CHAPTER 3

Transmission of Chagas Disease (American Trypanosomiasis) by Food

Karen Signori Pereira,[*] Flávio Luis Schmidt,[†] Rodrigo L. Barbosa,[‡] Ana M. A. Guaraldo,[§] Regina M. B. Franco,[‡] Viviane L. Dias,[§] and **Luiz A. C. Passos[§]**

Contents

[*] Departamento de Engenharia Bioquímica, Escola de Química, Centro de Tecnologia Bloco Sala E203, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[†] Departamento de Tecnologia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
[‡] Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
[§] Centro Multidisciplinar para Investigação Biológica na Área da Ciência em Animais de Laboratório (CEMIB), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil

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Abstract In April 2009, the centenary of the discovery of the American trypanosomiasis, or Chagas disease, was celebrated. A hundred years after the discovery, little has been invested in diagnostics and treatment because the disease affects mainly poor people in developing countries. However, some changes in the epidemiology of the disease are of great importance today. Chagas disease transmitted through food is a public health concern in all areas where there is a reservoir of *Trypanosoma cruzi* in wild animals (e.g., mammals and marsupials) and/or where infected triatomine bugs are in contact with human food source items (especially fruits and vegetables). Recently, several outbreaks of illness related to the ingestion of food contaminated with *T. cruzi* have been recorded in Brazil, Colombia, and Venezuela.

I. CHAGAS DISEASE (AMERICAN TRYPANOSOMIASIS)

American trypanosomiasis or Chagas disease, named after Carlos Chagas, who first described it in 1909, exists primarily only on the American Continent. It is caused by *Trypanosoma cruzi*, a flagellate protozoan parasite. Chagas disease represents the leading cause of cardiac lesions in young, economically productive adults in the endemic countries in Latin America (Moncayo and Silveira, 2009).

The minimal infective dose of the parasite needed to acquire Chagas disease is not established in humans. It is known that, for African trypanosomiasis (or sleeping sickness), the minimal infective dose is 300–450 metacyclic trypomastigotes (Alvarenga and Marsden, 1975).

According to the World Health Organization (WHO), it is estimated that about 10 million people are infected with Chagas in the Americas, 2 million of them in Brazil alone. More than 10,000 die each year as a result. Because Chagas disease affects mainly poor people in developing countries, diagnosis and treatment of this disease have not been well studied (WHO, 2009).

Historically, transmission has occurred predominantly in rural areas of Latin America, where poor housing conditions have promoted contact with infected vectors. Successful programs to reduce vector- and blood-borne transmission, as well as migration within and beyond endemic countries, have changed the epidemiology of the disease. Bern *et al.* (2007) present an estimate that 100,000 infected persons live in the

United States; most acquired the disease while residing in endemic areas. However, *T. cruzi*-infected vectors and animals are found in many parts of the United States, and rare cases of autochthonous transmission have been documented. Transfusion, organ transplantation, and mother-to-infant transmission are more likely infection routes in the United States (Bern *et al.*, 2007). According to Rodriguez-Morales *et al.* (2009), this disease is emerging in European countries: Spain, Switzerland, France, Italy, Germany, and England. Transmission of Chagas disease by the oral route could have implications for *T. cruzi* transmission in North America and Europe if contaminated food is imported from endemic areas.

A new epidemiological, economic, social, and political problem has been created with the internationalization of Chagas disease due to legal and illegal migration from the endemic countries of Latin America to non-endemic countries in North America, Europe, Asia, and Oceania, in particular the United States, Canada, Spain, France, Switzerland, Japan, emerging Asian countries, and Australia. These migrations have created new epidemiological and public health problems for the countries that have received the infected migrants. These problems include risks of transfusion and congenital transmission, as well as a need for medical care for Chagas patients and additional controls over blood banks in countries with little experience with this illness (Coura and Dias, 2009; Schmunis, 2007).

The costs of coping with the public health burden of chronic Chagas disease are enormous, as a result of the morbidity, mortality, hospitalization, and drug treatment of symptoms. There is no vaccine, and none is likely because the role of autoimmunity in pathogenesis is under dispute. No prophylactic drugs exist, and treatment for infection, although potentially lifesaving in the acute phase, entails prolonged administration and side effects, and is not guaranteed to eliminate *T. cruzi*. Preventing transmission is therefore an excellent investment for the governments of the countries of Latin America where this protozoan is endemic (Miles *et al.*, 2003).

II. DISCOVERY

Carlos Ribeiro Justiniano das Chagas (1878–1934) was a physician and researcher at ''Instituto Oswaldo Cruz'' in Rio de Janeiro city (Rio de Janeiro state—Brazil). On April 15, 1909, he reported on one of the most remarkable feats in public health and tropical medicine of the 20th century, by combining his knowledge of insect-transmitted malaria with a high level of clinical suspicion and shoe-leather epidemiology. Carlos Chagas made a unique discovery: he described a new infectious disease in all its aspects, from the causal pathogen, the vector—the bloodsucking

triatomine insect that transmits it—and the parasite's life cycle with its natural reservoirs to a description of the disease itself. The discovery occurred, together the researcher Oswaldo Cruz, while he was working in the village of Lassance, an inland town of the state of Minas Gerais (Brazil).

Carlos Chagas discovered a new flagellated pathogenic protozoan species, and named it *Trypanosoma* (*Schizotrypanzonum*) *cruzi*. The symptoms caused by this protozoan infection were first described for marmoset (*Callithrix penicillata*). After that, Berenice, a 2-year-old girl, was the first case of what would be considered a new human disease (Kropf and Sá, 2009; Scliar, 2002; Voelker, 2009; WHO, 2009).

III. *T. CRUZI* LIFE CYCLE

T. cruzi is a flagellate protozoan of the Kinetoplastida order and *Trypanosoma*tidae family, an exclusively parasitic taxon that infects a wide range of animals and plants. The life cycle includes the passage through two types of host. The intermediate host includes haematophagous hemipteran insects (e.g., *Triatoma infestans*, *Triatoma brasiliensis*, *Panstrongylus megistus*, *Rhodnius prolixus*, and others), also called ''kissing bug,'' ''benchuca,'' ''vinchuca,'' ''chinche,'' or ''barbeiro'' and the definitive host consists of mammals from various different classes, including humans. *T. cruzi* multiplies in the digestive tract of the insects and the infectious form is eliminated in their feces. It generally circulates in the blood of the mammals and lodges in different tissues (Coura, 2003).

The *T. cruzi* life cycle includes several stages: infective metacyclic trypomastigotes invade a definitive host (e.g., human). Inside the host, the trypomastigotes invade cells where they differentiate (loses its flagellum) into intracellular amastigotes by binary fission. Intracellular amastigotes will differentiate back into trypomastigotes and be released from the infected cell, entering the bloodstream. The bloodstream trypomastigotes do not replicate. These trypomastigotes can invade another cell and repeat the replicative process. Clinical manifestations can result from this infective cycle. In the bloodstream, trypomastigotes can be taken up by a triatomine bug (e.g., *T. infestans*) during feeding. Within the vector's midgut the parasite differentiates to an epimastigote and undergoes multiple rounds of binary fission. The epimastigotes quit dividing and differentiate back into metacyclic trypomastigotes in the hindgut (CDC, 2009).

An important discovery about *T. cruzi* life cycle occurred in the 1980s. Deane *et al.* (1986) have shown that the entire developmental cycle of *T. cruzi* (trypomastigotes–epimastigotes–metacyclic infective trypomastigotes) may take place within the lumen of the anal odoriferous glands of

opossums (*Didelphis marsupialis*), thus potentially bypassing the development in the insect vector (intermediate host). The epimastigotes multiplying extracellularly and metacyclic trypomastigotes are stages that correspond to the cycle of *T. cruzi* in the insect vector gut (Deane *et al.*, 1984, 1986; Urdaneta-Morales and Niromi, 1996). Thus, it is important to note that with marsupials, like opossums, excretions could play an important role in the transmission of Chagas disease.

IV. PHASES AND SYMPTOMS

Several transmission mechanisms exist for Chagas disease in humans including transfusions, congenital factors, oral or buccogastric transmission, and principally, via vectors. This last via occurs when humans are bitten by infected insects, mainly triatomines such as *T. infestans*, which defecate on the skin. When the individual scratches the location of the bite, the contaminated insect feces enter the bloodstream.

According to Coura and Dias (2009), the transmission mechanisms for Chagas infection can be divided into two groups: (i) the principal mechanisms, by means of vectors (triatomines), blood transfusion, oral transmission, contaminated food and placental, or birth canal transmission; and (ii) secondary mechanisms, by means of laboratory accidents, management of infected animals, organ transplants, sexual transmission, wounds, contact with sperm or menstrual fluid contaminated with *T. cruzi* and, hypothetically, deliberate criminal inoculation or contamination of food with the parasite (Coura and Dias, 2009).

Actually, in some South American regions (e.g., the Brazilian Amazon), *T. cruzi* infection by oral route is the most important mode of Chagas disease transmission.

V. DIAGNOSES AND TREATMENT

Chagas disease in humans can be divided in two main phases: the acute phase and the chronic phase. The acute phase may have no symptoms or have very mild symptoms. When present, the symptoms can include diarrhea, vomiting, headache, fever, edema, rashes, swollen lymph glands, enlarged liver or spleen, and myocarditis and/or meningoencephalitis. This phase is characterized by the presence of the protozoa in the patient's blood and can be severe and/or fatal in infants, children, and in people with weakened immune systems. In this phase, morbidity and clinical symptoms are directly associated with the parasitemia level. Chagas disease manifestations in the acute form are very common when *T. cruzi* is acquired by via the oral route.

Following the acute phase, most infected people enter into a chronic phase. The chronic phase of the infection is considered as an intermediate phase by some authors because the infection may remain silent for decades or even for life. The chronic phase is characterized by the presence of *T. cruzi* in the patient's organs like the heart and intestine. The symptoms of the chronic phase are cardiac complications (e.g., cardiomyopathy, heart failure, altered heart rate or rhythm, and cardiac arrest) and digestive complications, especially megaesophagus and megacolon.

Many people may remain asymptomatic for life and never develop Chagas-related symptoms (Britto, 2009; Coura, 2003, 2007; Rodriguez-Morales, 2008; Yoshida, 2008). An important characteristic is the severity of the disease, in some cases culminating in death.

VI. TRANSMISSION ROUTES

Because it is an infection with a very long natural history, the host–parasite relationships have become extremely complex, involving far-reaching changes on both sides, interfering with the development of the infection. Thus, the process of natural selection that acts continuously on the protozoa leads to a parasite diversity that influences the severity of the disease.

In the case of oral infection, data from the literature demonstrates the existence of trypomastigotes more suitable for transmission by this route. Studies with mice revealed that trypomastigotes of *T. cruzi* are able to invade the gastric mucosa, and cause systemic infection, because of the presence of glycoproteins on the surface of the trypomastigotes promoting penetration of the parasite. Biochemical evaluations indicated the presence of three major groups of glycoproteins: gp90, gp82, and gp30, each one with a difference in the level of affinity for mucin and resistance to the gastric juice (Yoshida, 2008).

According to some authors, the success in the establishment of infection by the oral route is associated with the expression of gp82, a surface glycoprotein, which binds to mucin and gastric epithelial cells. This molecule promotes the entrance of trypomastigotes through a cascade reaction that culminates with the mobilization of intracellular calcium (Ruiz *et al.*, 1998).

Strains of *T. cruzi* deficient in gp82 also can, in certain circumstances, invade cells *in vitro* perhaps by stimulating the expression of gp30, a glycoprotein that can induce the calcium signal. But studies in mice showed that the parasites that express this molecule as a priority, are less virulent by the oral route because gp30 has a lower affinity for gastric mucin. In addition, trypomastigotes also express gp90, a surface

glycoprotein that binds to host cells and acts as a molecule that suppresses invasion (Cortez *et al.*, 2006).

Strains of *T. cruzi* expressing high levels of gp90, beyond gp82 and gp30, weakly infect cells *in vitro*. However, the invasive efficiency of these strains can vary by the oral route, because, in contrast with gp82 and gp30 that resist degradation by pepsin present in the gastric juice, some isoforms of gp90 present in certain strains of the parasite have a higher degree of susceptibility to peptic digestion.

Thus, the genetic diversity of strains, allied to their ability to produce certain glycoproteins, corresponds, at least in part, to the severity of cases of illness in outbreaks of oral infection.

VII. CHAGAS DISEASE EXPERIMENTALLY TRANSMITTED BY THE ORAL ROUTE IN ANIMALS

In the first week after *T. cruzi* infection, trypomastigotes are detected in blood samples by microscopic examination, but parasitemia is rapidly controlled and becomes extremely low. The infection persists for the lifetime of humans and laboratory animals as either latent or pathogenic parasitism. It is extremely difficult to demonstrate circulating parasites during the chronic disease (Krettli, 2009).

Demonstration of the causal agent is the diagnostic procedure in acute Chagas disease. It almost always yields positive results, and can be achieved, first, by microscopic examination of fresh anticoagulated blood, or its buffy coat, for motile parasites; and of thin and thick blood smears stained with Giemsa, for the visualization of parasites. On the other hand, positive results can be achieved by isolation of the agent: inoculation in culture with specialized media (e.g., Novy, McNeal, and Nicolle (NNN medium), liver infusion tryptose (LIT medium)); inoculation into mice; and xenodiagnosis, where uninfected triatomine insects are fed on the patient's blood, and their gut contents examined for parasites 4 weeks later (CDC, 2009). Additionally, several molecular methods are developed to diagnose Chagas disease.

At the end of the 1960s, an international clinical trial was conducted to assess the efficacy of the drugs nifurtimox and benznidazole, used in the treatment of Chagas disease. Several researchers had recommended treatment during the acute phase and in the chronic phase. In 1983, a panel of experts reversed these recommendations, suggesting that treatment not be provided during the chronic phase of the infection. There were, however, pockets of persistent use of drugs, mostly benznidazole rather than nifurtimox, during the 1980s, and follow-up studies have shown effectiveness during the chronic phase (Sosa-Estani *et al.*, 2009).

Treatment can cure infection and reduce or prevent the progression to Chagas disease-related heart disease/cardiomyopathy. The search for better drugs to find a solution for the 8 million infected people is one challenge that must be addressed. In the meantime, benznidazole and nifurtimox continue to be the only drugs approved as effective treatments for *T. cruzi* (Sosa-Estani *et al.*, 2009). Their effectiveness, however, can vary with *T. cruzi* strain, and patient health conditions. Another problem is the severe side effects of these drugs, which increase with the patients' age. Benznidazole, the most available and widely used drug, can cause hypersensitivity reactions, bone marrow depression, thrombocytopenic purpura, agranulocytosis, and neuropathies (Von *et al.*, 2007).

VIII. TRANSMISSION OF CHAGAS DISEASE BY THE ORAL ROUTE IN HUMANS

The importance of transmission via the oral route has long been known in the case of susceptible omnivorous or insectivorous animals, which feed on vector insects and infected animal reservoirs (Dias, 2006; Neto *et al.*, 2000). Some authors consider the possibility that Carlos Chagas, in his first study together with Oswaldo Cruz, played an important role in the first demonstrated case of oral transmission of Chagas disease. Marmosets (*C. penicillata*) were placed in cages together with insects infected by the parasite, and also acquired the parasite. It is now known that transmission to these animals by insect bites is rare, and, at the time of the experiment, no entrance points were found on the animals; thus, the hypothesis of ingestion of the insects by the marmosets cannot be discarded (Coura, 1997; Dias, 2006).

According to Coura (1997), oral transmission of Chagas disease was first hypothesized and studied by Nattan-Larrier in 1921, using sanguineous protozoa. However, according to Ribeiro *et al.* (1987), Mayer and Rocha Lima were the first to observe the transmission of Chagas disease by ingestion of blood containing trypomastigotes. In 1931, the possibility of oral transmission was reinforced the idea of putting feces infected with the triatomine insect in the oral mucosa of animals, thus infecting them. The researchers Kofoid and Donat, and Cardoso were thus able to experimentally confirm the transmission of Chagas disease by oral route (Coura, 2006; Neto *et al.*, 2000).

Yaeger (1971), in Lousiana—United States, demonstrated that opossums acquired *T. cruzi* infection by eating two infected triatomine insects (*R. prolixus*). According to Yaeger (1971), mammals such as the opossum, armadillo, racoon, skunk, and various rodents which acquire *T. cruzi* infection frequently do so as a result of their insectivorous habits and occasionally through predation of infected mammals. Still in the United

States, Roellig *et al.* (2009) presented the first demonstration of the oral transmission of *T. cruzi* to raccoons (*Procyon lotor*), a natural reservoir host in the United States, by ingestion of trypomastigotes and infected insects.

In Venezuela, between 1960 and 1980, a series of experiments by Carlos Diaz Ungria *et al.* demonstrated the oral infection by *T. cruzi* in dogs, hamsters, and other rodents (Dias, 2006).

In Brazil, Ezequiel Dias in 1933 was the first to officially describe the oral transmission mode of Chagas disease. He observed armadillos feeding on the insect *P. megistus* in his laboratory. This researcher also confirmed the importance of the transmission of the protozoa to cats that fed on infected insects and mice (Ribeiro *et al.*, 1987). Additionally, Ribeiro *et al.* (1987) described the infection of several opossums (*Didelphis albiventris*) by oral means by feeding them either on infected triatomines or on mice experimentally infected by *T. cruzi*. Souza *et al.* (1997) infected mice by oral administration of blood samples contaminated with *T. cruzi.*

The parasite can also be transmitted experimentally via the oral route through ingestion of contaminated food.

Mayer's experiments, in 1961 (Jörg, 1992), demonstrated the infection of mice, dogs, and cats by ingestion of milk contaminated with excrements from infected *T. infestans* (a drop of excrements per 20 ml of milk).

Lainson *et al.* (1980) contaminated a variety of food with *T. cruzi* suspensions: pasteurized milk; a mix of boiled beans, flaked fish, minced beef, and rice; bottled mango juice; mix of cheese and guava fruit preserve; and dry *mandioca* flour. Batches of six mice were fed with each contaminated food. *T. cruzi* survived for at least 3 h, at 26–28 °C, in milk and in a mix of rice, beans, fish, and beef. All the 30 mice fed with the contaminated food became infected.

In 1985, experiments demonstrated that mice were infected with *T. cruzi* when they fed on food contaminated with excrement from opossum (*D. marsupialis*) (Jansen and Deane, 1985). Since then, many Brazilian scientists have also demonstrated the infection of mice with Chagas disease by ingestion of food contaminated with *T. cruzi*, especially sugarcane juice (Cardoso *et al.*, 2006; Castanho *et al.*, 2002; Pinto *et al.*, 1990; Soares *et al.*, 1987) and Amazonian palm berry juice or *açaí* (*Euterpe oleracea* Mart.) (Barbosa-Labello *et al.*, 2008; Dias *et al.*, 2008b; Neves and Valente, 2007; Neves *et al.*, 2007).

Calvo-Méndez *et al.* (1994) carried out an experiment aiming to experimentally prove the infection by *T. cruzi* from the ingestion of contaminated food. They showed that drinking water, pasteurized milk, raw and cooked minced beef, fresh cheese, and cooked rice, when inoculated with the feces of the insect *Triatoma pallidipennis* containing *T. cruzi*, were capable of infecting mice orally with Chagas' disease. There was a variation in efficiency with respect to infective capacity according to the food used, milk being shown to be the most effective medium for transmitting the protozoa.

Añez and Crisante (2008) studied the survival of *T. cruzi* in fruits and vegetables stored at 26 °C: banana (*Musa* sp. AAA), peach (*Drunus persicae*), ananas (*Ananas sativus*), sugarcane (*Saccharum officinarum*), papaya (*Carica papaya*), apple (*Malus sylvestris*), potato (*Solanum tuberosum*), carrot (*Daucus carota*), arracacha (*Arracacia xanthorrhiza*), tomato (*Lycopersicum esculentum*), and other banana (*Musa* sp. AAB). According to Añez and Crisante (2008), in eight fruit and vegetable samples, 73% of the parasites remained alive for a period between 6 and 72 h. It was estimated that the largest number of live parasites was found between 6 and 18 h postcontamination. Pineapple was the only fruit in which *T. cruzi* survival could not be observed.

IX. THE INFLUENCE OF THE *T. CRUZI* STRAIN IN THE TRANSMISSION OF CHAGAS DISEASE BY THE ORAL ROUTE

Chagas disease can be acquired via oral route through several ways: the ingestion of infected mother's milk, raw or undercooked meat from infected animals, food contaminated with infected triatomines and/or their feces, food contaminated with anal gland secretions of marsupials, and finally, by ingesting the triatomines themselves. Jörg (1992) even reported the occurrence of a fatal case of Chagas disease due to the ingestion of flagellates when pipetting an acellular culture medium containing the protozoa. The victim was Mario Fatala Chaben, a doctor specialized on serological diagnoses of Chagas disease.

The oral route of transmission of *T. cruzi* is considered as the primary mechanism of transmission of Chagas disease to humans. According to Jörg (1992), in Argentina, in 1936, Salvador Mazza reported the first case of transmitting Chagas disease to humans via mother's milk. In the same article (Jörg, 1992), the author describes two cases of acute Chagas disease in humans transmitted by the ingestion of contaminated food. The first case occurred in 1948 in a baby, by ingestion of a potion made with sugarcane juice and armadillo's blood probably infected with *T. cruzi*. The second case (fatal) occurred in 1958. The victim was a 12-year-old boy who ingested uncooked meat of silvatic animals (vizcachas, aguties, and pacas) during 4 days while on an excursion.

X. ACUTE CHAGAS DISEASE OUTBREAKS ASSOCIATED WITH FOOD IN BRAZIL

According to Yoshida (2009), transmission of *T. cruzi* has steeply declined in South American countries (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay) especially because of control measures directed at elimination of the domiciliary vector (*T. infestans*). In 2006, Brazil was certified by

the Pan American Health Organization (PAHO) as free of Chagas disease vectorial transmission due to *T. infestans*.

On the other hand, actually, Brazilian health authorities have been alert to the occurrence of oral transmission, occurring as outbreaks, through the ingestion of food contaminated with vector-derived infective forms—mainly in the Brazilian Amazon region (Araújo *et al.*, 2009). However, the transmission of Chagas disease by the ingestion of food contaminated with *T. cruzi* and the occurrence of outbreaks have occurred since the1960s. Figure 3.1 presents a map of Brazil highlighting the regions involved in the episodes of acute Chagas disease orally transmitted, by ingestion of contaminated food.

The first scientific report of an orally transmitted outbreak of Chagas' disease in Brazil was made in 1968 (Nery-Guimarães *et al.*, 1968). This occurred in the district of Teutônia, municipality of Estrela (Rio Grande do Sul state) in the year 1965, between March 13 and March 22. Seventeen people from an Agricultural School (workers, students, and lecturers that usually had meals there) fell sick. The initial unconfirmed diagnosis was typhoid fever. Other possible diagnoses like infectious hepatitis, toxoplasmosis, infectious mononucleosis, and food poisoning were also discarded. Then, some of the infected people presented with clinical symptoms of acute myocarditis, and, based on clinical observations,

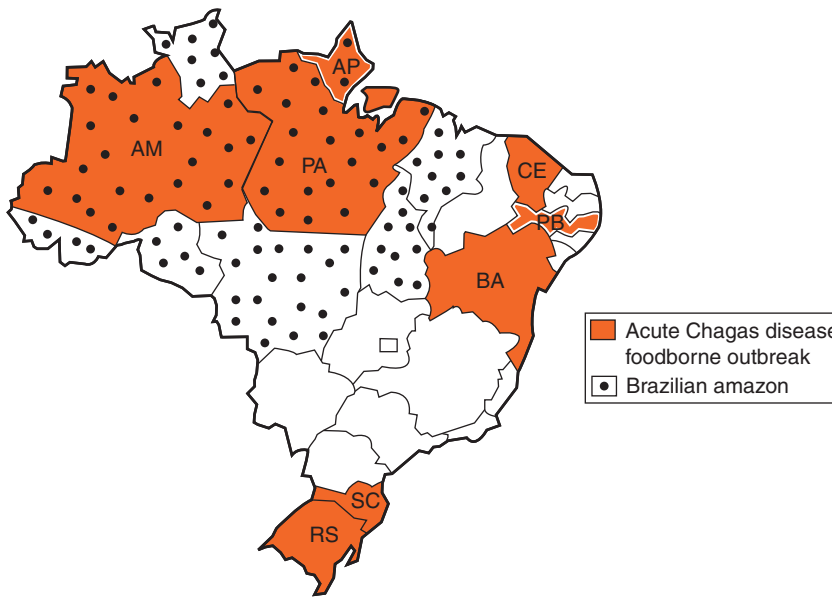

FIGURE 3.1 Brazilian map and states that registered acute Chagas disease foodborne outbreaks: Amapá (AP), Amazonas (AM), Bahia (BA), Ceará (CE), Pará (PA), Paraíba (PB), Rio Grande do Sul (RS), and Santa Catarina (SC).

electrocardiogram, and radiography, the hypothesis of chagasic myocarditis was considered. The diagnosis was established by isolation of *T. cruzi* in blood samples, complement fixation test, and evidence of the protozoa in cardiac tissue of necropsies. A serological and entomological study was carried out. No triatomines were isolated in the grounds of the school, but an opossum (*Didelphis azarae*) infected by *T. cruzi* was found. Thus, one of the hypothesis raised for the occurrence of this acute Chagas disease via oral outbreak was ingestion of food contaminated with the urine of marsupials (*D. azarae*) usually entering the houses and naturally infected in the area (Nery-Guimarães *et al.*, 1968; Silva *et al.*, 1968).

The second register of acute Chagas disease probably transmitted via the oral route occurred in Belém city (Pará state—Brazil). Four cases of Chagas disease were recorded in a single family. Three people presented with symptoms of the disease in the acute phase including diarrhea, vomiting, headache, and fever. The first hypothesis was that they had malaria, but, during the blood examination, *T. cruzi* was found. One case evolved to a death (a 15-year-old boy). Investigations were unable to find any reduviid insects in the house and surrounding dwelling places and suggested that other methods of transmission might be considered, for instance *per os* (Lainson *et al.*, 1980; Shaw *et al.*, 1969).

A third Brazilian acute Chagas disease outbreak occurred in Catolé do Rocha (Paraíba state) in October 1986. Twenty-six people, 7–22 days after a meeting at a farm, presented with the illness characterized by fever, bilateral cyclic and lower limb edema, mild hepatosplenomegaly, lymphadenopathy, and, occasionally, a skin rash. Patients were initially diagnosed as having typhoid fever. However, they were not toxemic, so acute toxoplasmosis was suspected. Both diagnoses were discarded based on clinical and laboratory evidences. An 11-year-old boy exhibited atrial premature complexes and a 74-year-old patient developed acute heart failure. In two patients hospitalized in São Paulo city, acute Chagas disease was diagnosed by the demonstration of circulating *T. cruzi*. At autopsy in a fatal case, acute Chagas cardiomyopathy was demonstrated. An epidemiological survey showed a low rate of infection with *T. cruzi* in triatomines (*T. brasiliensis*, *Triatoma pseudomaculata*, *P. megistus*), but a high rate of infection with *T. cruzi* was found in opossums (*D. albiventris*): all the 11 tested were positive. None of the 35 sheep and 15 cows from the farm tested positive for *T. cruzi* (Shikanai-Yasuda *et al.*, 1991). Preliminary epidemiological studies indicated the possible contamination of the food and/or utensils with infected excrement from the opossum. The meal had consisted of beef and lamb barbecue, a stew made using sheep entrails, cooked pork, salad, and sugarcane juice (Shikanai-Yasuda, 1987). Contamination of the sugarcane juice with triatomines and/or their feces seemed improbable, since the population density of these insects appeared to be low, as also their

level of infection by parasites, requiring a large number of them to contaminate the quantity of sugarcane juice consumed (Marcondes *et al.*, 1987; Shikanai-Yasuda *et al.*, 1991). On the other hand, preliminary studies of the survival of *T. cruzi* in sugarcane juice corroborated the possibility of this having been the transmitting vehicle in this case of ACD in Paraíba (Pinto *et al.*, 1990; Soares *et al.*, 1987).

Still in Brazil, almost 20 years after the outbreak occurred in Catolé do Rocha, in 2005, a large-scale outbreak of acute Chagas disease was reported to be associated with the consumption of sugarcane juice from a kiosk on the Brazilian Motorway BR-101 in the municipality of Navegantes in the State of Santa Catarina. On this occasion, 25 cases of the disease were confirmed and three led to death (SVS, 2005a). Two main hypotheses were raised to explain the contamination of the sugarcane juice with the protozoa. The first hypothesis was the grinding of the sugarcane together with triatomine insects infected by the protozoa. The second hypothesis was the contamination of the sugarcane with the feces of wild animals such as the opossum, hosts of *T. cruzi*. The following findings corroborated these hypotheses: 10 infected vectors were found in a palm tree near the kiosk and 30 in the dense wild forestland behind it. One infected vector (*Triatoma tibiamaculata*) was found in the kiosk, and finally an infected female opossum was found with four infected babies (Ianni and Mady, 2006). On March 31 of the same year, a report from the Evandro Chagas Institute (Pará state) confirmed the cause of an outbreak of an acute fever-producing disease in Igarapé da Fortaleza in the city of Santana (Amapá state), which had occurred in December 2004, as being acute Chagas disease. Twenty-seven cases of the disease were confirmed in this outbreak, and the common point between them was the consumption of *açaí* (*E. oleracea* Mart.) juice from the same sales outlet (SVS, 2005b).

Between 1968 and 2005, a total of 437 cases of acute Chagas disease were reported in the Brazilian Amazon region. Of these cases, 311 were related to 62 outbreaks in which the suspected mode of transmission was consumption of *açaí* (Nóbrega *et al.*, 2009; Valente *et al.*, 2006). Pinto *et al.* (2003) reported the occurrence of a family microepidemic of acute trypanosomiasis probably transmitted orally, involving 12 people, of whom two died, in the municipality of Igarapé-Miri (PA) in July 2002. In an outbreak of acute Chagas disease affecting 17 people belonging to three families, which occurred in the locality of Rio Bispo municipality of Mazagão (Amapá state) in October 1996, researchers were able to elucidate the transmission mechanism: ingestion of *açaí* juice infected with the feces of wild triatomines. *Açaí* juice was prepared at night and the insects attracted to the electric lights fell into the juice being prepared in the machine and were ground up with the fruit pulp (Valente *et al.*, 1999, 2002, 2006, 2009).

According to Nóbrega *et al.* (2009), in 2006, a total of 178 cases of acute Chagas disease were reported from the Amazonian state of Pará, Brazil. Eleven occurred in Barcarena city and were confirmed by visualization of parasites on blood smears. The researchers conducted a retrospective cohort study of an outbreak that involved five staff members at the health post who participated in the meeting on September 15. In this outbreak, vectorborne, transfusional, transplant-associated, and transplacental transmission were excluded. A shared meal was the only event linking case-patients, and cohort and case–control studies demonstrated an association between *açaí* consumption at this meal and infection. These findings indicate an outbreak of orally transmitted disease from contaminated *açaí* palm fruit. In the district of Mojuí dos Campos in the municipality of Santarém (Pará state), 17 cases of acute Chagas disease were confirmed with one death, infection probably being caused by the ingestion of *bacaba* (*Oenocarpus bacaba* Mart.) or white *açaí* juice (SVS, 2006).

In 2007 and 2008, in Brazil, the number of acute Chagas disease cases was 161 and 123, respectively, according to the Brazilian Ministry of Health. In 2009, with still incomplete reporting, 11 cases were registered (SVS, 2009). Most of them occurred in the Amazon region and involved transmission by the oral route.

Although *açaí* juice is frequently associated with oral transmission of acute Chagas disease in Brazil, other kinds of food have been implicated in some outbreaks. In Macaúbas city (Bahia state), in 2006, seven individuals developed sudden signs of cardiac and systemic impairment, with lethality of 28.6%. Serological tests were positive at least in one test in the five patients examined. No inoculation point in either the skin or the periocular region, caused by direct vector transmission, could be detected in any of the individuals involved in this outbreak. Seven people were members of a single family (father, mother, and five children). Transmission probably occurred via the oral route, through soft drinks and/or water that had been inadequately stored. Because they were kept in open containers, they could have become contaminated with the excrement of infected triatomines (Dias *et al.*, 2006, 2008b). In the same year (2006), in Redeção city (Ceará state), an outbreak of acute Chagas disease involving eight people from the same family was registered. Epidemiological studies pointed to transmission by the oral route and the vehicle probably was a soup made with water from a reservoir in precarious hygienic conditions (Oliveira *et al.*, 2007). In 2007, in Belém (Pará state), three people were confirmed for Chagas disease, in the acute form, transmitted orally. Epidemiological study concluded that raw shrimp could be associated with this outbreak, and may have been contaminated with

feces of infected triatomines, marsupials, or rodents while in transport, storage, or on display in the marketplace (Freitas *et al.*, 2008).

XI. OTHER ACUTE CHAGAS DISEASE OUTBREAKS ASSOCIATED WITH FOOD

The largest orally transmitted Chagas disease outbreak occurred on Chacao city (metropolitan Caracas, Venezuela) in December 2007. One hundred and twenty-eight acute Chagas disease cases were confirmed and all these were clustered in a municipality school ''Andrés Bello.'' From that, 12 patients were hospitalized, and one died at the onset of the outbreak. Patients complained of fever lasting more than 7 days, abdominal pain, headache, dry cough, and myalgias. In some cases, symptoms were followed by diarrhea, facial edema, dyspnea, and tachycardia. About 75% of the cases were younger than 18 years. This pattern of acute Chagas disease outbreak is typical of an orally transmitted event. Epidemiological studies concluded that the source of infection was a contaminated fresh guava juice produced under faulty hygienic conditions. Infected vectors were collected from the surroundings of the juice manufacturing locale, and one female worker involved in the elaboration of the beverage was found seropositive for *T. cruzi* (Noya *et al.*, 2008; Rodriguez-Morales, 2008; Villalobos, 2007).

Also in Venezuela (Chichiriviche de la Costa, in the western part of the state of Vargas), in April 2009, another outbreak occurred. Three teachers and 47students from the morning shift of the ''Romulo Monasterios'' state school became ill. Three children died. The hypothesis was that these acute cases of Chagas diseases were transmitted through the ingestion of contaminated guava juice (ISID, 2009).

In Colombia, there are two well-registered outbreaks of acute Chagas disease probably transmitted by contaminated food. The first occurred in 1999 in Guamal city (Magdalena state) involving 13 cases of patients with fever and acute myocarditis; five of them died. The epidemiological studies showed that a typical fermented beverage consumed in the region, and contaminated with triatomine feces, was the source of infection: ''vino de palma'' (palm wine). Moreover, *T. cruzi*-infected triatomines (*Panstrongylus geniculatus*) were found in palms in the outbreak area (Hernandéz *et al.*, 2009; Nicholls, 2006).The second outbreak occurred in 2008 in Bucaramanga city and involved 10 patients, of whom one was asymptomatic. Three of them belonged to the same family, and the other three were employed at Palonegro airport in Lebrija city; two of them died (22- and 21-year-old men). Epidemiological studies found that a common point with the nine symptomatic patients was the ingestion of orange juice from the same place (Hernandéz *et al.*, 2009).

XII. *T. CRUZI* CONTROL (IN FOOD)

The contamination of food with *T. cruzi* can occur mainly in two ways, as shown in Fig. 3.2.

Food contamination can occur via insect feces in situations where the insect(s) can deposit their feces (infected by *T. cruzi*) in food and/or on food preparation surfaces. When exposed to the environment, feces infected with *T. cruzi* undergo rapid dehydration with consequent death of parasite. Soares *et al.* (1986) demonstrated that at low humidity, both motility and infectivity were lost within 30 min. At high humidity, the mobility and infectivity were preserved up to 30 min at 33 °C. Also, Soares and Marsden (1978) proved that *T. cruzi* can remain infective in dead vector insects stored at temperatures of 10 °C for 6 days and between 26 and 30 °C for at least 2 months.

Additionally, food contamination can occur by squashing of the insect(s) with food ingredients, especially for fruits, in which case entire insects are squashed in the process of pressing fruit juice. This is the main hypothesis for several outbreaks that occurred in Brazilian Amazon associated with *açaí* juice (Valente *et al.*, 2002). In *açai* fruit, *T. cruzi* can be viable, at room temperature, for up to 9 h after contamination (Neves *et al.*, 2007) and in *açaí* pulp for up to 28 h after contamination (Dias *et al.*, 2008b).

No less important in the epidemiology of food-borne Chagas disease is the contamination of food, equipment, and the food-processing environment by excretions of infected opossums. Moreover, for two Chagas disease food-borne outbreaks associated with sugarcane juice in Brazil, some researchers believe that this was the mode of contamination (Ianni and Mady, 2006; Shikanai-Yasuda *et al.*, 1991).

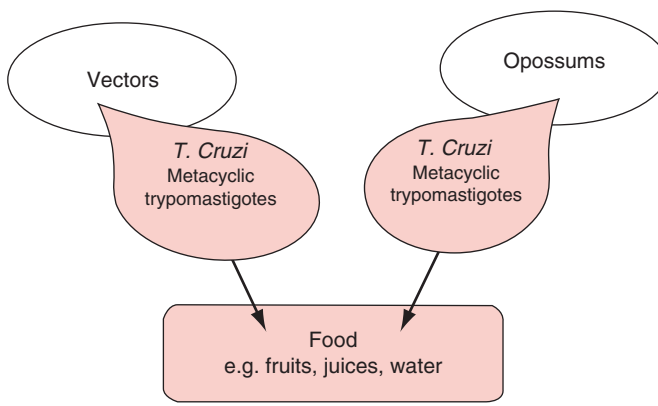

FIGURE 3.2 Simplified scheme representing the ways of food contamination by *T. cruzi*.

So, there are many potential sources of food contamination and not only *açaí* juice or sugarcane juice must be considered as high-risk food. Any human food source items (e.g., fruits) can be contaminated, in areas where there is a reservoir of *T. cruzi* in wild animals and/or infected triatomine insects, if unsafe food-manufacturing practices (e.g., harvest, transport, storage, and handling) are used.

In the Brazilian Amazon, *açaí* pulp can also be contaminated because of a lack of hygiene in the harvesting, transport, and/or processing of the fruits. The *T. cruzi*-infected insects are transported to the processing machine together with the fruits, in baskets or sacks (Valente *et al.*, 2002).

The basic procedures for sanitization of fresh food and the environment with chemical agents are considered effective for the destruction of *T. cruzi* cells: 1% sodium hypochlorite (1 h), gentian violet 1:4000 (24 h), and 70% ethanol (Dias, 2006).

According to Dias (2006), cooking above 45 °C and pasteurization are capable of killing *T. cruzi* cells. However, the meat of wild animals should be cooked above 60 °C, since *T. cruzi* amastigotes cells can survive in the tissues of these animals at such a temperature (Neto *et al.*, 2000). Ferreira *et al.* (2001) working with experimentally contaminated human milk found that heating at 62.5 °C for 30 min was sufficient for inactivation of trypomastigote forms of *T. cruzi*.

The use of microwaves was also suggested as a hypothesis for inactivation of *T. cruzi* in human milk. Ferreira *et al.* (2003) were able to inactivate trypomastigotes present in human milk when heating to 63 °C (7 min, 45% power) in a domestic microwave oven (2450 MHz, 700 W).

In contrast, methods such as freezing and refrigeration have not been shown to be effective in preventing the Chagas disease transmission by the oral route in mice. According to Neves *et al.* (2007), *T. cruzi* can be viable for up to 12 h at temperatures of 5 °C. The infecting protozoan remained in plasma after freezing at −20 °C for 3 and 24 h (Amato Neto *et al.*, 1975). Data about the efficacy of freezing to kill *T. cruzi* cells in *açaí* pulp are controversial. Barbosa-Labello *et al.* (2009) demonstrated that *T. cruzi* maintained its virulence even after staying in contact with the frozen pulp for up to 26 h. On the other hand, according to Neves *et al.* (2007), *T. cruzi* is killed after 2 h at −20 °C.

The use of ionizing radiation as a way to sterilize the environment and prevent oral transmission of this parasite also showed no practical application (Dias, 2006). The use of gamma rays on infected blood at an exposure of 5000 rad was not sufficient to inactivate the parasite, and studies with doses of 90 krad showed a loss of virulence, but not complete killing of the parasite (Amato Neto *et al.*, 1996; Salata *et al.*, 1973). Takeda *et al.* (1986) suggested that the gamma radiation dose to kill *T. cruzi* could be between 200 and 300 krad (Takeda *et al.*, 1986).

Thus, efforts should be concentrated on work to prevent the contamination of these high-risk food (e.g., fruit and vegetable beverages), using procedures such as Standardized Operational Procedures (SOPs), Integrated Pest Management (IPM), Good Manufacturing Practices (GMP), and eventually Hazard Analysis and Critical Control Points (Pereira *et al.*, 2009). Accordingly, in Brazil, the Health Ministry and Agricultural Ministry established rules for *açaí* manufacture and processing; for example, publication of a Technical Regulation on health and hygiene procedures for handling food and drinks prepared with *açaí* (Brasil, 2005), and a method of *açaí* pulp pasteurization (ANVISA, 2008; Freire, 2007).

XIII. FINAL CONSIDERATIONS

Unfortunately, until now there is no methodology for analysis of *T. cruzi* in food matrices. In the reported outbreaks, it was not possible to isolate the parasite from the food. *T. cruzi* analysis and detection still involves biological assays. Also, the use of culture media (NNN and/or LIT medium) for *T. cruzi* cells cultivation is still limited.

Despite the efforts being made by some research groups to develop a molecular method for identification of parasites in food in developing countries, the main objective of a control program of oral transmission of *T. cruzi* remains to be prevention, because the processing of many food that may pose some risk is the main source of income for the population.

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