected cases, no statistically significant difference ($p = 0.363$) was found in relation to the drug used, age, or date of treatment. From the 176 treatment, 79% had one or more adverse effects which predominated in adults (97.8%) as compared to children (75.5%), and the risk of having side effects was significantly higher for NFX in comparison with BNZ. Four adults and a child treated with NFX had severe side effects (pulmonary infarction, facial paralysis, neutropenia, blurred vision, and bone marrow hypoplasia) warranting hospitalization and drug suspension. Abdominal pain, hyporexia, weight loss, headache, nausea, and lymphocytosis were frequent reports for NFX, whereas skin rash, neurosensory effects, hyporexia, fatigue, pyrosis, abdominal pain, and eosinophilia were observed with BNZ. Frequency and severity of side effects during treatment of acute oral infection by *T. cruzi* demand direct supervision and close follow-up, even in those asymptomatic patients, to prevent life-threatening situations [52].
3. Posttreatment follow-up. The efficacy of BNZ and NFX varies with the stage of the disease, the patient's age, geographic area, and the *T. cruzi* isolate involved. A drawback of the studies assessing their efficacy in any phase, age, or geographical area is the lack of a marker to define cure. Current recommendations rely on the switch of serology from positive to negative, but this may take many years, limiting its use in clinical practice. Detection of *T. cruzi* DNA in peripheral blood only serves as a tool to identify treatment failure because a negative result does not mean absence of infection [48]. Nonetheless, based on serology, these drugs are more efficient during acute phase in which negative seroconver-

sion varies between 50% and 80% in comparison with 8% and 20% efficacy in chronic phase patients [48, 53]. Also efficacy is different in children and adults. Treatment with BZ for 60 days in children aged 6–12 years with early or indeterminate phase of ChD in Brazil and Argentina had a cure efficacy of 56% and 62%, monitored by seroconversion after 36 and 48 months after treatment, respectively [54, 55]. Treatment with NFX for 90 days of 100 patients with acute oral ChD showed a therapeutic failure that ranged from 69% to 78%, after 3 years of follow-up in Venezuela [56]. A similar situation was for 60 days of BNZ to 80 persons infected by the oral route from Chichiriviche outbreak during the same observational time (Alarcón de Noya et al., unpublished data). In spite of the treatment in our studies being administered under supervision, during acute phase, to children, the effectiveness of these two drugs seems to be lower than the treatment administrated during the acute phase of the cutaneous mechanism of infection as that described by Cançado in 2002, who observed cure in 76% of patients with acute phase after 13- to 21-year follow-up [57].

6 Prophylaxis and Control Measures in Foodborne Chagas Disease

The control measures that are recommended to prevent ChD orally acquired are those implemented by the general population in their own home and those implemented by the Ministry of Health of each country, ranging from legal regulations for the production, packaging, preservation, and distribution of food, particularly those that are to be consumed without cooking for public institutions (school canteens, nursing homes, factory canteens, or public and private companies) [58].

Individual and family prophylaxis measures are based on avoiding the triatomine-food contact, through physical barriers, preventing food from being exposed to contact with infected vectors and marsupials. The first one is based on preventing the entrance of the triatomines through the use of mosquito nets in windows and doors, ceilings, and sealed walls, as well as the spraying of walls with residual insecticides such as fenitrothion. The second one is by improving the housing with fritted walls and the use of paintings with insecticides that prevent the domiciliation of vectors, as has been observed with *Panstrongylus geniculatus*, originally a wild species. Likewise, protect the food either by covering it or placing it in hermetic containers especially at night, avoiding the exposure to the evacuations of the triatomines or the urine and excretions of *Didelphis marsupialis.* Being the schools where the biggest outbreaks have occurred, precautions must be taken on the hygiene of beverages and solid foods that are served in canteens, as in nursing homes as well as in military barracks, restaurants, etc. The supervisory role not only by the directors of the schools and companies but also the users or their representatives (parent associations, unions, etc.) is essential [58].

Other special prophylactic measures are based on avoiding the consumption of palm fruits such as açai or manaca for the production of juices in the Amazon region, since in the clusters of this fruit, the triatomines frequently inhabit. The triatomines are crushed and their content released into the drink during preparation, sometimes

being consumed far from the production site. This way has been called distantiae transmission [59], and contamination of artisanal açai juices is the most frequent mechanism of transmission in the Brazilian Amazon [60], where the majority of oral outbreaks have occurred in America.

The viability of trypomastigotes in beverages for human consumption varies according to the type of fruit, which has been studied by Añez et al. [61], who observed that parasites survive several hours in milk, orange, pineapple, papaya, sugarcane, and guava juices, after preparation and subjected to cooling in a refrigerator. Pasteurization of juices would be one of the prophylactic measures, particularly in those juices of industrial production and even artisanal.

It has been shown that blood trypomastigotes also have infective capacity by the oral route [62]; the consumption of meats or blood derivatives of raw wild animals, especially hunting, should be avoided.

Finally, the role of education from the school level to the general public on food hygiene is very particular about the risks of street foods which hygienic norms and preparation procedures are unknown.

References

1. Yoshida N. Molecular mechanisms of *Trypanosoma cruzi* infection by oral route. Mem Inst Oswaldo Cruz. 2009;104:101–7.
2. Deane MP, Lenzi HL, Jansen A. *Trypanosoma cruzi*: vertebrate and invertebrate cycles in the same mammal host, the opossum Didelphis marsupialis. Mem Inst Oswaldo Cruz. 1964;79:113–5.
3. Guhl F, Jaramillo C, Yockteng R, Cárdenas-Arroyo F. *Trypanosoma cruzi* DNA in human mummies. Lancet. 1997;349:1370.
4. Chagas C. Nova tripanosomiase humana, Estudo sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi*. Gen.Sp. Agente etiologico de uma nova entidade morbida do homem. Mem Inst Oswaldo Cruz. 1909;1:159–218.
5. Rassi A Jr, Rassi A, Marin Neto JA. Chagas disease. Lancet. 2010;375:1388–402.
6. Robertson LJ, Devleesschauwer B, Alarcón de Noya B, Noya González O, Torgerson PR. *Trypanosoma cruzi*: time for international recognition as a foodborne parasite. PLoS Negl Trop Dis. 2016;10(6):1–6.
7. Ruiz-Guevara R, Noya O, Alarcón de Noya B. Documented outbreaks of foodborne Chagas disease. In: Alarcón de Noya B, Noya O, Robertson LJ, editors. *Trypanosoma cruzi* as a foodborne pathogen, Springer briefs in food, health and nutrition. New York, NY: Springer; 2015. p. 53–71.
8. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Muñoz-Calderón A, Noya O. Update on oral Chagas disease outbreaks in Venezuela: epidemiological, clinical and diagnostic approaches. Mem Inst Oswaldo Cruz. 2015;110(3):377–86.
9. Ruiz-Guevara R, Muñoz-Calderón A, Alarcón de Noya B, Redondo C, Pulido L, Beitia Y, Mauriello L, Rojas D, Muñoz J, Navarro E, Reyes-Lugo M. Brote familiar de Enfermedad de Chagas por transmisión oral en Yaguapita, Estado Miranda, Venezuela. Bol. Mal. Salud Amb. En prensa. 2018.
10. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Zavala-Jaspe R, Suárez JA, Abate T, Naranjo L, Paiva M, Rivas L, Castro J, Márques J, Mendoza I, Acquatella H, Torres J, Noya O. Large urban outbreak of orally-acquired acute Chagas disease, at a school in Caracas, Venezuela. J Infect Dis. 2010;201:1308–15.

11. Añez N, Crisante G, Parada H. Nuevos casos de enfermedad de Chagas en el Occidente de Venezuela. Salus. 2007;11:87–90.
12. OMS/OPS/TDR. Reporte sobre la Enfermedad de Chagas. Grupo de Trabajo Científico. Eds. Felipe Guhl and Janis Lazdins. 2007. pp 1–96.
13. Pinto AYN, Valente SA, Valente VC, Ferreira AG, Coura JR. Fase aguda da doença de Chagas na Amazônia brasileira. Estudo de 233 casos do Pará, Amapá e Maranhão observados entre 1988 e 2005. Rev Soc Bras Med Trop. 2008;41:602–14.
14. Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. Clin Infect Dis. 2012;54:845–52.
15. Briceño-León R. La Enfermedad de Chagas en las Américas: una perspectiva de ecosalud. Cad Saude Publica. 2009;25:S71–82.
16. World Bank. 2013. http//ata.worldbank.org
17. Noya O, Alarcón de Noya B, Robertson L. Epidemiological factors related in foodborne transmission of Chagas disease. In: Alarcón de Noya B, Noya O, Robertson LJ, editors. *Trypanosoma cruzi* as a foodborne pathogen, Springer Briefs in Food, Health and Nutrition. New York, NY: Springer; 2015. p. 41–51.
18. Reyes-Lugo M. *Panstrongylus geniculatus* Latreille 1811 (Hemiptera: Reduviidae: Triatominae), vector de la enfermedad de Chagas en el ambiente domiciliario del centro-norte de Venezuela. Rev Biomed. 2009;20:180–205.
19. Coura JR, Albajar-Viñas P, Junqueira ACV. Ecoepidemiology, short history and control of Chagas disease in the endemic countries and the new challenge for nonendemic countries. Mem Inst Oswaldo Cruz. 2014;109:856–62.
20. Alarcón de Noya B, Noya O. Clinical aspects in foodborne Chagas disease. In: Alarcón de Noya B, Noya O, Robertson LJ, editors. *Trypanosoma cruzi* as a foodborne pathogen, Springer Briefs in Food, Health and Nutrition. New York, NY: Springer; 2015. p. 33–40.
21. Guhl F. Enfermedad de Chagas: realidad y perspectivas. Rev Biomed. 2009;20:228–3.
22. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas disease in the United States. Clin Microbiol Rev. 2011;24:655–81.
23. Díaz-Bello Z, Zavala-Jaspe R, Reyes-Lugo M, Colmenares C, Noya-Alarcón O, Noya O, Herrera L, Alarcón de Noya B. Urban *Trypanosoma cruzi* oral transmission: from a zoonotic founder focus to the largest microepidemic of Chagas disease. SOJ Microbiol Infect Dis. 2016;4(1):1–9.
24. Alarcón de Noya B, Noya-González O. An ecological overview on the factors that drives to *Trypanosoma cruzi* oral transmission. Acta Trop. 2015;151:94–102.
25. Shaub GA. *Trypanosoma cruzi*: quantitative studies of development of two strains in small intestine and rectum of the vector *Triatoma infestans*. Exp Parasitol. 1989;68:260–27.
26. Martín A, Alarcón de Noya B, Montero R, Rojas C, Garrido E, Ruiz-Guevara R, Díaz-Bello Z. Epidemia de Chagas agudo adquirido por vía oral en una escuela de Caracas: descripción del caso índice. Arch Venez Ped Puericult. 2009;72(3):97–100.
27. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Noya O. Enfermedad de Chagas de transmisión oral: vinculación del caso índice con una microepidemia urbana en Venezuela. Bol Mal Salud Amb. 2010;60:135–8.
28. Morocoima A, Tineo Brito EJ, Ferrer E, Herrera L, Nuñez M. Enfermedad de Chagas en el estado Anzoátegui, Venezuela: registro de un caso agudo y caracterización parasitológica y molecular del aislado. Bol Mal Salud Amb. 2008;48(2):121–6.
29. Zuleta-Dueñas LP, López-Quiroga ÁJ, Torres-Torres F, Castañeda-Porras O. Posible transmisión oral de la enfermedad de Chagas en trabajadores del sector de los hidrocarburos en Casanare, Colombia 2014. Biomédica. 2017;37:218–32.
30. Alarcón de Noya B, Colmenares C, Díaz-Bello Z, Ruiz-Guevara R, Medina K, Muñoz-Calderón A, Mauriello L, Cabrera E, Montiel L, Losada S, Martínez J, Espinosa R, Abate T. Orally-transmitted Chagas disease: epidemiological, clinical, serological and molecular outcomes of a school microepidemic in Chichiriviche de la Costa, Venezuela. Parasite Epid Control. 2016;1:188–98.
31. Ríos JF, Arboleda M, Montoya AN, Alarcón EP, Parra-Henao GJ. Probable outbreak of oral transmission of Chagas disease in Turbo, Antioquia. Biomédica. 2011;31:185–95.

32. Hernández LM, Ramírez A, Cucunubá Z, Zambrano P. Brote de Chagas agudo en Lebrija, Santander, 2008. Rev Observ Salud Púb Santander. 2009;4:28–33.
33. Noya O, Ruiz-Guevara R, Díaz-Bello Z, Alarcón de Noya B. Epidemiología y clínica de la transmisión oral de *Trypanosoma cruzi*. Rev Esp Epidem: XI workshop on chagas disease, Barcelona Spain. 2015. pp. 23–34.
34. Bastos CJC, Aras R, Mota G, Reis F, Dias JP, Silva de Jesús R, Silva Freire MG, de Araújo E, Prazeres J, MF RG. Clinical outcomes of thirteen patients with acute Chagas Disease acquired through oral transmission from two urban outbreaks in northeastern Brazil. PLoS Negl Trop Dis. 2010;4(6):e711.
35. Marques J, Mendoza I, Noya B, Acquatella H, Palacios I, Marques-Mejias M. ECG manifestations of the biggest outbreak of Chagas disease due to oral infection in Latin-America. Arq Bras Cardiol. 2013;101:249–54.
36. Alarcón de Noya B, Veas J, Ruiz-Guevara R, Martín A, Rojas C, Machado I, Telo C, Henao L, Díaz-Bello Z, Noya O. Evaluación clínica y de laboratorio de pacientes hospitalizados durante el primer brote urbano de Enfermedad de Chagas de transmisión oral en Venezuela. Rev Patol Trop. 2013;42(2):177–86.
37. Suárez J, B de Suárez C, Alarcón de Noya B, Espinosa R, Chiurillo MA, Villaroel A, De Martín F, Paiva M, Díaz-Bello Z, Valderrama E, Estrada D, Vivas E. Enfermedad de Chagas sistémico en fase aguda por transmisión oral: diagnóstico integral de un caso autopsiado. Gac Méd Caracas. 2010;118(3):212–22.
38. Alarcón de Noya B, Pérez-Chacón G, Díaz-Bello Z, Dickson S, Muñoz-Calderón A, Hernández C, Pérez Y, Mauriello L, Moronta E. Description of an oral Chagas disease outbreak in Venezuela, including a vertically transmitted case. Mem Inst Oswaldo Cruz. 2017;112:569–71.
39. Pinto AY, Valente VC, Valente SA, Figueiras AC. Congenital Chagas disease due to acute maternal *Trypanosoma cruzi* infection transmitted by the oral route. Rev Pan-Amaz Saude. 2011;2(1):89–94.
40. Andrade JP, Marin-Neto JA, Paola AA, Vilas-Boas F, Oliveira GM, Bacal F, Bocchi EA, Almeida DR, Fragata Filho AA, Moreira da MC, Xavier SS, Oliveira Junior WA, Dias JC, Sociedade Brasileira de Cardiologia. I Diretriz Latino Americana para o Diagnóstico e Tratamento da Cardiopatia Chagásica. Arq Bras Cardiol. 2011;97(2 Suppl 3):1–48.
41. WHO. Control of Chagas disease. Second report of the WHO expert committee. WHO technical report series no 905. Geneva: World Health Organization; 2002.
42. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Zavala-Jaspe R, Abate T, Contreras R, Losada S, Artigas D, Mauriello L, Ruiz-Guevara R, Noya O. The performance of laboratory tests in the management of a large outbreak of orally transmitted Chagas disease. Mem Inst Oswaldo Cruz. 2012;107:893–8.
43. Freilij H, Muller L, González-Cappa M. Direct micromethod for diagnosis of acute and congenital Chagas' disease. J Clin Microbiol. 1983;18:327–30.
44. Díaz-Bello Z, Thomas MC, López MC, Zavala-Jaspe R, Noya O, Alarcón de Noya B, Abate T. *Trypanosoma cruzi* genotyping supports a common source of infection in a school-related oral outbreak of acute Chagas disease in Venezuela. Epidemiol Infect. 2014;142(1):156–62.
45. Rueda K, Trujillo JE, Carranza JC, Vallejo GA. Transmisión oral de *Trypanosoma cruzi*: una nueva situación epidemiológica de la enfermedad de Chagas en Colombia y otros países suramericanos. Biomedica. 2014;34:631–41.
46. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, Díaz-Bello Z, Velazquez E, Muñoz-Calderon A, Juiz NA, Basile J, Garcia L, Riarte a NJR, Ocampo SB, Yadon ZE, Torrico F, Alarcón de Noya B, Ribeiro I, Schijman AG. Analytical performance of a multiplex real-time PCR assay using TaqMan probes for quiantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl Trop Dis. 2013;7(1):e2000.
47. Ramírez JC, Cura CI, da Cruz Moreira O, Lages-Silva E, Juiz N, Velázquez E, Ramírez JD, Alberti A, Pavia P, Flores-Chávez MD, Muñoz-Calderón A, Pérez-Morales D, Santalla J, Marcos da Matta Guedes P, Peneau J, Marcet P, Padilla C, Cruz-Robles D, Valencia E, Crisante GE, Greif G, Zulantay I, Costales JA, Alvarez-Martínez M, Martínez NE, Villarroel

R, Villarroel S, Sánchez Z, Bisio M, Parrado R, Maria da Cunha Galvão L, Jácome da Câmara AC, Espinoza B, Alarcón de Noya B, Puerta C, Riarte A, Diosque P, Sosa-Estani S, Guhl F, Ribeiro I, Aznar C, Britto C, Yadón ZE, Schijman AG. Analytical validation of quantitative real-time PCR methods for quantification of Trypanosoma cruzi DNA in Blood samples from Chagas disease patients. J Mol Diagn. 2015;17:605–15.

48. Sales PA, Molina I, Fonseca Murta SM, Sánchez-Montalvá A, Salvador F, Correa-Oliveira R, Martins Carneiro C. Experimental and clinical treatment of Chagas disease: a review. Am J Trop Med Hyg. 2017;97(5):1289–303.
49. Díaz ML, Leal S, Mantilla JC, Molina-Berríos A, López-Muñoz R, Solari A, Escobar P, González-Rugeles CI. Acute Chagas outbreaks: molecular and biological features of *Trypanosoma cruzi* isolates, and clinical aspects of acute cases in Santander, Colombia. Parasit Vectors. 2015;8:608–22.
50. Urbina JA, Docampo R. Specific chemotherapy of Chagas disease: controversies and advances. Trends Parasitol. 2003;19(11):495–501.
51. Coura JR, Castro SL. A critical review of Chagas disease chemotherapy. Mem Inst Oswaldo Cruz. 2002;97(1):3–24.
52. Alarcón de Noya B, Ruiz-Guevara R, Noya O, Castro J, Ossenkopp J, Díaz-Bello Z, Colmenares C, Suárez JA, Noya-Alarcón O, Naranjo L, Gutiérrez H, Quinci G, Torres J. Long-term comparative pharmacovigilance of orally transmitted Chagas disease: first report. Expert Rev Anti-Infect Ther. 2017;15(3):319–25.
53. Guedes PM, Silva GK, Gutierrez FR, João S, Silva JS. Current status of Chagas disease chemotherapy. Expert Rev Anti-Infect Ther. 2011;9(5):609–20.
54. de Andrade AL, Zicker F, de Oliveira RM, Almeida Silva S, Luquetti A, Travassos LR, Almeida IC, de Andrade SS, de Andrade JG, Martelli CM. Randomized trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. Lancet. 1996;348:1407–13.
55. Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas disease. Am J Trop Med Hyg. 1998;59:526–9.
56. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Muñoz A, Ruiz-Guevara R, Balzano L, Campelo R, Abate T, Maniscalchi MT, Ramírez JL, Noya O. Eficacia terapéutica en el primer brote de transmisión oral de la enfermedad de Chagas en Venezuela. Biomedica. 2011;31(3):64–5.
57. Cançado JR. Long term evaluation of etiological treatment of Chagas disease with benznidazole. Rev Inst Med Trop Sao Paulo. 2002;44:29–37.
58. Robertson L, Noya O. Prophylactic measures and implementation of control measures in foodborne Chagas disease. In: Alarcón de Noya B, Noya O, Robertson LJ, editors. *Trypanosoma cruzi* as a foodborne pathogen, Springer briefs in food, health and nutrition. New York, NY: Springer; 2015. p. 81–8.
59. Xavier SC, Roque ALR, Bilac D, de Araújo VAL, Neto SF, da Silva LFC, Jansen AM. *Distantiae* transmission of *Trypanosoma cruzi*: a new epidemiological feature of acute Chagas disease in Brazil. PLoS Negl Trop Dis. 2014;8(5):e2878.
60. Valente SAS, Vera da Costa Valente VC, Neto HF. Considerations on the epidemiology and transmission of Chagas disease in the Brazilian Amazon. Mem Inst Oswaldo Cruz. 1999;94(I):395–8.
61. Añez N, Crisante G, Romero M. Supervivencia e infectividad de formas metacíclicas de *Trypanosoma cruzi* en alimentos experimentalmente contaminados. Bol Malariol Salud Amb. 2009;49:91–6.
62. Dias GB, Gruendling AP, Marquez S, Gomes ML, Toledo MJ. Evolution of infection in mice inoculated by the oral route with different developmental forms of *Trypanosoma cruzi* I and II. Exp Parasitol. 2013;135:511–7.