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Trypanosoma cruzi as a Foodborne Pathogen

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This work is dedicated to:
Ramón Gustavo Alarcón.

Preface

Chagas disease is caused by a highly controversial, versatile, and startling parasite, *Trypanosoma cruzi. Controversial* because its pathogenic mechanisms are still a matter of discussion, particularly why some patients progress to a severe cardiomyopathy while others do not; treatment options are also a subject of debate. *Versatile* because new epidemiological circumstances result in it adapting to new transmission routes and make it ambiguous in its presentation, such that we are unable to delimit the disease to a single profile. *Startling* because we are continuously surprised by the various manifestations of the disease and the adaptations of the parasite to different vectors, reservoirs, and transmission circumstances.

Foodborne Chagas disease, which was demonstrated as long ago as 1967 and has resulted in numerous outbreaks, nevertheless remains unknown to a large proportion of health sector personnel, and its importance and impact seem to be underestimated by different public health officials. Although oral transmission has been postulated to be the usual transmission route between wild and domestic animals, there still seems some reluctance in academic sectors to admit the importance of this transmission mechanism. This is worrying as this transmission route is highly efficient, results in severe forms of disease due to the high parasite load of the initial inoculum and its rapid multiplication in the submucosa of the stomach, and has the potential to impact on large numbers of people when a common vehicle becomes contaminated. Outbreak scenarios, which usually concern small groups or families, but have been known to involve hundreds and have the potential to infect thousands, are difficult medical emergencies to handle. This is not only because of the large numbers of people that may be exposed, but also because of the potential for serious complications, such as cases of severe cardiac and pericardial effusions arrhythmias; if not diagnosed early and treated appropriately by well-trained specialists under optimal hospital conditions, then the mortality rate can be high.

Although research on foodborne Chagas disease is progressing, with studies on fields from epidemiology to molecular biology, there are still many neglected areas and several important topics lack data and research. This book seeks to draw attention to these areas where our knowledge is limited, as well as those areas where research is thorough, and kindle understanding and recognition of important facets

of this disease and transmission routes of infection. This book is the result of a collaborative effort between two groups with a special interest in this topic: Belkisyolé Alarcón de Noya and Oscar Noya from Instituto de Medicina Tropical, Facultad de Medicina, Universidad Central de Venezuela, are experts in Chagas disease and Lucy J. Robertson from the Norwegian University of Life Sciences has a close interest in a range of foodborne parasites, with a research focus on detection of contamination and interruption of foodborne transmission. In addition, some chapters are the work of contributors and colleagues from the Venezuelan team who were asked to contribute on the basis of their collaborative work and experience in the management of some of the outbreaks.

This brief thus attempts to summarize and highlight the main aspects of the biology of *Trypanosoma cruzi* and describe the main strategies of invasion, the epidemiology, most notably emphasizing the determinants of oral transmission, and the increase in prevalence. Recorded outbreaks and cases are described along with prophylactic measures. Original pictures depict geographical distributions, risk factors, and clinical manifestations. For teaching purposes, oral and vector-borne transmission routes are compared and final remarks are included. The purpose is both to elucidate general aspects of food as a transmission vehicle for parasites and to look at this in the particular context of *T. cruzi*. In addition, experiences in the management of outbreaks in Venezuela are provided and perspectives on those factors that are of relevance for oral transmission — that we believe should be firmly established as a dangerous transmission route, that may cause rapid morbidity and complications with high mortality.

Throughout the nine chapters of this book, we have attempted to keep technical details and jargon to a minimum such that the information is accessible to all, with particular hope that it can be read by health personnel who manage children and adults in the emergency room and also by public health officials and international food safety organizations, particularly those who still appear to doubt the importance of Chagas disease as a foodborne parasitic disease.

We would particularly like to thank the following additional contributing authors: Zoraida Díaz Bello, Cecilia Colmenares, and Raiza Ruiz-Guevara—all from Instituto de Medicina Tropical, Facultad de Medicina, Universidad Central de Venezuela.

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Chapter 1
Introduction: Some Historical and Geographical Aspects and the Relevance of Chagas Disease Among Foodborne Infections

Lucy J. Robertson

1.1 History and Geography of *Trypanosoma cruzi*

Trypanosoma is a genus of the Trypanosomatidae family, which is generally considered monophyletic (Momen 2001), and consists of a large group of flagellated protozoan blood parasites affecting more than 470 mammalian species worldwide (Guan et al. 2011). Of these various species *Trypanosoma brucei*, usually transmitted by the tsetse fly (*Glossina* spp.) and causing human African trypanosomiasis (sleeping sickness) across sub-Saharan Africa, is probably the most well-known. On the American continent *Trypanosoma cruzi* and *Trypanosoma rangeli* are the only trypanosomes that infect humans, and both species use the same vertebrate and invertebrate hosts.

T. cruzi is the aetiological agent of Chagas disease (ChD) (American trypanosomiasis), which was first discovered at the Instituto Oswaldo Cruz in Rio de Janeiro, Brazil in 1909 by Dr. Carlos Ribeiro Justiniano das Chagas (Chagas 1909; Guhl 2007), a Brazilian clinician and researcher (who also discovered *Pneumocystis*) who had been sent to Lassance to help with the control of a malaria outbreak. Chagas' original report provided meticulous details on the transmission cycle and the clinical manifestations of a human case (Chagas 1909; Rassi et al. 2010). He had observed the transmission stages in the intestine of the "assassin bugs", relatively large hematophagous insects infesting the rural dwellings where he was working, and conducted experiments to demonstrate transmission to marmoset monkeys that had been bitten by the infected insects. He suspected that transmission to humans probably also occurred and first described this in an infant girl; as well as describing over 25 acute cases and conducting autopsies on over 100 people with the chronic form of the disease, Chagas also proposed that armadillos might be a reservoir of the disease.

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American trypanosomiasis is a neotropic infection being one of the five major tropical diseases closely associated to "social diseases" of poverty together with malnutrition, diarrhea, tuberculosis and other parasitic diseases (Storino 2000). As of today, around 8–10 million people are infected by *T. cruzi*, mostly in Latin America (Bern 2015), and 108 million people have been calculated to be at risk in endemic areas (OPS 2006). According to the Pan American Health Organization (PAHO) and World Health Organization (WHO) around 12,000 people die from ChD annually; in 2008 around 11,000 deaths due to ChD were estimated for 2004 (WHO/FERG 2008; WHO 2010). ChD is considered endemic in 21 countries (Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guyana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Venezuela and Uruguay) that can be considered to be divided into three different ecological zones: Southern Cone, Andean and Amazon-Orinoquia region, and Mesoamerica. The epidemiological characteristics of ChD parasitic disease vary from one endemic area to another, as well as the prevalence rates, parasite characteristics, pathology, clinical, vectors and reservoirs (WHO 2002).

Initiatives in endemic countries, largely directed against the vectors, have reduced the number of cases in recent years, in contrast acute ChD has been observed in several countries in the last four decades. Furthermore, although ChD was once entirely confined to America, principally Latin America, it has now spread to other continents. In the last decade, due to the increased population relocation between Latin America and the rest of the world, ChD has spread to previously non-endemic areas, such as the United States and several European countries and the Western Pacific. Thus, there have been cases of ChD in countries with little disease knowledge or experience and where health control measures are insufficient, especially in blood banks and obstetric services (WHO 2008). Systematic review and meta-analysis of studies reporting prevalence of ChD in European countries indicated a high prevalence of infection in migrants from Latin America, particularly from Bolivia and Paraguay, living in Europe (Gascón et al. 2010; Requena-Méndez et al. 2015). Indeed, progressive movements of migration mean that ChD is becoming a considerable challenge for public health in many non-endemic countries. While in Europe, an increase in the number of cases has been observed in recent decades, other countries, such as Japan, are also reporting cases of ChD and the need to improve surveillance, diagnostic methods, and establish a treatment framework (Imai et al. 2015). The presence of ChD has long been recognized in the USA, and over 300,000 immigrants infected with *T. cruzi* were estimated to be living in the USA in 2005 (Bern and Montgomery 2009). Although most people infected with *T. cruzi* in the United States are immigrants from areas of endemicity in Latin America, a few transfusion/organ donation cases of infection have been reported and a handful of autochthonous vector-borne human infections have been reported from southern states (Bern et al. 2011). The presence of enzootic cycles of *T. cruzi* in the southern half of the United States, with reservoir hosts of woodrats, other rodents, raccoons, skunks, coyotes, opossums, and "armadillos", and at least 11 species of triatomine vectors, suggest that other autochthonous vector-borne human

infections may go undiagnosed (Bern et al. 2011). It has been suggested that hunting or camping activities in some areas of USA could place individuals at elevated risk for infection (Garcia et al. 2014).

According to Coura and Viñas (2010), regional differences in ChD-transmission cycles, prevalence, and control programs within Latin America, enable three separate infection groups to be recognized as follows:

- In Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Peru, Uruguay, and Venezuela, Chagas disease is characterized by domestic, peridomestic, and wild cycles, with zones of high prevalence of human infection, and the presence of myocardiopathy and, with the exception of Venezuela, digestive abnormalities. Vectorial and transfusional transmission controls are relatively well-established, with 100 % coverage of blood banks, (except in Bolivia).
- In Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, and Panama, ChD has similar domestic, peridomestic, and wild cycles, and patients present with chronic ChD cardiopathy, but exceptionally with digestive abnormalities. Vector and transfusional transmission controls are in place, but are more recent and less well established.
- In Belize, French Guiana, Guyana, Suriname, and Mexico, ChD primarily has a wild cycle, and documented information about acute and chronic clinical manifestations is relatively limited. Vectorial and transfusional transmission controls are in development.

American trypanosomiasis is a chronic, disabling, and debilitating infection; not only does it have a severe impact on the patients themselves, but in addition, the society, economy, and culture of the community suffer. Treating patients with ChD is expensive, and can exert a severe and unsustainable toll on public health systems, while the welfare state is burdened with the costs of early retirements, and patients becoming marginalized in society because of their apparent inability to work (Moncayo and Silveira 2009). The annual burden from *T. cruzi* infection has been estimated at 430,000 Disability-Adjusted Life Years (DALY), of which over 99 % were in America (WHO 2008). This is almost five times the amount of DALYs as reported from malaria from this region.

1.2 *Trypanosoma cruzi* as a Foodborne Pathogen

While ChD is still often considered mainly a vectorborne disease, primarily associated with rural locations and close contact with triatomines, as first observed by Dr. Chagas over a century ago, oral transmission seems to be increasing, especially in the Amazon, Orinoquia, and Andean regions. In some localized areas of Latin America, this endemic infection is actually considered to be emerging as alterations in factors such as human behavior and ecology result in the transmission cycle gaining a foothold in urban areas (Urdaneta-Morales 2014), as modifications to the environments surrounding towns and cities result in the establishment of an urban enzootic *T. cruzi* cycle (Reyes-Lugo 2009; Carrasco et al. 2014). In some situations

this may also exacerbate the importance of food as a transmission route (Rueda et al. 2014). It should be noted that oral transmission to reservoir hosts is also of importance, not just oral transmission to humans; oral transmission may lead to high infection rates in domestic mammals and other reservoir hosts, even when the vectors have a low preference for biting them, and this can ultimately result in elevated transmission to humans (Coffield et al 2013; Kribs-Zaleta 2014).

However, despite the clear impact that American trypanosomiasis has on human health and welfare, and despite the emergence of the oral route of transmission of *T. cruzi* as one of obvious importance, as demonstrated by multiple outbreaks and clusters of infection, beyond Latin America this knowledge appears to have been slow to impinge upon the consciousness of policy makers. For example, at the first formal meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG) as convened by WHO in 2007, the "Parasitic Diseases Task Force" within FERG did not mention *T. cruzi* in their list of "causative agents for which burden of disease estimates are to be derived" (WHO/FERG 2008; Kuchenmüller et al. 2009), and also was not mentioned in an update publication from 2014 (Torgerson et al. 2014). This is probably due to the majority of members of FERG not being parasitologists, and those that are parasitologists not having a connection with Latin America and probably not considering *T. cruzi* as a foodborne parasite. Although Torgerson et al (2014) claim that the FERG list of important pathogens was largely confirmed in another meeting, the FAO/WHO Joint Expert Meeting on Foodborne Parasitic Diseases (FAO/WHO 2014; Robertson et al. 2013) did in fact include *T. cruzi* as a foodborne parasite. In this latter initiative, an effort was made to rank the importance of different foodborne parasites from a global perspective using an expert risk-ranking approach. Using this strategy a list of 93 parasites with the potential for foodborne transmission were reduced to a list of 24, and these were ranked according to a list of several weighted criteria including global distribution, mortality rate, morbidity (acute and chronic), trade relevance etc. The relatively high positioning of *T. cruzi* in this ranking (tenth out of 24), indicates that this expert group were more aware of the potential severity of foodborne ChD, particularly as the nine potentially foodborne parasites that were ranked higher than *T. cruzi* (*Taenia solium*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Trichinella spiralis*, Opisthorchiidae, and *Ascaris* spp.), all have a considerably wider global distribution as foodborne parasites, and therefore would have ranked more highly on that criterion. It is noted that the information obtained from the risk ranking exercise represents only a "snapshot" based on the information available at the time, and that the process could be completely re-run at national or regional levels using data more specific to that particular country or region (FAO/WHO 2014). Indeed, a similar exercise based only on India has been shown to result in a similar, but different, pattern (Robertson et al., in press), and it could be assumed that a risk ranking concentrating only on Latin America might elevate the rank position of *T. cruzi* substantially. In the subsequent chapters of this book, we intend to give a more in depth view of *T. cruzi*, and, in particular, its role as a foodborne pathogen that is of significant importance to communities in endemic regions of Latin America.

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Chapter 2
Biological Aspects of American Trypanosomiasis

Zoraida Díaz Bello and Belkisyolé Alarcón de Noya

2.1 The Parasite (Taxonomy, Stages, Sub-populations)

Trypanosoma cruzi, the etiologic agent of Chagas Disease (ChD) or American Trypanosomiasis (Chagas 1911), is a flagellated and heteroxene protozoan of the phylum Sarcomastigophora, class Mastigophora, order Kinetoplastida. It belongs to the Trypanosomatidae family and genus *Trypanosoma*, within which the subgenus *Schizotrypanum* was adopted in order to designate trypanosomes that multiply intracellularly in vertebrates. The full taxonomic name is *Trypanosoma (Schizotrypanum) cruzi* (Hoare 1971). The fact that it is eliminated through the feces of their invertebrate hosts, include it among Stercoraria group.

Trypanosoma cruzi is characterized by the presence of only a single flagellum and a single mitochondrion, whose genome is organized in a complex and compact region into the mitochondrion itself and near the base of the flagellum called the kinetoplast. It has a complex lifecycle, being a digenetic organism that alternates between two hosts: one invertebrate, in the digestive tract of an insect vector, and the other vertebrate, in blood and tissue, changing its morphology and antigen expression according to the stage and site where it is residing. In order to adapt to the different internal microenvironments of the hosts, the parasite must undergo biological transformations, which cause structural and metabolic changes, allowing the possibility of infection (Corrêa et al. 1998).

Trypanosoma cruzi has three morphologically identified evolutionary stages (Hoare and Wallace 1966; Hoare 1972; Brener 1973, 1997; Bourguignon et al. 2006):

- Amastigote: is the intracellular form of *T. cruzi*, found in vertebrate host tissues. It lacks outer flagellum and undulating membrane with only rotational movement and measures 2–6.5 μm in diameter. It multiplies longitudinally by

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binary fission every 12 h, becoming the blood trypomastigotes approximately 11–13 h prior to cell rupture. Amastigote forms are able to infect new cells (Ley et al. 1988).

- Trypomastigote: is the extracellular infective form with no replicative capacity and is found in both invertebrate and vertebrate hosts. The metacyclic trypomastigote is located at the intestine ends and in the Malpighian tubules of the vector insect, as well as in the *Didelphis marsupialis* anal glands (Jansen et al. 1997). It is approximately 17 μm in length and resembles the thin forms of the blood trypomastigotes; it has a large central nucleus and a prominent kinetoplast that includes high density DNA in a terminal posterior position. The sanguineous trypomastigote can be found in the blood and other body fluids (cerebrospinal fluid, lymph) of vertebrates. The flagellum emerges from the basal corpuscle near the kinetoplast, representing one third of the protozoan length. The undulating membrane is narrow and between 12 and 20 μm in length, including the free flagellum (Alvarenga and Bronfen 1997; Siqueira-Batista et al. 2007).
- Epimastigote: replicates in the triatomine digestive tract and axenic cultures. It has an elongated shape, and the flagellum originates near and ahead of the nucleus, emerging as a short undulating membrane. The spherical nucleolus occupies a central position in the nucleus (Lopez-Velazquez et al. 2005). This mobile stage measures 20–40 × 2 μm, presenting an intense replication activity by longitudinal binary division, forming red spots. This form does not withstand temperatures of 37 °C temperature and has considerably better development at 20–28 °C.

Trypanosoma cruzi is a set of sub-populations with noticeable heterogeneity in nature and high genetic variability (Mattei et al. 1977; Miles 1983; Nogueira and Coura 1989; Andrade and Magalhaes 1996; Macedo and Penna 1998; Briones et al. 1999). One of the first attempts to identify the different populations was the biological characterization performed by Brener (1977), who described strains Y and CL as reference strains, being representatives of the intraspecific variability. Various criteria have been used in the characterization and grouping of the different strains of *T. cruzi* includes haplotypes, discrete typing units (DTU), ribosomal DNA, schizodemes, zymodemes, biodemes, chemotherapy sensitivity, virulence and pathogenicity, clinical and pathological characteristics, disease forms, infected vertebrate host species and geographical origin.

Trypanosoma cruzi has a multiclonal population structure, with a non-random association between alleles at different loci (Tibayrenc et al. 1986; Tibayrenc and Ayala 1988). The first evidence of the complexity of the structure of *T. cruzi* was derived from phenotypic studies. Using biochemical methods, gene expression products were evaluated through analysis of isofunctional enzymes that allowed differentiation between subpopulations, known as zymodemes. In the cycles of wild and domestic transmission of *T. cruzi* different isolates were closely associated with a particular isozyme pattern, the evaluation of which included up to 18 enzymes (Andrade and Magalhaes 1996; Miles et al. 1977). These patterns were described as:

- **Zymodeme 1 (Z1)** found in sylvatic transmission cycles, associated primarily with arboreal mammals, especially of the genus *Didelphis* to vectors with ecological niches restricted to wild environment.
- **Zymodeme 2 (Z2)** associated with domestic transmission cycles related to vectors and domestic mammals.
- **Zymodeme 3 (Z3)** mainly found in wild habitats associated with terrestrial mammals, such as armadillos (*Dasypus*) and the vector settlers in their burrows (Miles 1983).

DNA analysis of *T. cruzi* has revealed the existence of schizodemes (parasite subpopulations separated by the fragment pattern determined by kDNA using restriction enzymes) indicating that the zymodemes are associated with particular biological behavior of parasites.

Ribosomal RNA genes, which are classical evolution markers, and miniexon genes used for tripanosomatidae taxonomy, have shown a clear dimorphism between isolates, dividing the species into two groups. Therefore, the great diversity of isolates of *T. cruzi* was regrouped into two main divisions that represent intraspecific variations (Miles et al. 1977), categorized as type I (Z1, isoenzymes 1–25) and type II (Z2, Z3, isoenzymes 26–43). Subsequent studies suggested that group II should be subdivided into five lineages (IIa-IIe) or DTUs (Discrete Typing Units) (Brisse et al. 2000). A polymorphism present in the nucleotide sequences of two genes, in six representative strains of each of the proposed lineages (TCI and TcIIa-e) resulted in a distribution to six groups (Brisse et al. 2000), substituting the system of division into two groups and five subgroups of one type. By studying a large number of loci it was found that multiple polymorphisms are correlated with type I and II classification (Sturm et al. 2003). This analysis also showed that the only homogeneous groups, are group I and subgroup TcIIb, while others (IIa/IIc and IId/IIe) are hybrids. This is consistent with the hypothesis that genetic recombination has occurred between the different lines of *T. cruzi* (Brisse et al. 2003). The most recent phylogenetic studies showed that the proposed Tc II subdivision would not be appropriate. In order to adapt the classification of *T. cruzi* by consensus and based on new knowledge, the second nomenclature revision meeting was held, in which the 6 DTU name was changed: *T. cruzi* I to VI (Zingales et al. 2009).

The TCI parasites have a multiplicity of individual characteristics in relation to transmission cycles, host preferences, clinical or geographical distribution (Herrera et al. 2007) that led to the consideration of an intra-DTU I diversity, classified into five haplotypes: Ia, Ib, Ic, Id, and Ie (Falla et al. 2009; O'Connor et al. 2007; Cura et al. 2010) that were analyzed by genes "spliced-leader" SL-IR intergenic region polymorphism. In Colombia, an association between Tc Id with wild populations of *Rhodnius prolixus* and wild reservoirs has been found (Herrera et al. 2007).

Multiple genotypes of *T. cruzi* have been isolated from patients with orally acquired heart disease: genotypes TcI (Tc Ia, Ib Tc, Tc Ic and Id Tc) in Venezuela (Muñoz-Calderón et al. 2013; Díaz-Bello et al. 2014); Tc I and IV in Colombia (Ramírez et al. 2013) and Tc I, II and VI in Brazil (Andrade et al. 2011).

2.2 The Vectors

ChD vectors are usually the triatomines, insects belonging to the order Hemiptera, suborder Heteroptera, group Gymnocerata, family Reduviidae, and subfamily Triatominae. One hundred thirty species are known worldwide. Most of these species live in America (from the United States to southern Argentina). Triatomines are 5–30 mm in length with a typical body shape and head. The body color is brown or black, and depending on the species, may show yellow, orange, or red bands on connexivum and legs.

Triatomines are known by a variety of different local names, depending on the country. These include: pitos, chinches, besadores o besucones in Colombia; barbeiros in Brazil; vinchucas in Chile and Argentina; chipos in Venezuela, or chinche picuda or besucona in Mexico (Lent and Wygodzinsky 1979).

Triatomines are blood feeders, requiring blood for lifecycle development. Both sexes are hematophagous, and at all active stages of the lifecycle (all nymph instars and adults). Sensory organs help in locating the source of blood, the antennae are particularly sensitive to carbon dioxide emission sites, the mouth is heat sensitive to mammalian body parts, and eye, nose and vibration stimuli or contact enable the insects to orientate themselves towards the supply source. The increase in abdominal pressure when the insect feeds causes the excretion of excess water from the blood meal and the discharge of the remains of previous meals through rectal emission via diuresis. Rectal parasites accumulate when triatomines are infected with *T. cruzi*; thus, diuresis mechanisms enable the liberation of these flagellates through the triatomine feces. The ingested blood volume correlates with the development of triatomine. If the amount of ingested blood is greater than necessary, then ecdysis takes place. This is a metamorphosis that occurs in these insects moving from one developmental stage to another.

The distribution of insects in the geographic space depends on factors such as temperature, humidity, and the presence of power supply. In macro-environmental terms, there is a well defined geographic distribution for each species in America, whereas in the micro-environment, physical and environmental conditions determine whether a species can effectively colonize a particular niche, and the temperature and humidity affect the movements and dispersion of several species. This is of relevance to spread of infection, and high temperature, along with poor nutritional status, stimulates dispersion thereby increasing the incidence of ChD (Zeledón and Rabinovich 1981).

The triatomine species adapted to the home environment have population attributes that allow them to colonize, grow numerically, and survive in human dwellings. These are characteristics that classifies them as K-strategists, that is species that are adapted for the efficient exploitation of a stable environment, such as mammal nests or a human home, and whose populations fluctuate at or near the carrying capacity (K) of the environment in which they reside. For this reason, some triatomine species can reach high densities and their populations exhibit minimal seasonal fluctuations (Lehane et al. 1992).

The presence of some vector species in modern Latin American cities has been noted. *Triatoma dimidiata* is present in the capitals of Central America and Guayaquil in Ecuador; *Triatoma infestans* in cities of Uruguay; Recife, Belem and Salvador in Brazil; *Panstrongylus megistus* in El Salvador (WHO 2002); *P. geniculatus* in Caracas, Venezuela (Herrera and Urdaneta-Morales 1992; Herrera and Urdaneta-Morales 1997; Reyes-Lugo and Rodríguez-Acosta 2000; Díaz-Bello et al. 2003; Carrasco et al. 2005) and *T. maculata* colonizing houses in Maracaibo, Venezuela (Torres 1982).

The main environments (or ecotopes) where triatomines can be found are palm trees, offering a wide variety of birds and mammals as potential sources of blood. Virtually all species of *Rhodnius* and some of *Triatoma* and *Panstrongylus* inhabit large areas of Latin America, including the Amazon. Ecotopes include: hollow trees (often occupied by mammals or birds), piles of rocks (where it is common to find rodents and some species of *Triatoma*), bird nests, caves inhabited by bats, underground mammal burrows, fallen dead trees, terrestrial bromeliads (such as pinecones) or epiphytes, and peridomestic structures of human housing, where pets and some opportunistic mammals, such as rodents, may also live (WHO 2002).

Among these species, *Triatoma infestans* is the most important from an epidemiological perspective. Its distribution includes Bolivia, Argentina, Chile, Brazil, Uruguay, Paraguay, and southern Peru. International control measures that were implemented in the beginning of the 1990s (Southern Cone initiative) have contributed to the elimination of this species over large areas where it was introduced artificially (being brought there by people in household goods). *Rhodnius prolixus* is the main vector of ChD in Venezuela, Colombia and most Central American countries. Other species of epidemiological interest are *Triatoma dimidiata* (Mexico, Central America, Colombia, Ecuador, and Peru) and *Panstrongylus megistus* (Brazil). In addition to these species, some others have managed to adapt to human dwellings in more restricted geographical areas where they have become important vectors (Lent and Wygodzinsky 1979; Ryckman 1986; Carcavallo et al. 1998; Galvão et al. 2003).

Adult insects of some species fly to human dwellings, attracted by electric light, in search of food; *Panstrongylus geniculatus*, *Rhodnius pictipes*, *R. robustus*, *Eratyrus mucronatus*, *Triatoma carrioni*, and *T. venous* are some of the potentially dangerous species (WHO 2002).

For a given species of triatomine to be an efficient vector of *T. cruzi* to humans, it must have several features or characteristics. These include: the ability to colonize human environments, wide geographic dispersion, anthropophily, a rapid defecation reflex, the ability to be infected by a vertebrate host and to excrete abundant infectious parasite forms (metacyclogenesis), and the density of the domestic colonies being suitable for transmission. This last point needs further clarification; when the density of the colony of triatomines in a home is low, each person receives few bites per night. In contrast, in very dense colonies, the amount of blood that each insect manages to take is usually small, which means that very few insects defecate during or immediately after biting. Thus, intermediate densities are considered more dangerous in terms of transmission of *T. cruzi* (WHO 2002). The amount of

blood taken by each triatomine to complete the lifecycle is important, because the probability of becoming infected increases with the amount of blood meal intake. It has been shown that in certain circumstances, household triatomine infestation can cause chronic anemia in habitants due to blood loss (WHO 2002). They can also interfere with the production of eggs and chicks, thereby contributing to malnutrition of residents when they feed on the blood of poultry kept around houses.

It should also be noted that insects in the *Cimex* genus (bed bugs) have also been recently suggested as potential vectors for *T. cruzi* (Salazar et al 2015). This indicates the possibility for a globally distributed vector that has seen a population explosion in recent years.

2.3 Reservoirs

Animal reservoir hosts of *T. cruzi* overlap in their wide geographical distribution with triatomines, between 45th parallel north of the United States of America and 46° south latitude in Patagonia, Argentina (WHO 2002; Bern et al. 2011). American trypanosomiasis was originally a zoonosis affecting, almost exclusively, many triatomine and wild animals in natural foci where humans and pets were absent. Contact between people and the vector in rural settlements, which man himself built, brought about significant changes in the natural habitats, and provided domiciliary and peridomestic cycles (WHO 1991). In the wild cycle, parasitized mammals essentially sustain the ecological basis of *T. cruzi* (Dias 1992; Herrera 2010, 2014). Not all species are equally important as participants in the wild Chagas enzootia, and in any ecological region usually one or two potential reservoirs serve as primary hosts (Herrera 2010, 2014).

Studies on the common opossum, *Didelphis marsupialis*, have demonstrated metacyclic trypomastigotes of *T. cruzi* in their anal glands. This is important, because this reservoir could excrete the parasite infective stages through their glandular secretions, as well as through their feces and urine (Deane et al. 1986; Jansen et al. 1991). The opossum is apparently able to introduce wild *T. cruzi* strains into rural housing and the urban jungle. The relocation of wild reservoir hosts among wild and home areas is related to sylvatic and peridomestic cycles.

Natural reservoirs of *T. cruzi* are distributed throughout America. The most important reservoir is *Didelphis marsupialis* (“common opossum”, “weasels”, “rabipelados”, “múcuras”, “shrimps”, “carachupas”, etc.), which, due to its close coexistence with triatomines and proximity to human habitation, in combination with high mobility and longevity, introduce *T. cruzi* to the domestic cycle. Rodents are also important wild reservoirs.

In the standard wild lifecycle, *T. cruzi* (concentrated in the rectal bulb in the terminal part of the digestive tube of the insect), enters the host following defecation of the vector and deposition of the infected feces on the skin or near mucosa during or soon after the blood meal; the bite of the insect results in a skin abrasion that allows the parasite entry under the skin. Other modes of infection can be to insectivorous

mammals that eat parasitized triatomines, or some carnivorous mammals can become infected from preying upon other parasitized mammals (Carpintero 1994).

The wild cycle can be observed even within major cities. In an apartment building, a jungle focus origin was confirmed by the presence of some mammals in the vegetation, such as *Didelphis aurita*, *Didelphis marsupialis, Metachirus nudicaudatus* and triatomine, *P. carioca*, all of which were infected with *T. cruzi* (Bejarano 1959). In a neighboring building, *P. megistus* parasitized by *T. cruzi* were identified (Bejarano 1959).

To date, there have been over 180 species and 25 families of wild, domestic, or peridomestic mammals reported to have been infected with *T. cruzi*. The importance of the animal reservoir role in both the sylvatic and domestic cycles, depends on the species to which it belongs, its wild, peridomestic, or ecotope habitat, the degree and extent of their geographic dispersion, the level of contact with vectors, the vectors' trophic preferences, and the various characteristics of the host-reservoir-parasite relationship (WHO 2002).

The natural link between wild and domestic cycles is basically derived from the association between domestic and wild mammals, some of which occasionally enter the human habitat and live in their homes. Dogs, cats, and other domestic mammals, such as goats, sheep, rabbits, pigs, and sometimes horses and cattle, can all act as food sources for triatomines; once infected with *T. cruzi*, they may establish as parasite reservoirs. Additionally, synanthropic opossums, skunks, ferrets, bats, and man himself can be added to the above list of hosts. Birds, such as chickens and other poultry, although not supporting the development of *T. cruzi*, can act as a source of food for triatomines that afterwards may have contact with infected mammals. Thus, even poultry can indirectly contribute towards the maintenance of the parasite cycle (Carpintero 1994). Dogs, opossums, and rodents are nevertheless probably the most important reservoir hosts within the peridomestic cycle (WHO 1991), while opossums and armadillo*s* are the most important reservoirs in the sylvatic cycle (WHO 2002; Barretto 1979).

In the domestic cycle (which is closely linked to the peridomestic cycle), the most important reservoir host is the human. The close relationship, and the triatomine contact with people, in the reduced intradomicilliary space is important; it should be noted that these vectors usually bite people at night (WHO 2002). However, ChD non-human domestic reservoirs are of varying importance. Infection rates can be high, ranging between 10 and 60 % in uncontrolled areas in Latin America, but these prevalences decrease substantially when domestic vectors are controlled. The most important domestic reservoir is the dog, followed by domestic cats, and rodents (Pinto Dias 2000). There is no doubt that in the human habitat, there is a close relationship between man, domesticated mammals, and *T. cruzi* vectors. Both humans, and the various domestic animals that live with people, provide a continuous supply of blood and where the domiciled triatomine density is high, man-vector contact is more frequent. Although small pets are often infected with *T. cruzi*, relatively few suffer a high rate of infection. However, the larger domestic animals such as pigs, cattle and horses, are less frequently infected (Carpintero 1994). The infection rate of cats and dogs in endemic countries (Argentina, Venezuela, Chile and Brazil) is variable, ranging between <5 and

100 % for dogs and <1 to >60 % for cats; infections in dogs and cats have been reported from 15 countries (Carpintero 1994).

With respect to mice (*Mus musculus*), infections have been reported from five countries, with an index of infection of 10–30 %, and infected mice may, as prey animals, transmit the disease further to cats. *T. cruzi* infected rats (*Rattus rattus* and *Rattus norvegicus)* have been reported in nine countries, from the United States of America to Argentina. The infection rate of *R. rattus* ranges from <10 to 100 % in the southwestern region of Bahia, Brazil. Due to the high population density of rats compared with the mouse, and also due to having closer contact with humans and home triatomines, rats constitute an important *T. cruzi* reservoir. In Panama and Costa Rica, *R. rattus* seems to be the main house reservoir (Zeledón 1974). In Peru and Bolivia, where guinea pigs are commonly bred as a human food source inside the house, high rates of infection with *T. cruzi* have been reported; from approximately 20 % in Peru and from around 10 % to over 60 % in Bolivia (Gürtler et al. 1990).

It is important to note that even in the best possible living conditions, humans may act as a reservoir even in the total absence of triatomines and nonhuman reservoirs (WHO 1991).

2.4 Enzootic and Zoonotic Cycles

There are two American trypanosomiasis cycles:

- Wild enzootic. The parasite circulates among species of triatomines not associated with the domestic setting and wild mammals, usually causing no apparent pathology in mammalian hosts. For the triatomines involved in the wild enzootic cycle, different habitats are involved, including niches in which palm trees, treetops, rock crevices, and animal burrows among others. This fauna on which these triatomines feed include numerous mammals such as monkeys, birds, reptiles, rodents, marsupials (Barretto 1979).
- Drought, floods, deforestation, and urbanization are all factors that may result in the migration of the food source mammals. The consequent gradual decline in feeding sources, for the triatomines broadens their flying area in search of food. Adult triatomines are attracted to light, heat, or odors from human dwellings, and may result in the triatomines moving towards domestic hosts. In addition, insects may reach human dwellings by being transported passively from jungle areas where building materials or crops are collected. In homes with people and pets, the triatomines are able to obtain blood meals, and therefore increase the likelihood of transmission of *T. cruzi* to people (Barretto 1979).
- Enzootic diseases in human populations that often affect man and his pets develop zoo-anthroponotic foci (Barretto 1979). Some authors have suggested the existence of a peridomestic cycle, in which the parasites circulate among domestic and synanthropic mammals (rats, mice, marsupials, bats) and vec-

tors with ability to adapt to these animals' shelters due to their zooanthropophilic habits (Zeledón 1974). Humans are *T. cruzi* hosts in the domestic cycle. Mans' life expectancy is over 70 years of age, and the fact that parasitaemia can be positive for over 40 years, gives man a particularly important role as *T. cruzi* host-reservoir (Pinto Dias 2000).

The amount of infected *T. cruzi* reservoirs varies according to geographical location, local epidemiologic factors, triatomine density, and rate of infection with *T. cruzi*. Parasite circulation in the domestic cycle is dynamic and reservoirs are infected early if there is contact with infected triatomines (WHO 2002).

Kissing bugs from wild environments must "adapt" to the domestic environment in order to colonize and spread the parasite to the inhabitants of the house. Triatomine domestication is a complex process with significant genotypic and phenotypic consequences (Scholfield et al. 1999). The transition from the wild to domestic environment is mainly facilitated by domestic habitat providing increased stability for triatomines, based on the continuous playback ease with a lack of predation and a permanent food source (people and pets) (WHO 2002).

In general, we can see that each of the cycles, wild, peridomestic, and domestic (to which, from 1979, urban areas without triatomines should also be added), are not closed. Even when they are isolated, there seems to be an epidemiological chain of adjustment according to the parasite's own biological characteristics, that impact not only on itself, but also interact with other factors, adding to the complexity. This may exacerbate the endemicity, and will also depend on ecological, economic, political and cultural factors of the relevant human society (Carpintero 1994).

Below are listed the main wild cycle interactions that are associated with different human infection possibilities:

- *T. cruzi*-infected wild triatomines infect humans or domestic carnivores in the wild environment.
- *T. cruzi*-parasitized wild triatomines invade human dwellings, and thereby infect man or domestic carnivores (dogs and cats).
- *T. cruzi*-infected wild mammals infect humans during butchering processes, when human wounds are exposed to infected blood or raw viscera.
- *T. cruzi*-parasitized wild mammals enter houses or peridomiciles, and adapt to being a domestic triatomine infection source; human and domestic animals are infected subsequently.
- Domestic carnivores are contaminated by feeding on *T. cruzi*-parasitized wild mammals, that have been captured from the wild environment or that have invaded the house (Herrera 2014).

The existence of human ChD can be thought of as accidental, beginning when humans exposed themselves to natural origin foci and caused ecological imbalances, such that infected triatomines were forced to occupy human dwellings; here they found shelter and a sufficient food supply in domestic animals and humans. Thus, man may be considered to have an active part in the American Trypanosomiasis epidemiological chain.

From the traditional transmission cycles described here, we have moved towards oral transmission, which is closely related to the fact that American trypanosomiasis is primarily a zoonosis. A likely mechanism of natural transmission could be the ingestion of triatomines infected by wild animals or even by domestic animals. The risk factors present in a community where oral transmission may occur are characterized by a cluster of jumbled houses, often precariously built, with the presence of triatomines, rodents, and domestic animals infected with *T. cruzi*, living indoors with people of low cultural and educational level. Dwellings and food preparation areas do not reach minimum standards of hygiene, and during storage and food handling can result in contamination of food. This is the outset of the cycle for oral transmission.

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Chapter 3
Mechanisms of Infection in Chagas Disease

Cecilia Colmenares, Belkisyolé Alarcón de Noya, and Oscar Noya González

3.1 Transmission via Cutaneous Vector Transmission, Blood Transfusion, Transplantation, Congenital Transmission and Laboratory Accidents

One of the most obvious reasons for the persistence of Chagas disease (ChD) is the difficulty in controlling *T. cruzi* and its global spread due to the variety of possible transmission mechanisms. *T. cruzi* infection in man is often described as a contaminated wound caused by the insect bite, or ocular mucosa infection, by blood-sucking triatomines containing *T. cruzi* metacyclic trypomastigotes (Rassi et al. 2010). The parasite intracellular multiplication and invasion to dermal or ocular tissues, including the respective inflammatory reaction, are indicated by the characteristic *T. cruzi* entrance signs ("chagoma" and "Sign of Romaña"), respectively (Rassi et al. 2010).

Trypanosoma cruzi can also be transmitted from mother to unborn child through the placenta and the larger the placenta size, the greater the likelihood of infection. Carlier and Truyens (2010) proposed three possible parasite penetration routes from mother to fetus: direct penetration through the placenta by the passage of blood through the umbilical cord (hematogenous transplacental route), by the rupture of intrauterine amastigote nests that may rupture as the uterus stretches, also disrupting these nests disrupt and releasing amastigotes into the bloodstream (transuterine transmission), and through the presence of trypomastigotes in the amniotic fluid and their ingestion by the fetus. The incidence of congenital ChD varies between endemic countries, and depends on the prevalence of infection in pregnant women (Carlier

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and Truyens 2010). However, in non-endemic countries it is considered as one of the common infection routes (Gascón et al. 2010).

The blood and organs of people with ChD may contain blood trypomastigotes and amastigotes of *T. cruzi*, respectively. If these are transfused or transplanted to healthy people, through blood transfusion (Bern et al. 2011) or organ transplants (kidney, liver, bone marrow, heart, etc.), then the burden of *T. cruzi* may also be passed to the otherwise healthy person (Favaloro et al. 2015). ChD screening is mandatory in blood banks in Latin America and, depending on the extent of screening conducted, the risk of infection varies by country (Schmunis 1999).

Accidental *T. cruzi* infection may occur in the personnel of laboratories where biological samples from people with ChD, *T. cruzi* cultures, or infected animals (rodents or marsurpials) are handled. Such cases tend to be rare, but a few reports have been published (Hofflin et al. 1987; Kinoshita-Yanaga et al. 2009).

3.2 Mechanisms of Oral Transmission

Trypanosoma cruzi can infect both animals and humans by the parasites being ingested from various sources. Oral *T. cruzi* transmission has been speculated to be the standard route of infection in the enzootic cycle of this parasite, taking place through susceptible mammals feeding on infected vectors and reservoirs (Coura 2006; Eickhoff et al. 2013). It is more likely that animals (rodents, armadillos, marsupials, dogs) ingest infected triatomines that cohabit in the same environment (caves, burrows, hollow trees) or feed on other infected animals, than that the vectors defecate infected feces into the bite wounds they have made in animals by feeding on them, given that these hosts often have very thick skin or abundant hair.

Although man may be less likely to ingest infected triatomines on purpose, oral transmission from ingestion of *T. cruzi* can occur via several possible routes:

- Through food contaminated with the feces of infected triatomines. Fruit juices are the major food transmission vehicle and may become contaminated with the feces of infected triatomines that wander into the kitchens of unprotected homes. These homes are usually located in areas where man has invaded the environment and where the vector-wild reservoir cycle occurs (Valente et al. 2009; Añéz et al. 2013). This mechanism has been experimentally demonstrated (Lainson et al. 1980; Añez et al. 2009; Cardoso et al. 2006) and is probably associated with the outbreak of ChD that occurred in Amapá State in Brazil in 1999 (Valente et al. 2009).
- Accidental consumption of *T. cruzi*-infected vectors. Diaz-Ungría (1968 and 1969) showed experimental infection in various animals due to the ingestion of *T. cruzi* infected triatomines (biological vectors) as well as flies (*Musca domestica*) that had fed on the feces of infected triatomines (mechanical vectors). These insects may become inadvertently incorporated into food products, including fruit juices and other drinks, and may be subsequently ingested accidentally. A worldwide bug can be added to this list since *Cimex lecturalensis* (bed bug)

has been confirmed as a potential vector, since it becomes infected after feeding on mice with *T. cruzi*. In turn, it can infect mice through oral or skin route (Salazar et al. 2015).
- The metacyclic trypomastigotes are not only present in the rectum of infected triatomines, but also in the secretions of marsupials' scent glands and are highly infective to mammals (Deane et al. 1984). In open kitchens that are exposed to the incursion of opossums (*Didelphis marsupialis*), fluids and solid food can become contaminated by their secretions.
- Ingestion of raw or undercooked wild animal meat. Jörg (1992) reports two cases of oral transmission associated with consumption of raw animal meat; the first case is from 1948, and describes infection occurring in a baby following ingestion of a potion made with sugar cane juice and armadillo's blood, presumably infected with *T. cruzi*. The second case describes the fatal infection of a young boy following consumption of raw meats from various wild animals including viscachas (Family Chinchillidae), agoutis (Family Caviidae), and pacas (Family Cuniculidae) while on a 4-day excursion. Thomas et al. (2007) found infection in *Phyllostomus* sp. (bats) following ingestion of *T. cruzi*-infected mice, but Roellig et al. (2009) did not detect infection using the same mechanism in raccoons. The contradictory results of these experiments may reflect the animal models used (both infected and healthy), the inoculum, and the chronic or acute condition of the animal that was ingested.
- Consumption of wild animals' blood during religious rituals or traditional medicine in some rural and jungle areas of the continent (OPS 2009; Alarcón de Noya et al. 2010a).
- Lactogenic transmission (Mazza et al. 1936). Despite confirmed experimental infection in mice and finding trypomastigotes in milk, infection through this route does not seem to be very effective (Miles 1972; Norman and López-Velez 2013).

3.3 The Oral Route of Infection and Host-Parasite Interactions

Numerous reports of large-scale, acute phase ChD outbreaks through the ingestion of contaminated food or drink (Dias et al. 2008; Beltrão et al. 2009; Nobrega et al. 2009; Pereira et al 2009; Alarcón de Noya et al. 2010b) have demonstrated the importance of the oral route of infection by *Trypanosoma cruzi* (Eickhoff et al. 2010).

With this oral transmission route, infection with metacyclic forms starts when the parasites bind to and invade the host cells. The metacyclic forms thereafter differentiate from amastigotes and become trypomastigotes after intracellular replication. These parasite forms are released into the blood circulation when the host cells breaks and thereby spread to the various organs and tissues (Zanforlin et al. 2013).

Pinto-Dias (2006) collected experimental evidence in small animals at the beginning of the twentieth century, using blood with trypomastigotes that confirmed the

oral transmission route (Nattan-Larrier 1921; Brumpt 1931; Kofoid and Donat 1933; Dias 1940; Marsden 1967). Between 1960 and 1980 an enormous wealth of valuable data was gathered by Diaz-Ungría and colleagues in Venezuela, who worked with dogs, guinea pigs, and rodents, infected orally with local *T. cruzi* strains obtained from naturally infected triatomines. In order to conduct this research they used milk or physiological saline as the vehicle to manage trypomastigotes, resulting in an incubation period that was longer than the one produced transcutaneously and required large inocula (~100,000 parasites). In other experiments that they conducted, mice were infected through the mucosa of the esophagus or by using intestinal-release gelatin capsules containing triatomine feces with infective metacyclic trypomastigotes (IMT) resulting in a much higher parasitemia. Histological studies showed signs of parasite penetration in the oral, esophageal, gastric, and intestinal mucosa, highlighting an important local reaction with eosinophilia, infiltrated lymphocytes and monocytes, and an important satellite adenopathy, with formation of mesenteritis and primary foci in liver interstitium. The parasites were subsequently dispersed around the body by the porta system (Diaz-Ungría 1968; Diaz-Ungría and Bracho 1970; Diaz-Ungría and Zeuss 1971). This group also conducted experimental infections of dogs with house flies that had been fed on feces of triatomines infected with *T. cruzi*, demonstrating the potential for flies to have a role as mechanical vectors (Diaz-Ungría 1969).

Hoft et al. (1996) carried out histopathological studies in mice (BALB/cAnNHsd), which show the anatomical route of infection after oral challenges with 10,000–50,000 parasites when examined 2 weeks post-infection, examining fine cuts of different organs without finding invasion or evidence of swelling or parasite reproduction in the oropharynx mucosa and esophagus. Four days after the oral challenge with IMT, mobilization of a mixture of inflammatory cells occurs, including polymorphonuclear and mononuclear cells. After 2 weeks, the cell composition of the inflammatory infiltrates has altered, consisting now of predominantly of mononuclear cells. At this time, the specific T and B-lymphocytes against *T. cruzi* can be detected in the gastric mucosal tissue. The inflammatory response is associated with tissue destruction, aiding parasite dissemination; disruption of the stomach serosal surface allows the release of trypomastigotes from infected cells such that they can invade adjacent structures within the peritoneal cavity. After a second oral challenge, a faster oral inflammatory response, which is associated with the parasite-specific increase in immunity, occurs. This can limit parasite replication and is able to hinder the secondary systemic spread of *T. cruzi.* The immunohistochemical detection of parasite replication in the proximal glandular stomach mucosa, and not in other areas throughout the gastrointestinal tract, provides firm confirmation of the conclusions reached by observation of sections stained with hematoxylin and eosin, that *T. cruzi* triggers infection through a highly focal invasion of gastric mucosa. Experiments comparing infected mice with controls have demonstrated that gastric infection stimulates the lymphocytes B response and the production of parasite-specific IgA and IgG, which are usually restricted to gastric mucosa lymphocyte cells (including 50–100 times more IgA and IgG secreting cells). During the first month after gastric invasion by *T. cruzi*, the parasite-specific lymphocytes'

B response is predominantly induced in the mucosal tissue, and not in the regional draining lymph nodes or spleen. This lymphocyte B antigen-specific mucosal concentration may reflect a greater parasite replication load in the mucosa, resulting in increased local antigen stimulation. However, it is believed that inductive sites for mucosal immune responses in the gastrointestinal tract are limited to Peyer's patches in the small intestine (McGhee et al. 1992). However, no evidence of replication or germinal center formation in the Peyer's patches of mice, nor parasite-specific antibodies response in ELISPOT assays with lymphocytes obtained from Peyer's patches after oral challenge with IMT, has been observed (Hoft et al. 1996).

Investigations on the molecular mechanisms of *T. cruzi* infection through the oral route confirm these findings and elucidate further the molecular interactions between the parasite and host cells. Various components of mammalian cells and parasites participate in the invasion by *T. cruzi*, inducing transduction pathway signals by activation of cytosolic Ca^{2+} elevation in both systems (Docampo and Moreno 1996; Burleigh and Andrews 1998; Yoshida 2006). Actin cytoskeleton disorganization of the Ca^{2+}-dependent host cell, followed by interaction with *T. cruzi*, enables mobilization of the lysosomes to cell peripheries, where fusion with the plasma membrane contributes to the biogenesis of the parasitophorous vacuole. Inhibition of these events prevents parasite internalization (Tardieux et al. 1992; Rodriguez et al. 1995; Martins et al. 2011; Maeda et al. 2012).

Maeda et al. (2012) described experiments that demonstrate cell signaling during invasion by *T. cruzi*. Tests were conducted with both IMT generated *in vitro* trypomastigotes and cell-culture-derived trypomastigotes (CCT) and used to simulate vector insects' environment and blood, respectively. The results showed that in these evolving forms of the parasite, different molecule sets and different strategies to induce Ca^{2+} signaling in vertebrate cells, together with lysosome exocytosis, are necessary for parasite internalization (Maeda et al. 2012). With respect to infection by IMT, the first step in this cascade of events is bonding of *T. cruzi* to the host cell, for which the parasite shows surface glycoproteins in metacyclic forms, such as GP90, GP82, GP30, and GP35/50. These molecules are differentially expressed according to the different parasite strains and their binding to the target cell is mediated by receptors that trigger the signaling pathways, that may result in efficient parasite absorption (Yoshida 2006; Maeda et al. 2012).

Prior to invasion, the parasite binds to gastric mucin. *In vivo* the GP82 molecule is associated with the infection and invasion of the mucosal epithelium by stage-specific binding (Neira et al. 2003). Gp82, identified by monoclonal antibody 3F6, is a specific-surface molecule of metacyclic trypomastigotes (MT) (Teixeira and Yoshida 1986) and is responsible for the high infectivity to host cells of different *T. cruzi* strains (Ramirez et al. 1993; Cortez et al. 2012a); it is a highly conserved molecule among parasite strains of divergent genetic groups (Maeda et al. 2011).

A synthetic peptide (p7) that resides in the center domain of GP82, corresponds to the sequence of the binding point to gastric mucin. Mice receiving oral p7, prior to MT infection, demonstrated reduced parasite reproduction in the gastric mucosa and developed low parasitemia in comparison with control mice. These results suggest

that the interaction of GP82 with gastric mucin can affect the invasion of the stomach mucosal epithelium with *T. cruzi* during oral infection (Staquicini et al. 2010).

GP82 triggers the signaling cascades of target cells, resulting in the mobilization of cytosolic Ca^{2+}, which is detectable in HeLa and Vero cells, but cannot be detected in cell lines that are resistant to *T. cruzi* K562 (Ruiz et al. 1998). In MT entering the host cells via the GP82 receptor, activation of phospholipase C (PLC) occurs. This generates diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG stimulates protein kinase C (PKC) and IP3, thereby promoting Ca^{2+} release from IP3-sensitive compartments. Phosphatidylinositol 3-kinase (PI3K) and protein tyrosine kinase (PTK) are also activated, mediating P175 phosphorylation. In the host cell, recognition of GP82 by its receptor initiates PI3K activation, the mammalian target of sirolimus or rapamycin (mTOR) and PLC, generating DAG and IP3. DAG stimulates PKC and IP3 resulting in the release of Ca^{2+} from the endoplasmic reticulum (Maeda et al. 2012). The Ca^{2+} elevation induced by GP82 promotes MT invasion by disorganizing the actin cytoskeleton and mobilizing the lysosomes which, together, culminate in exocytosis (Cortez et al. 2006; Martins et al. 2011). Experimental evidence suggests that GP82 selectively binds to gastric mucin rather than to submaxillary gland mucin (Yoshida 2009). This specificity may explain why parasites have not been found in either the oropharynx or the esophagus of orally inoculated mice (Hoft et al. 1996).

GP30 is a MT specific surface molecule that is recognized by the mAb 3F6 and expressed in GP82 deficient-strains of *T. cruzi*; it is also involved in cell invasion (Cortez et al. 2003, 2012a). GP30 binds to the target cells, inducing the Ca^{2+} and lysosomal exocytosis response, presumably through activation of signaling pathways involving PI3K, mTOR, and PKC. GP30 and GP82 seem to be recognized by the same receptor as the inhibition tests suggest (Cortez et al. 2003, 2012a).

The MT of different *T. cruzi* strains express varying levels of the different isoforms of the stage-specific surface molecule GP90, which works as a negative regulator of parasite infectivity (Málaga and Yoshida 2001). Expression of high levels of GP90 is associated with the ability of reducing entrance to the target cell (Yoshida 2006), and is thereby a negative modulator of MT invasion; furthermore, GP90 does not stimulate Ca^{2+} signals for host cell binding (Ruiz et al. 1998; Maeda et al. 2012). However, *T. cruzi* strains that express high levels of GP82, GP30 and GP90 demonstrate infective ability, and this is determined by the susceptibility of the GP90 isoform to peptide digestion. If a pepsin-resistant isoform of GP90 is expressed, it would be poorly infectious, in contrast, if a pepsin-resistant GP90 is expressed and exposed to gastric juice, a high infectivity would be expected (Yoshida 2009).

MT surface molecules GP35/50 are also found in parasite epimastigotes (Yoshida et al. 1989). They are recognized by a 10D8 monoclonal antibody, and are expressed in *T. cruzi* strains with poor infectivity. GP 35/50 are glycoproteins similar to mucin and are able to protect the parasite against the actions of gastric juices action during oral uptake by potential hosts (Yoshida 2009). They are highly glycosylated, and are rich in sialic acid and galactose residues that interact with the target cell through its carbohydrate moiety (Yoshida et al. 1989; Mortara et al. 1992; Schenkman et al. 1993). Although the binding of GP35/50 to host cells results in a rise in intracellular Ca^{2+}, this is to a lesser extent than with GP82 (Ruiz et al. 1998). Removal of sialic

acid from GP35/50 enhances the triggering of the Ca^{2+} response in the host cell and promotes MT invasion (Yoshida et al. 1997; Maeda et al. 2012). Expression of GP35/50 may be accompanied by high levels of the GP90 isoform that is resistant to peptide digestion (Covarrubias et al. 2007); this complicates the understanding of the roles of the different molecules in the *in vivo* infection (Yoshida 2009).

Components secreted by MT during the internalization of the parasite, such as proteins that are rich in serine, alanine, and proline (SAP proteins), bind to the target cell depending on the receptor and induce Ca^{2+} signals. These also participate in the MT infection mediated by GP82, but are not involved in the invasion mediated by GP35/50 (Baida et al. 2006; Maeda et al. 2012). SAP proteins are released into the extracellular medium by epimastigotes and MT as soluble factors or as components of secretory vesicles. Different variants of SAP have been found in extracellular amastigotes and in culture-derived tripomastigotes. The role of SAP during MT penetration is based on the interaction of the 54 amino acid fragment of SAP-EC with target cells and the induction of lysosomal exocytosis. The SAP proteins probably act synergistically with GP82 during cell invasion by regulating intracellular Ca^{2+} signaling (Zanforlin et al. 2013).

In addition to the molecular interactions, the various *T. cruzi* biodemes may influence oral infection. Comparison of a Peruvian biodeme strain type I and a Colombian biodeme strain type III, indicated a clear difference in infectivity when inoculated intragastrically, although both strains are highly pathogenic when animals are infected intraperitoneally. While intragastric inoculation of the Peruvian strain resulted in low parasitemia, little parasitism of macrophages, and mild to moderate tissue damage, in contrast the Colombian strain produced an intense myotropism involving the myocardium and skeletal muscle. Thus, mice infected with the Colombian strain via the intragastric route demonstrated tissue damage comparable to that observed in mice infected via intraperitoneal route, although with an evolution delay and a relatively low-level parasitemia (Camandaroba et al. 2002).

Thus it can be clearly seen that the route of infection affects the success of the parasite in the vertebrate host. Eickhoff et al. (2013) compared MT infectivity in mice with exposure through oral challenge or the cutaneous route, simulating the natural infection; *T. cruzi* was significantly more infectious by the oral route. On the basis of these results Eickhoff et al. (2013) suggested that most sylvatic transmission probably occurs when mammals ingest infected insect vectors.

As well as the exposure route, the severity of infection also depends upon the number of ingested MT, the parasitic resistance of the gastrointestinal mucosa, the regulation of invasion by local factors (gastric juices and glycoproteins), and the host's innate immune response (Shikanai-Yasuda and Carvalho 2012). Protection against infection in human disease with oral transmission has not been possible to consider because the infected population in oral outbreaks has generally been susceptible, and control populations do not show chronic disease (Shikanai-Yasuda et al. 1991; Pinto et al. 2008; Hernández et al. 2009).

Studies on animals have shown a TH2 immune response in the mucosa, as well as a TH1 systemic immune response capable of inducing protection against oral

challenge, but only the TH1 response protects against systemic infection (Hoft and Eickhoff 2002; Shikanai-Yasuda and Carvalho 2012). Due to the critical role of GP82 in parasite invasion of the epithelial mucosa, this glycoprotein is an ideal target for the mucosal immune response and has therefore been studied as a potential vaccine candidate against this type of infection. Experiments have been performed in the mouse model in which the protective mucosa is induced by immunization with GP82 prior to parasite challenge with MT. The results show that MT opsonization, when mixed with a specific antibody against GP82 prior to the mucosal challenge, significantly reduces parasite infectivity. GP82 antigen facilitates the induction of optimal protection at the gastric level, but other antigens are necessary to induce an efficient systemic protection.

The prevalence of *T. cruzi* infection in humans increases with the presence of infected dogs in human homes (Cohen and Gurtler 2001), and immunization with GP82 alone or as part of a multi-component vaccine, could be used to prevent infection in dogs and other pets, thereby reducing the risk of infection in humans (Eickhoff et al. 2010).

CCT can be used to simulate what happens with blood forms that express different molecules and use different pathways during their interactions with the host cell. Due to the action of oligopeptidase B (OPB), the parasite produces Ca^{2+} agonist that binds to its receptor and triggers the activation of PLC. IP3 then mediates Ca^{2+} release from the endoplasmic reticulum. Bradykinin, produced from kininogen by the CCT cruizipaina action, binds to the bradykinin receptor and triggers the activation of PLC. Tc85 is a CCT surface molecule, a member of the GP85/trans-sialidases superfamily with affinity to the extracellular matrix, is responsible for the host cell invasion, although our knowledge on its role in the cell signaling induction is limited (Maeda et al 2012; Yoshida 2006; Cortez et al. 2012b). Tc85 has an affinity for laminin, a high-molecular weight protein of the extracellular matrix, and this, may facilitate parasite spread through the organs and tissues (Cortez et al. 2012b).

The differences between the MT and CCT in their ability to cross the gastric mucin and in their resistance to the action of pepsin, both contribute towards explaining the low ability of trypomastigotes in blood to infect orally. MT forms and blood trypomastigotes seem to use a variety closely related molecules to interact with the various compartments that could occur in natural infection, in order to reach their target cells and to optimize survival within the host (Cortez et al. 2012b). It has been shown that both blood trypomastigotes and metacyclic trypomastigotes are transmitted orally, but their body distribution in the vertebrate, at least experimentally, varies according to whether they are metacyclic or sanguineous trypomastigotes (Dias et al. 2013).

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Chapter 4
Clinical Aspects in Foodborne Chagas Disease

Belkisyolé Alarcón de Noya and Oscar Noya González

4.1 Particular Clinical Aspects of Orally Acquired Infection

The acute phase of orally transmitted Chagas Disease (OChD) is hard to diagnose since it is difficult to associate the disease with an infection by a vector.

Several clinical symptoms can be analyzed in order to identify an infectious syndrome and its association with acute ChD transmitted by the oral route.

- *Incubation period*. Incubation periods following oral transmission are comparable to vector transmission, being 3–22 days and 4–15 days, respectively. Both of these are shorter than observed with transfusion transmission, 30–112 days (Shikanai-Yasuda and Carvalho 2012; Noya et al. 2015). In the Chacao microepidemic in Venezuela (Alarcón de Noya et al. 2010a), the estimated maximum incubation period was 11 days; a teacher with acute symptoms started working at the school on October 25th 2007, the teacher and most of the patients started to show symptoms simultaneously 11 days later.
- *Long-lasting fever* is a common symptom, occurring with 80–100 % frequency and reported in all oral outbreaks. Often the temperature is high (Pinto et al. 2008; Hernández et al. 2009; Alarcón de Noya et al. 2010a; Bastos et al. 2010; Santalla-Vargas et al. 2011; Rueda et al. 2014; Alarcón de Noya et al. 2015; Noya et al. 2015) (Table 4.1). Most oral outbreaks cases were associated with a viral process as no infectious source was identified. Fever may be accompanied by chills and sweating; in some cases these symptoms have resulted in an initial misdiagnosis of jungle fever or malaria (Martín et al. 2009).

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Table 4.1 Clinical manifestations (%) in foodborne acute Chagas disease outbreaks in five cohorts from Venezuela, Brazil, and Colombia

	Venezuela		Brazil	Colombia	
	Chacao, N = 103	Chichiriviche, N = 89	N = 181	Turbo, N = 11	Lebrija, N = 10
Fever	87.5	87.5	100	100	80
Headache	41.7	25	93.4	63.6	–
Facial edema	47.9	28.4	59.1	36.3	70
Abdominal pain	38.5	21.6	45.3	45	50
Nausea/vomiting	27.1	15.9	–	18.2	–
Palpitations	32.3	13.6	–	–	–
Dyspnea	27.1	11.3	56.9	27	30
Myalgia/arthralgia	44.8	11.3	85.6	63.6	–
Chest pain	11.5	11.3	–	–	60
Anasarca	3.1	–	13.8	9	–
Asthenia	72.9	10.2	–	45	–
Lymphadenopathies	–	7.9	11	–	–
Hyporexia	–	7.9	–	9	–
Painful nodules	1	–	15.5	–	–
Cough	24	6.8	–	–	–
Lower limb edema	24	4.5	57.5	–	30
Hepatomegaly	–	2.2	21	–	20
Splenomegaly	–	–	11	–	–
Rash	–	–	29.8	–	–
Diarrhea	15.6	2.2	6.6	–	30
Jaundice	–	–	2.8	–	20
Weight loss	–	1.1	–	–	–
Pallor	–	–	71.8	18	–
Seizures	–	7.9	11	–	–
References	Alarcón de Noya et al. (2010a, b)	IMT-UD	Pinto et al. (2008)	Ríos et al. (2011)	Hernández et al. (2009)

Table modified from Noya et al. (2015)
IMT-UD Instituto de Medicina Tropical, unpublished data

- *Edema* is also a common symptom and may be confined to the face (facial edema) as occurs in 50–70 % of cases, but also the lower limbs have been reported affected in 24–57 % cases, and generalized anasarca in 3–14 % of cases (Pinto et al. 2008; Hernández et al. 2009; Alarcón de Noya et al. 2010a; Rueda et al. 2014; Noya et al. 2015) (Table 4.1, Fig. 4.1).
- *Other general symptoms associated with fever* such as headache, malaise, weakness, arthralgia, and rash included among other concomitant symptoms. This symptom profile raises suspicions about a single process, similar to dengue, although fever is prolonged and there is no platelet involvement.
- *Digestive symptoms* including abdominal pain, gastritis, gastrointestinal bleeding, diarrhea have been noted in a smaller percentage of patients (Shaw et al. 1969).

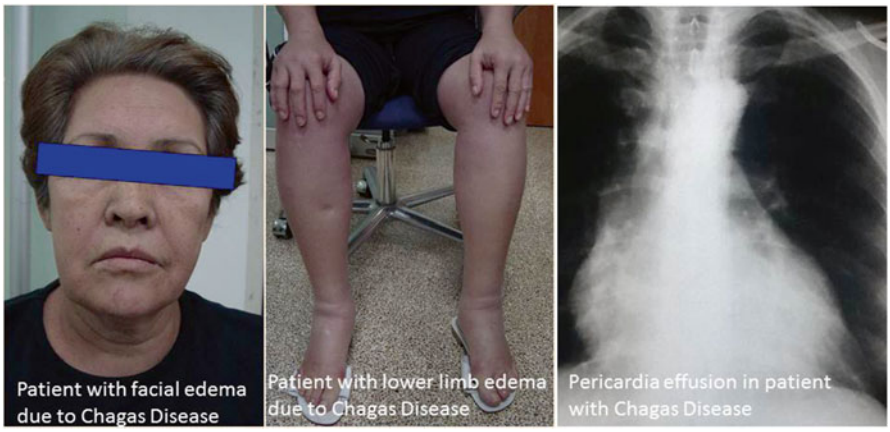

Fig. 4.1 Some clinical characteristics in patients with acute orally transmitted Chagas disease

- *Cardiac disorders* are a frequent sign, with electrocardiographic abnormalities common, and showing ST-T changes in 37.8–100 % of the cases (Marques et al. 2013; Bastos et al. 2010). With respect to known outbreaks, right branch blockage was present in 1.9 % of the patients in Venezuela (Marques et al. 2013), 8.3–25 % in Brazil (Shikanai-Yasuda et al. 1991; Bastos et al. 2010) and 40 % in Lebrija, Colombia (Hernández et al. 2009). Atrial or ventricular fibrillation occurred in 3 % in Venezuela (Marques et al. 2013), 8.3 % in Ibipitanga-Bahia, Brazil (Bastos et al. 2010;) and 20 % in Lebrija, Colombia (Hernández et al. 2009). In the outbreak of Chichiriviche, Venezuela, echocardiography was performed routinely on all patients, whether symptomatic or not (n = 89), and left ventricular hypertrophy was found in 14.6 % and pericardial effusion in 71.9 % cases (Fig. 4.1).
- *Myocarditis occurs in severe forms of disease* if acute infection is not diagnosed sufficiently early. Large pericardial effusions can compress the heart and impair its ability to pump blood. This condition, called cardiac tamponade, is potentially life-threatening and is a common cause of death in acute cases of OChD.
- *Absence of T. cruzi dermal entrance signs* ("chagoma" and "Sign of Romaña") along with lack of knowledge on the vector's existence are also indications of oral transmission. When infection occurs through contaminated food, remote infection (Alarcón de Noya et al. 2010a; Xavier et al. 2014) is difficult to associate with the symptoms from the causative *T. cruzi* agent transmitted by blood-sucking triatomine.
- *Symptom severity and mortality* varies and depends on time between the onset of symptoms, the etiologic diagnosis, and treatment delivery. In Venezuela, out of 227 cases, 198 (87 %) patients were symptomatic, of which 116 were severe - 51 % of the total number of cases (Alarcón de Noya et al. 2015). Noya et al. (2015) compiled acute cases of oral transmission in countries reported and in which mortality ranges from 0 to 13.8 % per country; however, the highest outbreak mortality occurred in Magdalena, Colombia, where 5/13 (38.5 %)

people died (Cáceres et al. 1999). Out of the three adults who died in Venezuela during OChD outbreaks, two were women, one of whom was pregnant with parasite systemic dissemination (Suárez et al. 2010) and the second women had given birth two months previously and was breastfeeding her child.

- *Occurrence of more than one case* may be more common when food is the transmission vehicle due to the likelihood of common exposures. After an acute case of ChD has been diagnosed for which oral transmission is suspected, it is crucial to start an immediate search for other cases within the patient's environment, from the family to the social environment (school, community) (Alarcón de Noya et al. 2010b). The largest outbreak described in the literature (Alarcón de Noya et al. 2010a) was discovered by work from the index case (Martín et al. 2009). In this instance the detection and identification of blood trypomastigotes was a surprise because malaria had been suspected and the blood was being screened for *Plasmodium* parasites. Clusters of oral ChD, in which several family members are affected, are frequent (Soto et al. 2014). This is due to the shared exposure route.
- *Simultaneous symptoms* frequently occur when oral infection affects a whole group of people; the incubation period has been shown to be similar in such instances, regardless of symptom intensity. Since it is very helpful to estimate the incubation period and the probable date of exposure to the parasite, it is important to specify the time of symptom onset.
- *Laboratory tests* are generally nonspecific. In the first outbreak in Venezuela, laboratory tests were only performed on hospitalized patients; results showed elevated troponin (73 %), elevated sedimentation rate (57 %), C-reactive protein (87.5 %), increased lactic dehydrogenase (89 %) and leukocytosis (42 %) (Alarcón de Noya et al. 2013). However, in the second school outbreak (Alarcón de Noya and Martínez 2009), laboratory tests were performed in most patients (43–87 out of 88 affected). Anemia (78 %), elevated troponin (50.6 %), leukocytosis (69 %) (>10,000), AST (aspartate transaminase) increase (26 %), ALT (alanine aminotransferase) (51.6 %) and creatinine (2.3 %) were observed. In Bolivia, 14 patients showed lymphocytosis (77 %), elevated transaminases (84 %) and bilirubin (Santalla-Vargas et al. 2011).
- *Differential diagnosis protocols* for conditions such as dengue, viral hepatitis, malaria, chikungunya fever, mononucleosis should also include oral infection with *T. cruzi*. This is also true for patients with prolonged fever syndrome of unknown etiology.

Figure 4.2 summarizes the events of clinical evolution.

4.2 Laboratory Diagnosis

Parasitological techniques are indicated in order to identify the presence of the causative agent, *Trypanosoma cruzi*, for patients suspected to be suffering from the acute phase of ChD. Simple methods, such as fresh blood test and microhematocrit

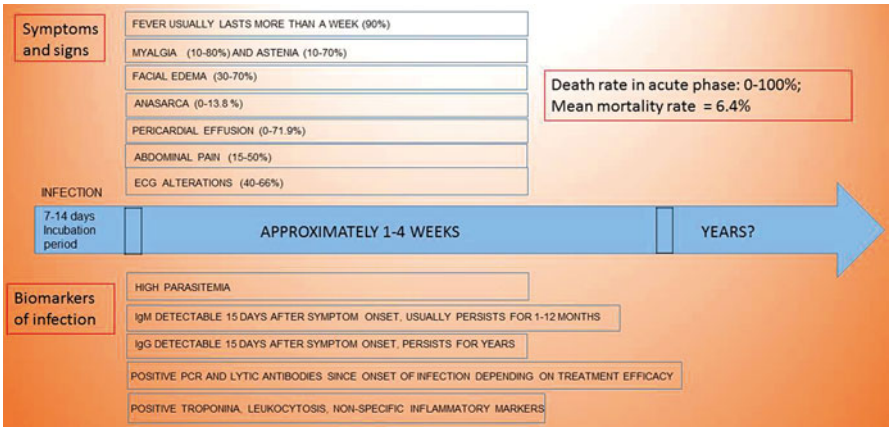

Fig. 4.2 Timeline of infection, symptoms, and biomarkers of oral Chagas disease

technique (Freilij et al. 1983) demonstrate the mobility of the parasite, enabling immediate diagnosis. However, when cases are very numerous, such as in the two Venezuelan school outbreaks (Alarcón de Noya et al. 2012; Alarcón de Noya and Martínez 2009), the approach to the population at risk should be carried out using high sensitivity serological methods, such as ELISA, for the detection of IgG and IgM. This strategy allows rapid identification of infected individuals as it is not always possible to directly confirm the parasite existence, even in symptomatic individuals. However, due to the absence of good, standardized commercial kits serological tests are not considered to be completely reliable in endemic areas for diagnosis of acute asymptomatic infection and also standard diagnostic protocols may be difficult to implement outside of large urban centers. Furthermore, serodiagnosis in infants born to seropositive mothers is of low positive predictive value because of the passive transfer of maternal anti-*T. cruzi* IgGs.

Although several methods can be applied (Ríos et al. 2011; Alarcón de Noya et al. 2012; Benítez et al. 2013), direct parasite confirmation is carried out through cultures, animal inoculation, and indirect techniques using molecular biology methods (Alarcón de Noya et al. 2012; Santalla-Vargas et al. 2011; Hernández et al. 2009). Real-time PCR was used to confirm diagnosis in the outbreak of OChD in French Guiana; in six patients PCR identification was used, but parasites had not been detected by direct microscopic examination (Blanchet et al. 2014).

Although PCR diagnosis of ChD has not been introduced routinely as a first-step diagnostic tool in most diagnostic laboratories in endemic countries, and the possibility of running such protocols is unlikely to be feasible in most primary health centers outside urban areas, comparative data with serological methods (IFAT and ELISA) and hemoculture indicate that it can be an excellent support methodology (Gilber et al 2013).

4.3 Treatment

Benznidazole and Niturtimox are the two drugs currently used for treatment of ChD. They are administered for 60 and 90 days, respectively, and are both associated with side-effects and limitations that sometimes result in suspension of treatment. Their effectiveness is higher in acute cases and children. However, a 5-year follow-up of acute cases, most of them orally transmitted, in a cohort of 179 individuals in Brazil yielded negative serology results in only 26.7 % (Pinto et al. 2013). Similarly, in the 4-year follow-up of patients from the Chacao outbreak in Venezuela who had been treated with Nifurtimox, 70 % continued to be positive to specific IgG and anti-*T. cruzi* lytic antibodies, some with positive PCR also suggesting a therapeutic failure (Alarcón de Noya et al. 2011).

Furthermore, treatment with these drugs is contraindicated during pregnancy, if the patient has severe renal or hepatic insufficiency, and in immunosuppressed patients with severe granulocitopenia and aplastic anemia. Newer medicines with better risk-benefit profiles and fewer contraindications for use are clearly necessary. Nevertheless, despite chemotherapy research, there are presently no alternative drugs to current ones. Efforts to achieve more effective drugs with fewer side-effects should be reinforced.

4.4 Concluding Comments on Diagnosis and Treatment

Timely diagnosis and effective trypanocidal treatment are known to reduce the likelihood of disease progression, and also to prevent congenital transmission. Furthermore, in order to contain Chagas disease as a public health problem, proper diagnosis, including of asymptomatic cases, appropriate case management, and effective treatment are all essential components. It should be noted that in an outbreak situation, there may be considerable pressures put upon health services, and the population that has been potentially exposed may also be highly concerned. In such situations, effective and rapid communication is also essential, as well as ensuring accurate diagnostics, follow-up, and appropriate treatment.

Despite decades of experience, the challenge of ensuring diagnosis and access to treatment for millions of infected people remains. Although resolutions such as those from the Pan American Health Organization (PAHO) and World Health Organization (WHO) on the prevention, care, and control of Chagas disease, have resulted in several countries in the Americas strengthening control activities, and these measures must be acknowledged as contributing to significant progress towards the goals of control and elimination, further initiatives, and the political will and finances to conduct them, are necessary. For example, on such activity has been the convening of a group of experts under the auspices of PAHO, in collaboration with the Drugs for Neglected Diseases initiative (DNDi), Médecins sans Frontières (MSF), and the Special Programme for Research and Training in Tropical Diseases

(TDR). The intention was to review the evidence and initiate discussions with the purpose of fostering the development of much needed tools required for point-of-care diagnosis for patients in the acute phase of infection, point-of-care diagnosis for asymptomatic or symptomatic patients in the chronic phase, and assessment of response to antiparasitic treatment in the chronic phase (Porrás et al. 2015).

There is no doubt that long-term commitment and a range of cross-disciplinary approaches are necessary for the control of ChD, including OChD. Amongst these approaches, development and implementation of more appropriate diagnostic tools and more effective treatment options are important goals.

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Chapter 5
Epidemiological Factors Related to Foodborne Transmission of Chagas Disease

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5.1 Introduction

The various different mechanisms of transmission of Chagas disease (ChD) have been demonstrated since the first description by Carlos Chagas in 1909 when he associated the human disease with dermal contamination with the intestinal contents of triatomines containing metacyclic trypomastigotes. Thereafter, other mechanisms of transmission were discovered such as the congenital route, infecting the unborn child during pregnancy, transfusions, transplantations, laboratory accidents, and the oral route (Rassi et al. 2010).

Whereas oral transmission appears to be the usual infection route between wild and domestic fauna, as fur and thick skin create a barrier for *Trypanosoma cruzi* cutaneous penetration, it has not been recognized as particularly relevant for human infections until relatively recently when publications from several outbreaks in five Latin American countries proliferated (Noya et al. 2015). This happened during a period in which international health institutions, such as WHO and PAHO, were able to demonstrate a decreasing prevalence in ChD in the different American countries (OMS/OPS/TDR 2007). In fact, this success has been achieved through regional initiatives coordinated by PAHO, as it is the case of the Southern Cone Initiative, which, by using intradomicile pesticides, achieved a transmission reduction from about 700,000 cases per year in 1990 to around 41,200 in 2006 (OMS/OPS/TDR 2007; Senior 2007). However, in contrast to the decreased transmission shown in

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these figures, an increase in acute cases of orally acquired infection has been observed in the last two decades (Pinto et al. 2008; Shikanai-Yasuda and Carvalho 2012). This pattern can be particularly seen in Venezuela, from where only 11 acute cases of ChD acquired by skin transmission have been published during the last 15 years (Añez et al. 2007; Morocoima et al. 2008). Furthermore, among these 11 cases, four were members of the same family, and actually possibly infected by oral route (Añez et al. 2007; Alarcón de Noya et al. 2015). On the contrary, there have been 10 oral outbreaks during the same period when 249 people have been infected (Alarcón de Noya et al. 2015; Noya et al. 2015). At the same time, over 500 acute cases have been reported in Brazil, acquired through the same infection route (Pinto et al. 2008; Noya et al. 2015), since Nery-Guimarães et al. reported the first outbreak in 1968.

5.2 How Have Epidemiologic Factors Influenced the Resurgence of Oral Transmission?

5.2.1 Human Migration and Urban Developments

All around the South American continent, massive migration of people from rural areas to the cities has probably been one of the most influential factors in the changing epidemiology of this disease. It is worth noting the great demographic transformation of the American continent, in which the rural population decreased from 70.5 to 47 % in 1950, and in 2013 reduced further to 15 and 11.2 % in Brazil and Venezuela, respectively. In Argentina, the rural population accounts for only 10.5 % of the population, while in Latin America as a whole, only 21 % of the population remains in rural areas (Briceño-León 2009; World Bank 2013). While this fact probably helped to decrease the figures of incidence of ChD in rural areas, on the other hand people settled in the outskirts of cities, building precarious and unprotected houses.

Furthermore, urban development (middle or low class housing) led to deforestation and as the natural food sources of triatomines, such as wild mammals and birds, were depleted from their natural habitats, the consequent domiciliation of triatomines occurred (Coura et al. 2014).

5.2.2 Domiciliation of Triatomines

Progressive invasion and domestication of wild triatomines have occurred in rural and urban localities. The species *Triatoma dimidiata*, *Panstrongylus rufotuberculatus*, *Rhodnius stali*, *Eratyrus mucronatus* and *Panstrongylus geniculatus* have all been reported as being domiciled in urban areas (Schofield et al. 1999; Reyes-Lugo 2009). The efficient skin transmission vectors (*Rhodnius prolixus* and *Triatoma infestans*), which were domiciled in homes in rural areas, were affected by domiciliary spraying (Reyes-Lugo 2009) so our attention should now be directed towards

these secondary vectors that have a delayed defecation reflex but have become permanent residents of the urban houses (Reyes-Lugo 2009; Coura et al. 2014; Noya et al. 2015).

The case of *Panstrongylus geniculatus*, which is also the most widely distributed species in America, is illustrative of this epidemiological pattern (Reyes-Lugo 2009). Alterations in ecology have conditioned this species to establish and reproduce in homes, allowing food contamination, either directly by nymphs or adult triatomines through stool/urine deposition or by disintegration of the entire insect during blending of particular fruit juices, such as guava, mango, açai, orange, and palm fruits ("açai", "comou", etc.) in which the insect has become entrapped. Due to its delayed defecation reflex, this triatomine is not suited to being a skin-transmission vector, however, its wide geographic distribution and high prevalence of infection with *T. cruzi*, distinguishes it as the species most responsible for oral outbreaks in Colombia and Venezuela (Noya et al. 2015; Alarcón de Noya and Noya-González 2015). The explanatory elements of the new role of this species are the result of human-caused factors; these include the continuous and uncontrolled deforestation, this being the major risk for domiciliation of triatomines in the Amazon Region (Coura and Junqueira 2012); unprotected housing with no mosquito nets and unplastered walls in poor neighborhoods in city peripheries, providing triatomines with crevices between the bricks of the walls for protection; and attraction of triatomines to houses by increased use of artificial light at night in both domiciliary and street areas. Thus, the biological cycle has moved away from the wild environment to the peridomicile and domicile environment (Guhl 2009).

Although *Rhodnius prolixus* and *Triatoma infestans* vectors are domiciled in rural areas, and *Panstrongylus geniculatus* in urban areas, due to its wide geographical distribution and rapid domestication, the most important vector is *P. geniculatus* (Schofield et al. 1999; Reyes-Lugo and Rodríguez-Acosta 2000).

In cities such as Caracas, the high prevalence of infection of *P. geniculatus* with *T. cruzi* (Carrasco et al. 2005; Díaz-Bello et al. 2011), the wide distribution of this vector in neighborhoods, and its presence in homes during its immature stages (five nymph stages) are factors that lead to the direct contamination of food and beverages. Numerous anecdotal reports have been received from people who have seen "little kissing bugs" inside blenders. This appliance acts as a trap for *P. geniculatus* nymphal stages; they are unable to leave once they fall inside as they do not have wings to fly and they slide down by the smooth inner walls of blenders. Furthermore, in many cases their small size (first nymphal instar is around 2 mm) means that they may not be noticed by those who prepare food, particularly home-made juices, that may be consumed at home or obtained from street-vendors, and public meal centers (school canteens, nursing homes, military institutions, companies, etc.).

Another possibility for triatomines ending up in juices is that adult triatomines are attracted by the smell of boiled fruits and fall into containers left uncovered for cooling during the night. This explanation was also provided by cooks involved in the two largest oral outbreaks reported so far, in public schools in Chacao and Chichiriviche de la Costa town in Venezuela. In both cases, handcrafted guava juice, previously boiled and prepared the day before were the vehicles of infection. In these cases the implicated fruit juices were exposed in kitchens to where triatomines

had easy access from the surrounding areas (Alarcón de Noya et al. 2010; Alarcón de Noya and Martínez 2009).

Handcrafted fruit juices that are sold in cities, on the streets, in public canteens, and in other situations where sanitary supervision is lacking are at risk of being contaminated with triatomins feces, or with the entire insect. Fig. 5.1 illustrates some of the scenarios where there is a high contamination risk.

5.2.3 Reservoirs

During the *T. cruzi* lifecycle, a rich fauna of mammals may act as reservoirs (marsupials, monkeys, bats, horses, dogs, cats, rats, and other rodents, etc.). While many parasites are relatively host-specific, *T. cruzi* has a broader range of non-specific hosts, since it is capable of infecting a variety of mammals; indeed, a total of 180 species of 25 families of infected mammals has already been described as potential hosts for *T. cruzi* (Guhl 2009; Bern et al. 2011). Herrera (2010) highlights the important role of domestics and/or synanthropic animals in the biological cycle established near man when the original enzootic areas are affected by various human interventions.

The wide variety of species of host mammals, along with the various different species of triatomines with a range of habits, allows the geographic dispersal of the genetic diversity of *T. cruzi*. However, when the cycle occurs in cities (zoonotic urban focus), the variety of vector species is reduced, along with the range of species of potential host mammals (humans, rats, dogs and cats), consequently resulting in the concentration of a "pool" of various genotypes in a single host. This thereby neutralizes the "dilutor effect" of genetic diversity observed in wild environments (Keesing et al. 2010; Schmidt and Ostfeld 2001).

The rat, *Rattus rattus*, deserves special attention as three basic conditions have enabled it to become very efficient urban reservoir for *T. cruzi*. Firstly, rats are very susceptible to *T. cruzi* infection, which is maintained for a long period in them; 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* (Díaz-Bello et al. 2011; Herrera 2010; Urdaneta-Morales 2014).

5.2.4 Parasitic Load

Unlike with skin transmission, in which it has been determined that about 3000–4000 metacyclic tripomastigotes/μL are present in the fecal inoculum, of which only a portion of them succeed in penetrating the epidermis, an infesting *Triatoma infestans* can contain in its gut about 684,000 metacyclic trypomastigotes (Shaub 1989), and this number is able to infect, by the oral route, literally hundreds of people. Both blood and metacyclic trypomastigotes infect orally, but they differ in their histotropism. Recent studies show that the oral route is much more efficient

Fig. 5.1 Situations with risk factors associated with oral transmission of Chagas disease by the artisanal production of fruit juices. (**a**) Street vendor of sugar cane in downtown Caracas, Venezuela (BAN). (**b**) Street sales of juices in Mexico (ONG). (**c**) The kitchen where the juices in the Chacao outbreak were prepared (note the *parrot* in the *window* and an *aperture* in the *upper wall*) (ONG). (**d**) Fruit vendors at an open-air market. Juice sales in the vicinity (from the *red cool-box* on the *right hand side* of the picture) (ONG). (**e**) School kitchen with open access to the outside environment (ONG). (**f**) Roadside sales of "Manaca" and sugar cane juice sale on the south route in Venezuela (ONA). (Photos BAN: B. Alarcón de Noya, ONG: Oscar Noya-González, ONA: Oscar Noya-Alarcón)

than the skin route of transmission; the parasite load is larger and thereby the clinical severity (Dias et al. 2013). In addition, trypomastigotes can live between 6 and 72 h in handcrafted juices, depending on the type of fruit used (Añez et al. 2009).

5.2.5 *Factors Other Than the Components of the Lifecycle of* Trypanosoma cruzi

5.2.5.1 Vegetation

Decreasing vegetation due to deforestation causes serious environmental imbalance, and has also been one of the initial determining factors for the transient invasion of houses by triatomines and the subsequent domiciliation of these vectors (Reyes-Lugo 2009). An additional consequence to the domiciliation risk, is that since the newer neighborhoods are settled at the city limits, bordering on woodlands, there is an additional risk of man-triatomine contact, enabling recurrent invasions of non-domiciled adult triatomines. This risk factor is known as the "Edge Phenomenon" (Fagan et al. 1999), and it is of interest to consider the urban outbreaks in Caracas as shown in Fig. 5.2, where oral outbreaks occurred in areas adjacent to surrounding forests.

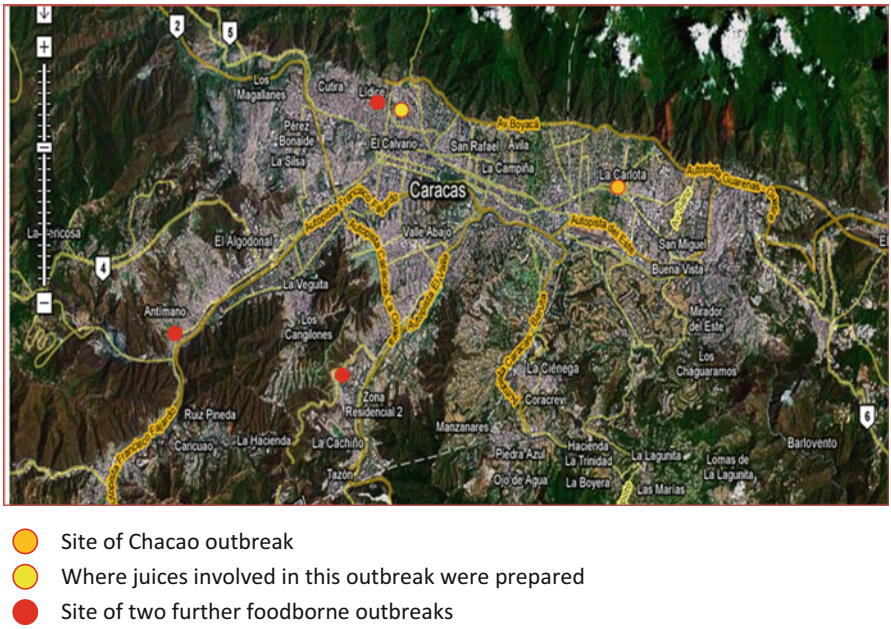

Fig. 5.2 Map of the city of Caracas-Venezuela demonstrating the occurrence of oral outbreaks and contamination of food near forested areas, emphasizing the role of the "Border phenomenon" in the transmission of Chagas disease

Investigation of factors associated with transient house infestation by *Triatoma dimidiata* in rural villages in Yucatan, Mexico indicated that the presence of dogs, chickens and potential refuges, such as rock piles, in the peridomicile as well as the proximity of houses to vegetation at the periphery of the village were of key importance (Dumonteil et al 2013).

However, in the Chacao school outbreak, the incriminated fruit drink was made at a site ("Initial Focus" or "Founder Focus"), which is located 6 km away from the school where the juice was consumed. This series of events can also be observed at other geographic locations, such as Belem do Pará, which is a city that has received fruits, food and "açai" drinks contaminated with infected triatomines that inhabit the palm bunches harvested in the Amazon River islands. This food contamination site is distant from the site of food consumption and is therefore known as "*Distantiae* transmission" (Xavier et al. 2014). A similar scenario may be envisioned in the cases of street-vendor juices and other foods, where the epidemiologic investigation to determine the source of infection, and thus locate the "initial focus", may be difficult to establish.

5.2.5.2 Climate

The larger outbreaks of orally transmitted acute cases happened during the dry months from March to May, which correspond to the end of the dry season (Noya et al. 2015; Feliciangeli et al. 2004; Shikanai-Yasuda et al. 1991). This temporal affect may be in part due to the presence of triatomine bugs being associated with high temperatures and low humidity, as has previously been reported from other studies (Carcavallo 1999; Lorenzo and Lazzari 1999; Dumonteil et al. 2002).

5.2.5.3 Brightness

Human population growth is accompanied by an increasing use of light in both houses and streetlights, and these are especially attractive for *Panstrongylus geniculatus* adult fliers (Reyes-Lugo 2009). Proximity of houses to public light sources was also found to be major risk factors for transient house infestation with *T. dimidiata* in rural villages in Yucatan, Mexico (Dumonteil et al 2013).

5.2.5.4 Housing

The houses most likely to be infested, either transiently or permanently, by triatomines are mostly inhabited by marginalized populations located in slums ("favelas," "villas miseria," "ranchos," etc.), in which most of the walls are not plastered, they have no mosquito nets, and there are numerous crevices in which vectors can enter, hide, and multiply (Noya et al. 2015). An enzootic focus can then be established in animals at home (dogs, cats, poultry, and rodents). Less frequently, other animal

species such as opossums, armadillos, and squirrels can also approach houses located in forest area borders and serve as additional reservoirs.

5.3 New Challenges

The acute phase of orally transmitted ChD is a difficult entity to diagnose clinically and some parasitological and epidemiological features determine the course of infection, modifying a largely vectorborne disease into a foodborne disease. Table 5.1 summarizes the differences between oral and cutaneous transmission in order to clarify the various factors that contribute to the success of the oral transmission.

Trypanosoma cruzi transmission, either oral or cutaneously, far from being controlled, is persisting, and even increasing. Climate changes are forecast to increase the presence of triatomines in new settlements (Garza et al. 2014), and results

Table 5.1 Epidemiological differences between skin transmission and oral transmission of Chagas disease

Oral (foodborne) transmission	Cutaneous transmission
Usually seen in outbreaks	Usually isolated cases
Transmission by any triatomine species	Transmission only by triatomines with fast reflex defecation
Metacyclic and sanguinous trypomastigotes are likely infectious	Only metacyclic trypomastigotes seem to infect
Parasitic load can be very high (entire infected triatomine gut)	Parasitic load limited by a defecation content
Distant contamination source "*Distantiae* transmission" is possible	Local transmission obligatory
Larger geographical population area may be affected	Geographic area restricted to the range of the vector species
Infection source: triatomines and *D. marsupialis* (contamination of food by excretions from reservoir host)	Triatomine vectors (or other biting vectors) only transmission source
Occurs in both rural and urban areas	More common and expected in rural areas
House type not necessarily associated	Commonly associated with adobe houses with thatched roof houses
Usually single infection	Reinfection is common
No records of cutaneous entrance signs	Frequently confirmed entrance cutaneous signs (Romaña sign and "Chagoma")
People of any social status may be exposed	Usually affects poor people in rural areas who are more likely to be exposed
Patients often deny having seen triatomines	Usually people admit to seeing or being bitten by triatomines
The most common infection route in animal species other than humans	Apparently a more frequent mechanism of human infection

predict a potential northern shift in the distribution of *Triatoma gerstaeckeri* and a northern and southern distributional shift of *Triatoma sanguisuga* from its current range due to climate change in the United States.

The potential role of other vectors susceptible to infection by *T. cruzi* is a further concern, as recent publications have shown the vectorial capacity of bedbugs, *Cimex lecturalensis*, an insect with worldwide distribution, that have been shown to be able to infect experimental animals either through the skin, but also by oral route (Salazar et al. 2015). This important finding could mean that *T. cruzi* can be transmitted through this biological vector in any of the continents outside the traditionally endemic area.

Global warming resulting in the spread of vectors, the emergence of new vectors, the migration of individuals infected with *T. cruzi* to non-endemic areas, and globalization of the food-chain may be all factors that ensure the continuation of oral transmission, both in endemic areas, but also in other regions beyond the traditionally endemic areas.

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Chapter 6
Documented Outbreaks of Foodborne Chagas Disease

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6.1 Introduction to Documentation of Foodborne Outbreaks of Chagas Disease

The first publication of an outbreak of orally transmitted Chagas Disease (OChD) in humans dates back to 1967 and occurred in Brazil (Nery-Guimarães et al. 1968). Since then successive outbreaks have occurred, and seem to be increasing year after year, not only in Brazil (Moncayo & Silveira 2009), but also in other countries of Latin America (Alarcón de Noya et al. 2015). The majority of outbreaks have occurred in the Amazon basin and in the Colombian-Venezuelan cordillera axis (Fig. 6.1).

Despite considerable efforts being expended in trying to complete an inventory of all oral outbreaks and the actual number of associated cases (Pinto et al. 2008; Valente 2011; Shikanai-Yasuda and Carvalho 2012; Rueda et al. 2014; Alarcón de Noya et al. 2015), compilation of this information is no easy task. This is due to several factors, including:

- Under registration of cases due to diagnostic confusion with other diseases such as dengue, mononucleosis, Chikungunya fever and malaria).
- Sub-optimal access to information, much of which is not published in scientific papers but occurs only in local epidemiological records, graduate theses, internal reports, etc.
- Duplication of information that appears both in reports from the Ministry of Health and also in papers by the researchers studying the outbreak.
- Lack of consolidation of clinical, epidemiological, and field research into a single publication, but divided into several reports with a lack of cross-referencing.

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Fig. 6.1 Geographical distribution of oral transmitted Chagas disease outbreaks in America, indicating eco-region, vectors and reservoirs involved, alimentary vehicle, and *Trypanosoma cruzi* DTU

Due to these difficulties, the data regarding morbidity and mortality, presence of vectors, prevalence of infection at the site of outbreaks, parasite discrete typing units (DTUs) responsible for each outbreak, and the occurrence of reservoir hosts often do not accompany the registration of outbreaks, diminishing the value of the available information.

By relying largely on published or accessible literature, the current compilation provided here is acknowledged to be incomplete and suffer from discrepancies. Nevertheless, we present data on documented outbreaks of foodborne infection with *Trypanosoma cruzi* until the middle of 2015. The record of outbreaks and cases of OChD presented here are given in relation to known ecoregions of America, according to World Wide Fund for Nature (WWF 2015), considering that there may

be some biotic and abiotic factors that might affect the predominance of cases, vectors, reservoirs, DTUs, etc.

The World Wide Fund for Nature (WWF) defines an ecoregion as "a large area of land or water with distinctive geographical features sharing common aspects regarding the vast majority of their species and ecological dynamics, environmental conditions and interactions between these factors" (WWF 2015). The boundaries of any given ecoregion are not fixed and well defined, but cover an area in which significant environmental and evolutionary processes interact more strongly. There are more than 800 terrestrial ecoregions grouped in the so-called "super" ecoregions and in the Neotropical South American Region where OChD cases have occurred as covering five large areas: Tropical and Subtropical Moist Broadleaf Forests; Tropical and Subtropical Dry Broadleaf Forests; Tropical and Subtropical Grasslands, Savannas and Shrublands; Montane Grasslands and Shrublands; and Mangroves (Tables 6.1 and 6.2, and Fig. 6.1).

6.2 Documented Outbreaks in Different Ecoregions

6.2.1 Tropical and Sub-tropical Moist Broadleaf Forests Area

6.2.1.1 Northern Andean Montane Forests

Eleven microepidemics of OChD have been described from this ecoregion that includes regions of both Venezuela and Colombia, between 1992 and 2014.

Venezuela: Three of these microepidemics occurred in Venezuela. One in El Bordo (Mérida) in 2012 (Añéz et al. 2013), another in Rubio in 2010 (Benítez et al. 2013) and the third in San Cristóbal (2014) (IMT-unpublished). In none of these three episodes, in which a total of 17 people were infected with two deaths, it was possible to determine the contaminated food, and the foodborne transmission route was ascertained by epidemiological approaches. In Rubio (Táchira), the reduviid species *Panstrongylus geniculatus* were captured at the place where the food was presumed to have been contaminated, but no descriptions of potential reservoirs were made Benítez et al. (2013). At the outbreak of Mérida, adult specimens of *P. geniculatus* (infected with *T. cruzi*), *Triatoma maculata*, *Rhodnius prolixus* and *Eratyrus mucronatus* were found in the home and potential domicile and peridomicile reservoirs were also identified: dogs, *Didelphis albiventris* (white-eared opossum) and rodents of the genera *Mus* and *Rattus* (Añéz et al. 2013). We have no information on the DTUs associated with the outbreaks in the Venezuelan Northern Andean Montane Forests (Table 6.1).

In addition, two oral family outbreaks, one in Miranda state and a second one in Merida state have recently occurred (Table 6.1). The first involved three women, one of them pregnant and whose fetus died. Trypomastigotes were seen in the amniotic fluid. The second outbreak involved 12 people from the same family group resulted in three deaths, possibly associated with delayed diagnosis and treatment. In this case poor hygiene conditions for family food handling and disposal were

Table 6.1 Locations of outbreaks of foodborne Chagas disease in relation to American ecoregions of Tropical and Subtropical Moist Broadleaf Forests

Ecoregions where oral Chagas disease has been described		Description of oral Chagas disease					
Area	Terrestrial ecoregions	Country	State or department	Community	Date	Mortality/ affected	Reference
Tropical and Subtropical Moist Broadleaf Forests	Northern Andean Montane Forests	Venezuela	Mérida	El Bordo	2012	1/5	Añéz et al. (2013)
				Febres Cordero	2015	3/12	ProMED mail (2015)
			Táchira	Rubio	2010	1/7	Benítez et al. (2013)
				San Cristóbal	2014	0/5	IMT-UP
		Colombia	Cesar	Aguachica	2010	1/12	ProMED mail (2010a)
			Antioquia	Turbo	2010	1/11	Ríos et al. (2011)
			Norte de Santander	Tibú	1992	NM/6	Bohórquez et al. (1992); Nicholls, (2006)
			Santander	Bucaramanga	2003	3/3	Referred in Rueda et al. (2014)
				Lebrija	2008	2/10	Hernández et al. (2009)
				Bucaramanga	2009	NM/5	Zambrano et al. (2010)
				Piedracuesta-Girón	2009	NM/5	Referred in Rueda et al. (2014)
				San Vicente del Chucurí	2010	0/3	Referred in Rueda et al. (2014)
	Coastal Venezuela Montane Forests	Venezuela	Caracas	Chacao	2007	1/103	Alarcón de Noya et al. (2010)
				San José	2008	0/3	IMT-UP
				Antímano	2010	1/21	ProMED mail (2010b)
				Coche	2012	0/4	ProMED mail (2012)
			Vargas	Chichiriviche	2009	5/88	Alarcón de Noya and Martínez (2009)
			Miranda	El Guapo	2014	0/3	IMT-UP
			Falcón	Mirimire	2013	1/8	IMT-UP
			Miranda	Guarenas	2015	1/4	IMT-UP
	Río Negro-Juruá Moist Forests	Brazil	Amazonas	Santa Izabel do Rio Negro	2010	0/21	Souza-Lima et al. (2013); FVS-AM (2011)

	Southwestern Amazonian Moist Forests	Brazil	Acre	Plácido de Castro	1988	0/1	Barata et al. (1988)
			Amazonas	Tefé	2004	0/9	Medeiros et al. (2008)
				Coarí	2007	0/25	FVS-AM (2011); SVS (2007)
				Carauari	2015	0/12	O Globo (2015)
			Pará	Belém (Canudos)	1968	1/4	Shaw et al. (1969)
				Icoarari	1992	NM/4	Crescente et al. (1992)
				Afuá	1992	NM/5	Valente et al. (1993)
				Abaetetuba	1998	0/13	Pinto et al. (2001a); Valente et al. (2001a)
				Pau D'Arco	1999	0/10	Valente et al. (2001b)
				Belém	2000	0/11	Pinto et al. (2001b)
				Santarém	2006	1/17	SVS (2006)
				Belém	2006	0/4	Pinto et al. (2011)
				Barcarena	2006	0/11	Nóbrega et al. (2009)
				Cachoeria de Arari	12	0/12	Valente (2011)
				Breves e Bagre	2007	0/25	Beltrão et al. (2009)
				Belém	2014	NM/10	ProMED Mail (2014a)
			Amapá	Macapá	1984	0/8	Rodrigues et al. (1988)
				Mazagão	1996	0/17	Valente et al. (2009)
				Santana	2004	0/27	Valente 2011
			Tocantins	Axixá do Tocantins	2009	0/4	Oliveira et al. (2011)
		Bolívia	Beni	Guayayamerín	2010	0/14	Santalla-Vargas et al. (2011)
	Atlantic Forests	Brazil	Santa Catarina	Navegantes	2005	5/24	Tatto et al. (2007)
			Rio Grande do Sul	Teutônia	1968	6/17	Nery-Guimarães et al. (1968)

IMT-UP Instituto de Medicina Tropical, *UCV* unpublished data, *NM* not mentioned, *FVS-AM* Fundação de Vigilância em Saúde do Amazonas, *SVS* Secretaria de Vigilância em Saúde

Table 6.2 Distribution of oral Chagas disease cases according to terrestrial ecoregions other than Tropical and Subtropical Moist Broadleaf Forests in America

Ecoregions where oral Chagas disease has been described		Description of oral Chagas disease					
Area	Terrestrial ecoregions	Country	State or department	Community	Date	Mortality/ affected	Reference
Tropical and Subtropical Dry Broadleaf Forests	Atlantic Dry Forests	Brazil	Maranhão	Tutóia	2006	0/2	Pinto et al. (2008)
			Ceará	Redenção	2006	0/8	Cavalcanti et al. (2009)
			Paraiba	Catolé da Rocha	1986	1/26	Shikanai-Yasuda (1987)
			Bahia	Riacho de Santana	1986	0/20	Maguire et al. (1986)
				Macaúbas	2006	2/7	Dias et al. (2008)
				Ibipitanga	2006	0/6	Bastos et al. (2010)
Tropical and Subtropical Grasslands, Savannas and Shrublands	Llanos Savannas	Colombia	Casanare	Paz de Ariporo	2014	1/31	ProMED Mail (2014b)
Montane Grasslands and Shrublands	Northern Andean Paramo	Colombia	Magdalena	Guamal	1999	5/13	Cáceres et al. (1999)
			Bolívar	Villanueva	2009	2/2	Referenced in Rueda et al. (2014)
Mangroves	Guianan-Amazon Mangroves	French Guiana	Cayenne	Iracoubo	2005	0/8	Blanchet et al. (2014)

reported as possible epidemiological factors associated with transmission in this outbreak (ProMED 2015).

Colombia: From the Andean region of Colombia at least seven outbreaks of OChD have been reported and one further poorly documented outbreak is also suspected to have occurred (Table 6.1); further details of these outbreaks are provided in chronological order below.

- In the community of Aguachica, Department of Cesar, 12 cases of acute ChD were reported with 1 death (8.3 %) in June 2010. This episode occurred among miners who consumed food that had been prepared in unhygienic conditions (ProMED Mail 2010a). *Rhodnius pallescens* was apparently involved in this outbreak, but no reservoirs were reported.
- In Turbo in the Department of Antioquia, a microepidemic of ChD with apparent oral transmission was described to have occurred in 2010 (Ríos et al. 2011). Eleven people were infected and the fatality rate was 9.1 % (1/11). The most likely source of infection was a shared meal in the same house. Although a few triatomines (*P. geniculatus*) were found and potential reservoir hots (the marsupial, *Caluromys lanatus*, the brown-eared woolly opossum) none were infected. All patients had fever and systemic symptoms
- In the town of Tibu in the Department of Norte de Santander, one microepidemic was reported in 1992. Although oral transmission could not be proven definitively, six soldiers presented simultaneously with acute chagasic myocarditis while another 24 of 144 had positive serology for *T. cruzi* with electrocardiographic abnormalities in more than half of them. The probable source of infection, mortality, vector and reservoir were reported (Bohórquez et al. 1992; Nicholls 2006).
- A small cluster of three cases of foodborne Chagas disease were reported from Bucaramanga in the Centro Nacional de Enlaces in 2003 (Rueda et al. 2014). All cases were fatal, but the source of infection and the vector were not reported.
- In Lebrija, in the Department of Santander, one microepidemic of OChD was documented in 2008. Ten people were affected, of whom two died of acute myocarditis. Two uninfected *Panstrongylus* triatomines were captured. The authors postulate the possibility of orange juice contaminated with marsupial droppings as being a possible source of infection (Hernández et al. 2009).
- A second cluster of cases of acute OChD was reported from Bucaramanga in the Centro Nacional de Enlaces in 2009. Five people from the same family were affected and one child died with severe myocarditis. The investigators captured two genera of triatomines, *Rhodnius* and *Panstrongylus*, which were positive for *T. cruzi*. Identification of the source of infection was not possible (Zambrano et al. 2010).
- Also in 2009, five cases of acute OChD were reported in Piedracuesta-Girón. Nevertheless, whether transmission was oral or vectorborne could not be definitively determined (Rueda et al. 2014).

- Another outbreak in 2010 occurred in San Vicente de Chucurí where three patients were diagnosed with acute OchD; again information on the source of infection, mortality, and vector involved were not reported (see Rueda et al. 2014).

Ramírez et al. (2013) evaluated eight isolates of *T. cruzi* from human OChD outbreaks in Tibu (Norte de Santander), Lebrija, Bucaramenga and San Vicente del Chucurí. Their data suggest that oral outbreaks from Colombia were due to wild genotypes of *T. cruzi* (TcIa and TcId).

6.2.1.2 Coastal Venezuela Montane Forests

At least seven outbreaks have been reported from this area (Caracas, Vargas, Miranda and Falcón). The predominant DTU for all the outbreaks from this ecoregion has been TcI (Muñoz-Calderón et al. 2013). These are summarised in chronological order in the list below.

- An outbreak of foodborne Chagas disease in Chacao in 2007 represented a significant challenge for health personnel as 1000 persons were exposed to the contaminated vehicle of infection at a school in Caracas. In this case, the children and staff were infected by guava juice prepared in association with a school meal (Alarcón de Noya et al. 2010); 103 people were infected, 75 % were symptomatic, 20.3 % required hospitalization, 59 % showed ECG abnormalities, and one child died. *Panstrongylus geniculatus* and rats were responsible for maintaining the cycle of *T. cruzi* in the founder focus. Rapid diagnosis and treatment (Alarcón de Noya et al. 2008; Alarcón de Noya et al. 2012) of this medical and epidemiological emergency prevented the morbidity being higher, and the mortality staying below 1 %; considering that this was the largest outbreak of OChD reported to date (Miles 2010). In 2008 in San Jose, north of Caracas, three people from the same family suffered simultaneously from acute OChD (Alarcón de Noya et al. 2015). *Panstrongylus* and rats were present around the house.
- In April 2009, another significant microepidemic of OChD was described in Chichiriviche School of Vargas State in Venezuela (Alarcón de Noya & Martínez 2009). In this outbreak, 89 were infected and five persons died, including a pregnant woman (Suárez et al. 2010). *P. geniculatus* were present all around the mountains of this touristic town where small mammals live.
- An outbreak of OChD occurred in Antímano (ProMED Mail 2010b), with 22 people reported infected and the death of one child. The common presumable source of infection was passion fruit juice (Alarcón de Noya et al. 2015). A landslide just after the outbreak prevented the epidemiological search for triatomines and reservoirs.
- In Coche (Caracas) in 2012, four workers in a food market were diagnosed with foodborne Chagas disease. The source of infection was not identified and no triatomines were captured, however rats were found to be infected with *T. cruzi* (ProMED-Mail 2012).

- In Miremire (Estado Falcón) a group of eight people acquired *OChD* in 2013. The postulated likely source of infection was mango juice that had been contaminated via *P. geniculatus*, some of which were subsequently captured in the house where the juice was prepared (Alarcón de Noya et al. 2015). One patient died during the first week of treatment.
- In 2014 (Alarcón de Noya et al. 2015) another cluster of three persons with OChD occurred in Miranda State, in the community of El Guapo. This time the source of infection was apparently, rose apple juice.

6.2.1.3 Rio Negro-Juruá Moist Forests

In this ecoregion, the Fundação de Saúde em Vigilância state of Amazonas (2011) reported a microepidemic of OChD that occurred in 2010 in Santa Izabel do Rio Negro (Amazonas State) with 21 cases without any deaths (14 cases diagnosed in the town and seven in Barcelos). Souza-Lima et al. (2013) also reported 17 such cases. As other outbreaks in the Amazon region the presume vehicle of infection was açai. However, for these outbreaks we lack information regarding vectors, reservoirs, and DTUs.

6.2.1.4 South-Western Amazonian Moist Forests

In this ecoregion, outbreaks of OChD have been reported in Brazil, Bolivia, and in the Amazon regions of Ecuador and Peru (Table 6.1 and Fig. 6.1).

Brazil
Acre State:

- Barata et al. (1988) made the first description of an autochthonous case of acute OChD in Colônia Plácido de Castro Acre in an infant (1 year and 7 months old) with fever, anemia and anasarca. During the epidemiological investigation, although no domiciliary vectors were found but *Rhodnius robustus* positive for *T. cruzi* was identified in peridomestic palm trees (Barata et al. 1988). After excluding other mechanism of infection, the possibility of oral transmission was suggested.

Amapá State:

- Valente et al. (2009) described an OChD microepidemic from 1996 in which 26 people were affected. *Rhodnius pictipes* and *P. geniculatus* were captured and found to be infected with *T. cruzi*. *Didelphis* sp. (large American opossums) and *Marmosa* sp. (mouse opossums) were suggested as being reservoirs. TcI and TcZ3 DTUs were identified in both in humans and vectors.
- Rodrigues et al. (1988) reported acute OChD in eight patients from two families residing in different neighborhoods in Macapa. Neither vectors nor animal reservoirs were found, but the possibility of contamination of food with urine from

Didelphis sp. was nevertheless suggested. From the same locality, Valente et al. (2003) reported ten cases of probable OchD. Triatomines tested were negative for *T. cruzi*. All persons had consumed açai that had been purchased from a dealer in the street of residence. It is postulated that vectors may have been brought in from other regions and crushed when preparing açai juice.

- In Igarapé da Fortaleza (Santana) 27 cases of OChD were reported from eight different families in 2004, but with no mortality (Valente 2011). The common feature of the patients was the consumption of açai from a common source (Valente 2011).

Amazonas State:

At least four outbreaks of OChD have been reported from Amazonas state since 2004.

- In Tefé (2004) nine persons were infected subsequent to ingestion of açai juice. One of those infected presented with an acute meningoencephalitis (Fundação de Vigilância em Saúde 2011, Medeiros et al. 2008). There is no other information about this microepidemic.
- Coarí. The Fundação de Vigilância em Saúde (FVS) do Amazonas (2011) provided brief official information about the Coarí outbreak in 2007 in which 29 people were infected.
- Carauari. In January 2015, the newspaper O Globo and the local press reported 12 cases of acute lethality OChD, apparently associated with açai, in the Municipality of Carauari on the left bank of Rio Jurua. There is no information on other factors involved in the transmission chain (vectors, reservoirs) or DTU.
- Other cases have been reported in the literature (e.g. 96 cases in 2006 and 88 in 2009) were from the Amazonas state and/or in the Amazon region in different years.

Pará State:

Of the 1087 cases of acute ChD reported in Brazil between the years 2000 and 2010, 70 % (765/1087) were associated with transmission by the foodborne route (ingestion of sugar cane juice, açai, bacaba, etc.). The northern region of Brazil was responsible for 89 % (971/1087) of these cases and the State of Pará by 80 % (781/971) of new cases, possibly due to "Plano Estadual de Intensificação das Ações de Controle da Doença Chagas" that sensitized health surveillance equipment for searching for and reporting of new cases (Sistema Nacional de Vigilância em Saúde, 2011).

According to Valente (2011) over 20 microepidemics of OChD have occurred in the state of Pará between 1968 and 2014, most of them having the consumption of açai (produced from the fruit of the *Euterpe oleracea* palm) and bacaba (produced from the fruit of the *Oenocarpus bacaba* palm—also known as Turu palm) as a common source of infection. Both fruits are obtained from racemes of native palm trees in the Amazon region and juices, soft drinks, sweets and icecreams are produced. This local industry represents an important source of income in communities of this region, and may contribute up to 42 % of the nutrients in some villages.

- In Abaetetuba several episodes of OChD (19 according to Valente 2011) have apparently occurred; one of these outbreaks occurred in 1998 and was linked to the consumption of acai, affecting 11 people from five families (Pinto et al. 2001a).
- Valente et al. (2001b) reported 10 cases of OChD in Pau D'Arco (1999). In this cluster of cases, *R. robustus* (nymphs and adults) positive for *T. cruzi* were found in bacaba palms.
- Another microepidemic occurred in Belen 2000 (Pinto et al. 2001a) with 11 people involved, but without any recorded mortality. The source of infection was not identified.
- The Secretaria de Vigilância em Saúde (SVS 2006) reported an outbreak of OChD in Santarem (2006). Of the 17 people affected in this microepidemic, also associated with bacaba juice intake, one person died.
- Valente (2011) describes a microepidemic of OChD in Cachoeria deArarién in which 12 people were ill in October 2006. DTU TcI was identified between humans and Z3 was detected in an armadillo (Roque et al. 2008).
- In mid-2006 a microepidemic of OChD occurred in Belém (Valente 2011). In this cluster of cases nine people in four families who lived in the same street were infected and all had consumed açai juice obtained from the same dealer.
- In the microepidemic of Barcarena (October 2006), 12 people (11 of whom worked in a health-center) apparently acquired OChD by consuming açai juice from the same source. Vectors and animal reservoirs were not identified (Nóbrega et al. 2009, Valente 2011).
- Beltrão et al. (2009) reported 25 cases of probable OChD in Breves and Bagre communities between July and August 2007. Vectors were not found and the probable vehicle of infection was açai.
- In November 2014, ProMed-Mail (2014a) reported the occurrence of ten cases of OChD in the Tenoné neighborhood in the city of Belen; the probable infection route was consumption of contaminated açai. No information on vectors or reservoirs was found.
- Other epidemiological studies of Valente et al. (2009) and Valente (2011) and others, suggest that the most important vectors in the Amazon region of Brazil are *R. pictipes* and *P. geniculatus*, while *Didelphis* spp. and *Marmosa* spp. are the main wild animal reservoirs. *T. cruzi* isolates obtained from humans, wild mammals, and vectors were TcI and TZ3.

Tocantins State:

- Oliveira et al. (2011) described an outbreak of OChD in Axixá do Tocantins in 2009. Four people from the same family were affected and palm vegetable was identified as being the probable source of infection. No triatomines were found.

Bolivia

Santalla-Vargas et al. (2011) reported 14 cases of acute OChD in five families in Guayaramerín, Department of Beni part of the Bolivian Amazon. All the patients had fever and other symptoms that appeared almost simultaneously in October

2010. After a thorough search for the common source of infection, it was determined that all patients had a history of consumption of majo juice, prepared at the residence of one of the families. The majo is a fruit produced by the *Oenocarpus bataua* (mingucha) palm and which is ground to produce fresh juice. No vectors were found in the homes of this group of patients. Potential reservoirs of infection and/or parasite genotypes are not mentioned. (Table 6.1 and Fig. 6.1).

6.2.1.5 Atlantic Forests

- A microepidemic of OChD occurred in Navegantes in the Brazilian state of Santa Catarina in 2005. In this outbreak 24 individuals were infected and among these there were five deaths. Sugarcane juice was the vehicle of infection (Tatto et al. 2007). The vector was identified as *Triatoma* sp. and *Didelphis* sp. was the main animal reservoir (Roque et al. 2008) with 93 % of this animal species found to be infected. In human cases the isolate of *T. cruzi* was TcII in humans, while a mixed pattern of TcI and TcII were found in triatomines and wild animal reservoirs (Steindel et al. 2008). In the State of Rio Grande do Sul, in Teutônia, Nery-Guimarães et al. (1968) reported and OChD outbreak of 17 people that was apparently related to food distributed at a school; in this outbreak six persons died of those infected.

6.2.2 Tropical and Sub-tropical Dry Broadleaf Forests

6.2.2.1 Atlantic Dry Forests

This ecoregion is part of the Tropical and Subtropical Dry Broadleaf area Forests and contains some of the Brazilian Northeast states where cases of OChD have been reported.

Maranhão State: two cases of OChD occurred in Tutóia in 2006 (Pinto et al. 2008) probably acquired by ingesting bacaba juice.

Ceará State: a microepidemic of OChD occurred in Redenção in 2006 involving eight members of the same family. A soup flavored with cilantro and pureed onions that had been harvested at home was considered the vehicle of infection (Cavalcanti et al. 2009). The vectors *Triatoma brasiliensis* and *Panstrongylus lutzi* were found, but both were negative for *T. cruzi* (Cavalcanti et al. 2009). The gray short-tailed opossum (*Monodelphis domestica*) and *Thrichomys laurentius* (a species of punaré or spiny rat) were identified as the major reservoirs in the maintenance of the parasite cycle and a TcI strain was isolated Roque et al. (2008).

Paraiba State: Shikanai-Yasuda (1987) described a microepidemic of OChD in the rural community of Catolé da Rocha. One of the 26 infected patients did not sur-

vive. *Triatoma brasiliensis* was found in the home of infected individuals. The authors also suggest the possibility that food had been contaminated from secretions from the anal glands of reservoir hosts, as a high prevalence of infection with *T. cruzi* was found in *Didelphis* sp. (opossums).

Bahia State: Three microepidemics of OChD have been reported from the central-western region of this state.

- Maguire et al. (1986) suggest the possibility of a microepidemic of OChD in 20 people in Riacho de Santana. Although a common source of infection was not identified, the emergence of *T. infestans* in the community was considered to be of relevance.
- An outbreak of OChD in Macaúbas in 2006 resulted in the deaths of two of seven infected people. The vehicle of infection was postulated to be water that had been contaminated with triatomine droppings (Dias et al. 2008); *Triatoma sordida* were found to be positive for *T. cruzi*.
- Six cases of acute OChD were reported in a family of 11 from the municipality of Ibipitanga. The vehicle of infection was apparently sugarcane juice and *Triatoma sordida* was incriminated as the vector as specimens infected by *T. cruzi* were also identified (Bastos et al. 2010).

6.2.3 Tropical and Sub-tropical Grasslands, Savannas, and Shrublands

6.2.3.1 Llanos Savannas

- ProMED (2014b) reported 31 cases of people with acute OChD from Paz de Aripoto (Department of Casanare in the Orinoquia region of Colombia), with one person deceased in May 2014. The source of infection, vectors, and potential reservoirs were not reported.

6.2.4 Montane Grasslands and Shrublands

6.2.4.1 Northern Andean Paramo

Clusters of OChD have been reported from this ecoregion, from two departments of Colombia in the Magdalena Department.

- The first outbreak occurred in the municipality of Guamal in 1999 with 13 cases reported, including five deaths (mortality of 38.5 %) (Cáceres et al. 1999). The common source of infection was a fermented drink made from palm wine that

had apparently been contaminated with feces from *P. geniculatus* infected with *T. cruzi* (Nicholls 2006).
- The second outbreak of OChD occurred in the Bolívar Department (Rueda et al. 2014) with two fatal cases in the town of Villanueva. Further details are not available.

6.2.5 Mangroves

6.2.5.1 Guianan-Amazon Mangroves

To this ecoregion belongs in French Guiana where Blanchet et al. (2014) and OPS (2008) reported a microepidemic of OChD affecting eight people from the same family in Iracoubo (coast of French Guiana, north of Cayenne). The infected people had ingested palm juice "comou", produced from the fruit of the *Oenocarpus bacaba* palm (Turu palm) and were evaluated between November 2005 and February 2006. No descriptions of possible vectors or reservoirs are available. The DTU identified in 6 patients was TcI.

6.3 Concluding Considerations

Outbreaks of foodborne American trypanosomiasis, or OChD, are characterized by the simultaneous onset of symptoms, usually in groups of individuals with a common history of consumption of particular items of food or drink obtained from the same source and without showing any cutaneous signs of exposure to triatomines.

In the ecoregion described as Northern Andean Montane Forests, which includes the Andean states of Venezuela and Colombia, 11 outbreaks of OChD have been reported. The vehicle of infection has usually been handcrafted fruit juices and other non-specific foods. The main vector is *Panstrongylus geniculatus*. This species of vector has already been described in at least four outbreaks of OChD: El Bordo (Mérida), Rubio (Táchira), Bucaramanga, and Lebrija (Santander), although *Rhodnius* sp. was also found in Bucaramanga and Aguachica (Cesar). Regarding the DTU in outbreaks from this ecoregion, the available information is reported by Ramirez et al. (2013) in Colombia in which TcIa and TcId identified in Aguachica, Tibu, Bucaramanga, Lebrija, and San Vicente del Cuchuri. In Venezuela DTU Tc1 was identified in isolates from patients involved in five outbreaks (Muñoz-Calderón et al. 2013). *Didelphis marsupialis* (common opossum) was described as a reservoir in outbreaks of El Bordo, Turbo, and Lebrija.

In the Northern Andean Paramo ecoregion, the vehicle of infection was palm wine and *Panstrongylus geniculatus* was the vector found at the outbreak of the Guamal (Magdalena).

The largest number of outbreaks and cases of OChD in Venezuela are reported from the Montane Forests Coastal Venezuelan ecoregion, occured where *P. geniculatus* is the main vector involved. TcI genotype was the DTU identified in Chacao and Chichiriviche outbreaks. In the founder focus of Chacao, reservoirs of infection were found were rats and dogs.

In the ecoregion River Rio Negro-Juruá Moist Forest one microepidemic of OchD has been reported from Santa Izabel do Rio Negro and information about the contributing factors involved in the maintenance of the lifecycle is lacking.

The Southwestern Amazonian Moist Forests ecoregion has hosted the most microepidemics of OChD. The main vehicles of infection were bacaba and açai juices. *Rhodnius robustus* was found in Plácido de Castro in Acre, Pau D'Arco, and Belém in Pará. In Mazagão, the vectors *Rhodnius picpites* and *Panstrongylus* sp. have been identified and *Didelphis* sp. indicated as a reservoir of infection. Isolates of *T. cruzi* in this ecoregion appear to be highly virulent (Barata et al. 1988), TcIe1 being the predominant DTU. In the areas of Bolivia belonging to this ecoregion, descriptions of vectors, reservoirs, and DTUs are lacking.

In the southern states of Brazil belonging to the Atlantic Forests ecoregion two OChD outbreaks have occurred (Santa Catarina and Rio Grande do Sur). In the microepidemic of Navegante in Santa Catarina (2005), the main incriminated vector was *Triatoma* sp. and *Didelphis* spp. the main wild reservoir. Two genotypes were described TcII in humans and TcI in triatomines.

The states that correspond to the Brazilian northeast are located in the Atlantic Dry Forests ecoregion. From here, four clusters of OchD have been described with the vehicles of infection identified as soup with fresh seasonings (Redencao), food contaminated with secretions of the anal glands of *Didelphis* (Catolé da Rocha), and water or sugarcane juice in Macaúbas and Ipitanga. The associated vectors are: *Triatoma brasiliensis* in Redenção (Ceará) and Catolé da Rocha (Paraíba), *Triatoma sordida* in Macaúbas and Ibipitanga, and *Triatoma infestans* in Riacho de Santana. TcI was the single most important genotype in Redenção (Ceará state). The Guianan ecoregion-Amazon Mangroves corresponds to French Guiana. The vehicle of infection was bacaba juice, which is similar to outbreaks reported in the Amazon region. Vectors and reservoirs were not determined and the parasitic DTU was TcI.

Based on these outbreaks reported to date it is clear that the Northern Andean Montane Forests, the Coastal Venezuela Montane Forests, and, particularly, the Southwestern Amazonian Moist Forests are the ecoregions that are more prone to foodborne transmission of *T. cruzi*, having already hosted at least 40 very important oral outbreaks. This indicates that the vegetation in these areas probably plays an important role in the diversity and density of triatomines, and thus the parasite. In addition, particular aspects of the ecology, host behavior, particular encroachment of humans into areas where wild reservoirs live, and "urbanization" of wild reservoir hosts, may exacerbate the possibilities of foodborne transmission of *T. cruzi* (Fergnani et al. 2013). The overview provided here could be used as a basis for dissecting out further the factors of importance regarding contamination and of food

vehicles by trypomastigotes of *T. cruzi*, and the subsequent consumption of the contaminated food by susceptible humans. Although the data may suggest that foodborne Chagas disease is increasing, this may not represent an absolute increase, but rather a decrease in vectorborne infection due to initiatives to decrease this transmission risk. Furthermore, our ability to recognize outbreaks of OChD may have improved, and earlier clusters of cases may actually have been foodborne but were previously erroneously considered to have been due to vectorborne transmission.

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Chapter 7
Food as a Transmission Vehicle for *Trypanosoma cruzi*

Lucy J. Robertson

7.1 Introduction to Food as a Transmission Vehicle for Parasites

A large number of parasites may be transmitted by the foodborne route. These include all groups of parasites: nematodes, cestodes, trematodes, and protozoa. Of these, some are transmitted as encysted stages in the tissue of a previous host, whether fish or meat, and, as such, the tissue of the previous host acts as a protection for the parasite from the digestive enzymes of the next host. Indeed, the tissue of the previous host must be digested away in order for the parasite to begin the next stage in its lifecycle. For parasites that are transmitted as contaminants on fresh produce, without the protective tissue of the previous host, protection is often in the form of the walls of the robust and relatively inert transmission stage, whether cyst, oocyst, metacercaria or egg, that have also protected the parasite from environmental pressures prior to transmission. Among these parasites, relevant examples are the oocysts of *Cryptosporidium* and *Cyclospora*, the eggs of *Ascaris* and other soil-transmitted helminths, the cysts of *Giardia* and *Entamoeba*, and the metacercaria of *Fasciola*. Foodborne transmission of *T. cruzi* is therefore unique in that the transmission stage, the metacyclic trypomastigote, is not inert with a thick cell wall, but a flagellated stage that undertakes active invasion of host cells. Thus, counter-intuitively, given the morphology of the parasite, this stage must have extraordinary resistance to physical and biochemical agents, and also, given the plethora of animals to which it is infective, be remarkably adaptable to a range of environments.

At the beginning of the process of digestion, the parasite will come into contact with the salivary enzymes that are the beginning of digestion for each animal

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species. For example, salivary amylase that hydrolyses starch into glucuose, is found in many, but not all, bird and mammal species, including humans. Thereafter, the ingested *T. cruzi* meets a range of different physiological conditions, depending on the animal that has ingested the parasite; with a great range in potential pH values that it will encounter, as well as enzymes and microbiota (Stevens and Hume 2004; Hungate 1966).

In addition to the intrinsic physiological conditions of the digestive tract of mammals that are susceptible to infection with *T. cruzi*, are the conditions of solid or liquid food, which enable infective trypomastigotes to survive.

As well as having to survive digestion, metacyclic trypomastigotes of *T. cruzi* must also survive in relatively hostile environments when they invade phagocytic cells. Indeed, studies have demonstrated that trypomastigotes are able to survive in the phagolysosomal vacuoles associated with *Coxiella burnetii*, the etiological agent of Q fever (Andreoli et al. 2006).

The structure of metacyclic trypomastigotes and, in particular, the composition of their surfaces, are key to their survival in these various host-associated hostile environments. Mucin-like gp35/50 molecules are abundant on the surface of metacyclic trypomastigotes and, since they are resistant to protease digestion, are partially responsible for protecting them from destruction during infection by the oral route (De Souza et al. 2010; Yoshida 2009). The cytoskeleton of trypanosomatids is also complex and stable, being composed of sub-pellicular microtubules (De Souza 1988) that confer an unusual resistance to mechanical lysis, and contribute with other molecules, to the stabilization of the protozoan shape. Furthermore, in contrast with the microtubules of mammalian cells, these are resistant to low temperature (Sant'Anna et al. 2005).

7.2 Detection and Occurrence of *Trypanosoma cruzi* in Different Food Matrices

Detection of contamination of different food matrices with parasitic transmission stages has become a standard procedure for a range of parasites, particularly those associated with meat and fish-borne transmission (such as *Trichinella* spp., *Taenia* spp., and anisakid larvae). These procedures are considered to be important for preventing potentially infectious food items entering the food-chain, and recognized and approved methods for conducting these investigations have been developed.

In addition, numerous surveys have been conducted for contamination of fresh produce with other parasite transmission stages such as *Ascaris* eggs, *Cryptosporidium* oocysts, and *Giardia* cysts. The information gained from such surveys may not be particularly appropriate for preventing infection, as often the produce will already have been consumed by the time the results of the analyses are available, but they provide useful information for the requirement for interventions and risk assessment. Furthermore, they have resulted in the development of methods

for when outbreaks or suspected contamination events occur and it is necessary to investigate whether a particular batch of produce has been contaminated or is the source of an outbreak or cluster of infections.

However, standard methods for investigating contamination of different food products with metacyclic trypomastigotes of *T. cruzi* have not been conducted, and surveys are usually limited to investigation of occurrence of infection in vectors or reservoir hosts that may be associated with infection, whilst determination of the specific food-product associated with an outbreak or cluster of infection is generally based on epidemiological methodologies.

Given that mandatory screening of blood products in many Latin American countries has probably resulted in the prevention of millions of new infections (Dias et al. 2002), it might be argued that the growing threat of foodborne transmission could also be met by a screening approach, particularly, for example of fruit juices and other drinks that have been associated with outbreaks of infection. However, it should be noted that screening of food has entirely different challenges to screening of blood products; the methods in place, for example, for screening vegetables and fruit for *Cryptosporidium* oocysts and *Giardia* cysts (e.g. ISO Standard Method 18744; Microbiology of the food chain—Detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits), have a notably low recovery efficiency and are expensive and cumbersome to conduct.

Although there are good arguments against routine screening of juices, development of a robust method that can be used to confirm or refute that a particular implicated product is the source of infection could be useful, not least to ensure that the product is removed from the market and other mitigation methods are implemented to prevent any repetition of the contamination.

Although it has been stated that the wider and more routine use of high-resolution analysis for molecular epidemiological tracking would be of great value to allow more-detailed epidemiological investigations (Miles 2010), generally it seems that the focus is on molecular investigations (particularly multilocus microsatellite typing) of *T. cruzi* isolates from larger quantities of patient samples, vectors, and reservoirs. The value of investigating different food matrices for contamination is clearly an area open for further exploration and investigation.

7.3 Survival of *Trypanosoma cruzi* in Different Food Matrices

As outlined, metacyclic trypomastigotes must be able to survive in a wide range of hostile environments inside the host, both in the digestive tract but also in the host cells that it invades. The specific characteristics that enable survival in these environments, may also confer an ability to persist and remain infective in food matrices prior to ingestion, and therefore exacerbate the potential for foodborne transmission.

Although the overwhelming majority of studies concerned with the viability and survival of *T. cruzi* trypomastigotes are concerned with chemotherapy and treat-

ment of infected patients, the survival of the trypomastigotes in various food matrices, such as fruit juice, should also be of interest.

7.3.1 *Methods for Determining Viability or Infectivity of Metacyclic Trypomastigotes*

In order to investigate the survival or inactivation of the parasites, an acceptable method of determining viability and/or infectivity is important. The simplest method is observation of motility, as has been used in studies by Añez and Crisante (2008) and Añez et al. (2009); use of a video camera to record movement may assist in classification of the motility (very active, active, slow), or the use of a dye such as trypan blue may be useful for visualization of the parasites (Barbosa et al. 2012). However, motility does not necessarily equate with infectivity, and other methods for assessing viability are useful. For example, MTT assays have been used for assessing trypomastigote viability (de Almeida Nogueira et al. 2013). In this assay, the presence of NAD(P)H-dependent cellular oxidoreductase enzymes is considered as an indicator of viability; these enzymes can reduce the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), to insoluble formazan, which has a purple color that can be measured spectrophotometrically. However, the gold standard method is infection, and infection of mice to determine infectivity is standard practice for such studies (Añez et al. 2009; Barbosa et al. 2012), with infection being detected by microscopy or PCR.

Some of these methods have been applied for investigating the survival of metacyclic trypomastigotes in various foods that have been associated with outbreaks has been investigated in various studies (Table 7.1).

7.3.2 *Sanitizing Regimes in the Fresh Produce Industry and Survival of* T. cruzi

The fresh produce and fresh juice industry uses a variety of sanitizing regimes to ensure the safety and quality of fresh produce and fresh juices. These post-harvest procedures for removing or inactivating pathogens on fresh produce include washing procedures for removal, whilst inactivation procedures include physical (temperature, pressure, irradiation, etc.) and chemical approaches, including various liquid and gaseous sanitization regimes. Not all the sanitizing regimes used in the food industry, and commonly used for decontamination of those food matrices mostly associated with foodborne Chagas disease, have been experimentally tested against *T. cruzi*. However, some investigations have been conducted.

Chilling and freezing are commonly used as preservation methods in the fresh produce industry. While these procedures may have a deleterious effect on contaminant pathogens, the primary aim of these procedures is to keep the produce fresh and

Table 7.1 Survival of *Trypanosoma cruzi* trypomastigotes in different food matrices

Food matrix	Survival period and temperature	Assessment methodology	Reference
Sugarcane juice	Viable for at least 4 h at room temperature and infective for at least 1 h at room temperature	Microscopy (observation of motility) and also infection of mice (as assessed by blood microscopy, mortality and xenodiagnosis	Pinto et al. (1990)
Sugarcane used to prepare juice	Infective for at least 24 h at room temperature	Infection of mice (assessed by blood microscopy)	Cardoso et al. (2006)
Various fruits/vegetables and their juices (banana, peach, pineapple, sugarcane, papaya, apple, potato, carrot, arracacha, tomato)	At room temperature (22–26 °C), trypomastigotes survived for 18 h in most matrices with the exception of peach and pineapple, in which no living trophozoites were observed after 6 h. The longest survival was observed with banana for 72 h	Microscopy (observation of motility)	Añez and Crisante (2008)
Various fruits and vegetables and their juices (banana, melon, papaya, tomato, potatoes and carrots) and coconut water and fresh milk	Active parasites observed for up to 10 h on/in all products, and for 18 h in coconut water	Microscopy (observation of motility)	Añez et al. (2009)
Fresh milk	Mice were infected after ingestion of milk between 1 and 6 h after contamination with metacyclic trypomastigotes	Infection of newborn mice as assessed by blood examination, including stained smears and molecular methods	Añez et al. (2009)
Açaí pulp	Active parasites and infectivity observed for up to 144 h at 4C; infectivity after 24 h at room temp. and also after 48 h at room temp followed by 72 h at 4 C. Freezing (−20 °C for 26 h) resulted in infection in 1 out of 2 mice	Microscopy (observation of motility) and infection of SCID mice using parasitaemia and mortality to assess infection	Barbosa et al. (2012)

edible for a prolonged period, thereby extending shelf-life. Freezing, in particular, is considered to be the most satisfactory method for preserving quality during long storage periods with extreme cold retarding the growth of microorganisms and slowing down the chemical changes that affect quality or cause food to spoil. Although chilling or cooling does not enable prolonged storage, it extends the

shelf-life of fresh produce by reducing the rate of physiological change (respiration, ethylene production, enzymatic processes and water loss) and by slowing the growth of microorganisms. There are various approaches in the fresh produce industry to both chilling (room cooling, forced air cooling, hydro-cooling, ice-cooling, top-icing, vacuum cooling), whilst for freezing, which is usually down to −18 °C or lower, approaches include air-blast freezers, tunnel freezers, belt freezers, fluid-bed freezers, contact freezers, immersion freezers, cryogenic freezers (produce exposed to an atmosphere below −60 °C), liquid nitrogen freezers (produce exposed to liquid nitrogen spray, with a boiling temperature of −196 °C) and liquid carbon dioxide freezers (produce exposed to liquid carbon dioxide at a temperature of −70 °C). Many pathogens survive refrigeration, and *T. cruzi* has been shown to be infective after at least 144 h at 4 °C (Barbosa et al. 2012). Although many other pathogens such as bacteria are inactivated by freezing, other pathogens such as some viruses and helminth eggs survive freezing. Although our data are limited, the experiments by Barbosa et al. (2012) and Passos et al. (2012) indicate that a proportion of trypomastigotes remain infective after 14 h (Passos et al. 2012) and 26 h (Barbosa et al. 2012) at −20 °C. However, it has been reported that freezing without chemical protection can destroy the organism, although no supporting data were provided (Miles 2010).

Although low temperatures, rather than high temperatures, are usually considered with respect to methods of preservation of fresh produce, some heat treatments are relevant, particularly with respect to the preparation of fruit juices that are an important vehicle in outbreaks of Chagas disease. Pasteurization is effective at against the majority of foodborne pathogens, and has been shown to be effective against trypomastigotes of *T. cruzi* in milk (Santos Ferreira et al. 2001). Furthermore, heating to 63 °C in a domestic microwave oven has also been shown to be effective at abrogating infectivity of *T. cruzi* trypomastigotes in milk (Santos Ferreira et al. 2003). One can also assume that pasteurization of fruit juices would have the same effect; in Brazil, açaí pulp that is to be exported to other regions of the Amazon or abroad is pasteurized with the intention of ensuring inactivation of any contaminating *T. cruzi* trypomastigotes (Dias et al. 2011). As meat, particularly from wild animals, can also be a transmission vehicle for *T. cruzi*, cooking should be at 60 °C or over, as amastigotes can survive in the tissues below this temperature (Neto et al. 2000).

The other physical treatments (e.g. high pressure, irradiation) and chemical treatments (chlorination, hydrogen peroxide, organic acids, ozone etc.) often used against pathogens in the fresh produce industry have not been widely tested against *T. cruzi*, although it has been reported that common disinfectants such as sodium hypochlorite (1 h), gentian violet (24 h) and 70 % ethanol are effective at inactivating *T. cruzi* (Dias 2006; Pereira et al. 2010). Use of gamma irradiation to inactivate the parasite in blood has been investigated, and has indicated that doses of over 200 krad would be necessary (Pereira et al. 2010), and has not been considered to be of application for prevention of oral transmission (Dias 2006).

7.4 Concluding Thoughts on Occurrence and Survival of *Trypanosoma cruzi* in Food

Published methods of analyzing for food substances for *T. cruzi* contamination are currently lacking, and development of such methods does not seem to be an area for active investigation at present. This means that surveys for contamination of different food substances, either to gain information for epidemiological purposes or for investigating outbreaks or transmission routes in clusters or individual cases, are lacking. Whilst information on occurrence of such contamination may not function directly as a preventative measure for infection, it could provide useful data useful for risk assessment and identification of risk foods and implementation of interventions. Methods that are currently available for analyzing vector samples could probably be adapted for food, but it should be noted that molecular methods may be affected by the matrix under investigation, and different food matrices may affect molecular detection.

Although, standards for manufacture and treating of juices, particularly açaí, have been established (Pereira et al. 2010), these are often largely aimed at the export market. These manufacturing standards involve regimens such as pasteurization that are very severe. Although these standards are important for exported food, particularly juices, they are not necessarily appropriate for local production and/or artisan products. Also it should be noted that although fruit juices have been a commonly associated vehicle of infection, other food products have also been associated with transmission of Chagas disease and may not be so easy to treat. Thus, implementation of preventive measures, preferably grounded in Hazard Analysis and Critical Control Point (HACCP), seems to be a more appropriate approach to attempting to interrupt the oral transmission route.

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Chapter 8
Prophylactic Measures and Implementation of Control Measures in Foodborne Chagas Disease

Lucy J. Robertson and Oscar Noya González

8.1 Introduction to Prophylactic Measures

Several factors affect the likelihood of the infective metacyclic trypomastigotes of *Trypanosoma cruzi* contaminating food due for human consumption, including the way in which the food is produced, harvested, transported, stored, prepared, and consumed. Being aware of these factors is an essential step in identifying critical control points during this chain, such that preventive measures to reduce the likelihood of contamination can be implemented.

Infective forms of *T. cruzi* that may contaminate food are contained in the digestive tract of infected triatomines, in their feces, and in the anal secretions of different genera of reservoir hosts such as opossums. Furthermore, based on experimental findings (Dias et al. 2013), trypomastigotes in the fresh blood of infected animals that comes into contact with foods could also be infective to man. Thus, the conservative and prophylactic approach is based on implementing a range of measures that ensure that *T. cruzi* does not come into contact with food and beverages, particularly those that are to be consumed without cooking.

A relevant part of vector control is to ensure that neither the domiciliated insect nor its feces are able to come into contact with food. Mechanical measures that block the entrance of triatomines to houses are one effective step, but also ensuring that food remains covered or enclosed in a sealed container provides another barrier

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should the first one be breached due to an inadvertent action. Awareness of the problem, ensuring minimum standards of cleanliness in food handling, and basic health education are also important in reducing the likelihood of contamination of food and beverages.

Such safety measurement can be considered both at the personal or consumer level, but also with respect to regulations concerned with the provision of food commercially. It should be noted that while several of the small clusters of foodborne Chagas disease have been associated with food prepared inside the home, there are also several larger outbreaks in which the contaminated vehicle of infection has been prepared for commercial sale.

8.2 Risk Assessment and Food Regulations

The primary legislation that is in place to control the spread of pathogens in food includes local Food Safety Acts or Regulations. Such Acts or Regulations tend not to have any international impact, and are directed towards specific perceived or identified problems. As it seems most likely that the greatest risk from foodborne *T. cruzi* infection is related to locally produced, artisan, or homemade fresh food products and juices, international regulations are probably of lesser importance. The foremost food safety management system that is applicable internationally is Hazard Analysis and Critical Control Point (HACCP), supported by Good Hygienic Practice (GHP), Sanitation Standard Operating Procedures (SSOPs), Integrated Pest Management (IPM), and Good Manufacturing Practice (GMP). The intention of HACCP is to provide a systematic, cost-effective, and efficacious approach for risk management and prevention, and is also of international application. HACCP allows food manufacturers to identify hazards and measures for their control, and determine critical control points. In contrast, risk assessment tends to be a more scientifically based development, in which hazards are identified and characterized, exposure is assessed, and these steps enable risk to be characterized. In a quantitative risk assessment, a comparative numerical figure may also be derived.

As the food matrix that has been most associated with foodborne Chagas disease is fruit juices of various types, it is important to consider how risk assessment could be usefully applied. Provided that fruit juices are kept stored in sealed containers after pasteurization (to prevent post-pasteurization contamination), then the greatest risk would appear to be from non-pasteurized juices produced in areas where *T. cruzi* infection is endemic among reservoir animal populations and/or vector populations. A qualitative risk assessment on unpasteurized fruit juice and cider was conducted by Mihajlovic et al. (2013). Although *T. cruzi* was mentioned throughout the assessment, the focus of the assessment was on Canada, and the authors concluded that the lack of information on the availability of unpasteurized juices from South America in Canada meant that the risk to Canadians could not be estimated accurately, although it was likely to be low (Mihajlovic et al. 2013). However, although this assessment did not directly address *T. cruzi*, the approach

used by the authors for the other pathogens that they considered (*Escherichia coli* in the classes belong to entero-hemorrhagic *E. coli* (EHEC) and entero-toxigenic *E. coli* (ETEC); *Salmonella* spp., and *Cryptosporidium* spp.) may also be of relevance for considering a similar approach for *T. cruzi* in endemic countries in South America. The authors use the Codex Principles and Guidelines for the Conduct of Microbiological Risk Assessments for their study, in which the following information is collected for the pathogen under consideration:

- Hazard Identification: identifies the hazards of concern (*T. cruzi*) associated with the matrix under consideration (unpasteurized juices).
- Hazard Characterization: provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from ingestion of the hazard in the matrix of interest. This involves not only obtaining symptoms and pathogenicity information, but also dose–response information, and also considering host susceptibility factors (evaluating whether one sector of the population more likely to be adversely affected than another, for example children, pregnant women, etc.
- Exposure Assessment: considers the likelihood of acquiring foodborne disease through consumption of this matrix in the region under consideration. This step involves considering the most likely routes of contamination (for example are the fruit contaminated at harvesting or is contamination more likely to occur during pressing), the likelihood of survival of the pathogen in the fruit and fruit juice, the effect of treatments including sanitization and physical treatments and also the effects of preservatives, testing of the product, and also the quantity of fruit juice consumed or available for consumption within a given population.
- Risk Characterization: by combining the information from the previous sections this step attempts to determine the likelihood of illness should the identified hazard be ingested.

By using this step-wise approach, not only can scientific support be obtained for risk management decisions regarding unpasteurized juices, but also data gaps can be identified for the particular hazard (*T. cruzi*) and food matrix (fruit juices). While the Canadian authors of this article do not consider *T. cruzi* in fruit juices in any detail, they make some important comments about the contamination of fruit juices in general with pathogenic microorganisms (Mihajlovic et al. 2013), that also can be applied with respect to *T. cruzi*.

Firstly, contamination may occur at either the raw produce stage (before or during harvesting) or during the manufacture of juices, with the potential for animals and insects to enter poorly sealed processing facilities and contaminate the processing equipment; adherence to GHP, SSOP and GMP is important, including cleaning and sanitizing equipment and ensuring that premises remained sealed in order to reduce the risk of contamination. With regards to açaí, two main pathways for contamination with triatomines, and hence, potentially, *T. cruzi*, have been identified (Pereira et al. 2009). Triatomines may be attracted to the lights of the machine or associated with the machine used to grind the açaí and hence become ground together with the fruit. Alternatively, the contamination can be due to lack of

hygiene during harvesting and transport, with the insects transported to the processing place, together with the fruits, in baskets or sacks (Valente et al. 2002). While described for açaí, any other fruit that is made into juice may be associated with contamination from triatomines by the same mechanisms.

Secondly, the survival potential within both the fruit for juicing, and the juice itself, is critical. The acidic nature of most unpasteurized fruit juices is not lethal for many pathogens, including *T. cruzi*.

Thirdly, unpasteurized juice is usually consumed without a treatment to destroy pathogenic microorganisms. Development of methods for detecting contamination would be useful from an epidemiological perspective and also for following up in outbreaks, but would be unlikely to be of practical daily use. However, currently such a methodology has not been developed and it is stated that in the outbreaks it has not been possible to isolate the parasites from the food; this is because outbreak identification is generally achieved two to three weeks after the consumption of the infected food (Pereira et al. 2010).

Interestingly, the meeting of Codex Committee on Food Hygiene (CCFH) in November 2014 in Lima, Peru (Codex 2014), in considering guidelines concerning application of principles of food hygiene to the control of foodborne parasites agreed to delete any measures for the control of *T. cruzi* in fruit juices. The basis for this was that foodborne infection with this parasite is limited to "unprocessed products in specific areas" and also is "not relevant to the products traded internationally". Nevertheless, various companies/online traders claim to sell unpasteurised açaí pulp, with the claim that pasteurisation is detrimental to acclaimed anti-oxidant properties. A study by Albarici and Pessoa (2012) indicated that pasteurization of the pulp actually protects the main compounds that are responsible for the antioxidant properties attributed to açaí pulp.

However, regardless of international trade, açaí, sugarcane, and fruit juices are ideal food matrices for oral outbreaks; because the crops for these drinks are often grown, harvested, and pressed locally, (with the pressing often occurring at nighttime under artificial light), in rural or periurban areas where sylvatic triatomines may be abundant, the requirement remains for reducing the risk of local outbreaks (Miles 2010). Furthermore, studies by Xavier et al. (2014) indicate that in Belém, the capital of Pará state in Brazil and from which the highest number of ACD cases associated with the consumption of açaí juice has been reported, the source of infection is not actually the immediate locality, but from the islands of Combu and Murutucu that are a couple of kilometres away and geographically separated by the river Guamá. Thus, although triatomines may not be endemic in a particular area where juice is made or fresh produce is bought and sold, they can be readily transported with the produce, thereby introducing the risk of infection in areas where the vector does not normally reside. In fact, the founding focus of the largest oral transmitted microepidemic was located 6 km away of the school where the outbreak took place (Alarcón de Noya et al. 2010).

Therefore, from a HACCP perspective, the critical control points (CCP) are those concerned with ensuring that the juice does not become contaminated, either during or post-production, and ensuring that fresh produce is free of potentially infected triatomines. Corrective measures could include treatment of the product

(pasteurization, for example) to ensure inactivation of the parasite. However, there is currently a lack of tools for determining critical limits for each CCP, and despite non-pasteurised juices not being traded internationally, foodborne transmission of *T. cruzi* would be more readily limited if a simple HACCP could be implemented and act as a template for small-scale producers of fruit juices in endemic areas. Measures that may be considered to ensure that the juice is unlikely to be contaminated under semi-industrial conditions include: vigilance or cleaning of crops to exclude insects; covering of machinery used for making the juice and not operating the presses directly beneath, or adjacent to, artificial light; ensuring that juice preparations are kept covered and inaccessible to either insects or potentially infected reservoir hosts, such as opossums. These are all relatively simple measures that could reduce the risk of contamination, while pasteurization would ensure inactivation if contamination was suspected to have occurred. However, practical factors regarding implementation of even these simple measures need to be considered, as pointed out by Xavier et al. (2014). The city of Belém in Brazil has approximately 3500 sites of açaí juice sale, most of which are temporary, and the people preparing the açaí juice also have a rapid turnover. Although there are periodical sanitary inspections of these settlements, new sites and workers appear on an annual basis. Nevertheless, as this is a seasonal crop, the implementation of compulsory health education programs regarding basic hygiene procedures in food handling, and particularly prevention of contamination during preparation of açaí pulp could be of real value. In addition, workers who collect the açaí can also become infected, constituting a case of occupational disease, as it can occur both during the collection of fruit bunches, and particularly if consumed for checking taste. This is supported by comments made by Coura (2013) who stated that given that eradication of Chagas disease is not possible, control of the different transmission routes using educational initiatives, including in particular oral transmission, should be the major aim.

A different situation occurs where the level of contamination of meals and juices is at places such as canteens, restaurants, and homes. In such scenarios, the potential for implementing institutional supervision is limited, and control at the production and distribution sites of a potentially contaminated meal or juice is almost impossible. In such cases, only publicly available educational programs, for example on the internet or TV, or at the school level, could be of value (see Section 8.4).

8.3 Vector Control

Controlling human Chagas disease is very difficult and currently impractical in many cases, because current drugs for treatment of infected individuals are not efficient and have undesirable side-effects. Furthermore, the zoonotic nature of the parasite means that there are reservoir hosts in many environments.

Therefore, there are only two readily accessible targets: (a) the control of triatomines using residual spraying indoor insecticides, and (b) improving housing (plastering of walls, use of screens on windows and doors, concrete flooring and construction of roofs with suitable materials).

Triatomines are particularly susceptible to chemicals due to their low reproductive rate and low genetic variability, which hinders the development of resistance to insecticides. Fenitrothion and pyrethroids are the most efficient and commonly used insecticides for indoor residual spraying (WHO 2002).

Spraying frequency of housing is usually restricted to annual cycles unless the reinfestation rate exceeds 5 %, in which case re-spraying should be performed on houses that are re-infested, plus all houses within a radius of 200 m. However, this control measure has sometimes been rejected by different populations, as, emotionally, the residents feel that their privacy has been invaded and, more practically, the house inhabitants fear the potential of toxic effects on themselves and their families, and also on their pets (WHO 2002).

8.4 Health Education (Communities, Schools, Health Personnel Awareness, Conferences, TV, Multi-media etc.)

A different and more complex issue is the contamination of meals and juices at places such as canteens, restaurants, and homes, where the possibility of institutional supervision is limited. In such cases, it is important to act at different educational levels, two of which are described in greater detail below.

- Restaurants, canteens (schools, prisons, factories, religious and charitable organizations, etc): educational initiatives should be oriented to personnel handling food. In such establishments should have general guidelines on mandatory rules to prevent food contamination, including sanitary measures; these should be readily and easily available, such as wall placards. Another important group is the parent associations and representatives of each school group, which must be vigilant on the quality and quantity of food that receive their children in school canteens. The interaction with these particular target groups seeks to prevent major microepidemics.
- General population, including children and adults: particular emphasis should be targeted towards mothers, who are the group of people who are most often concerned with meal preparation. This can be achieved locally with the support of social organizations of neighborhoods, especially from slums. Health personnel (doctors and nurses), together with teachers from each locality, must also be incorporated. The interaction with this target group seeks to prevent minor microepidemics, usually limited to those generated at home.

In both these cases the use of mosquito nets in windows and doors should be emphasized as measures for reducing invasion by triatomines and other pests.

All measures are directed towards preventing the exposure of food to the feces and urine of invading or domiciliated vectors in kitchens, as well as marsurpials that liberate metacyclic trypomastigotes in their anal secretions. This includes the protection of blenders, which can act as traps for triatomines. The nymph stages of insects may be particularly probablematic, they may climb on to blenders, which are

rough on the outside surfaces, and fall inside. They are unable to climb out again due to the smooth surfaces of the inner walls of the blender, and also, unlike adult vectors, they cannot fly out as nymph stages do not have wings.

8.5 Concluding Comments

The lifecycle of *T. cruzi* is relatively complicated, with a variety of possible vectors and reservoir hosts. This means there are many different possible points of attack. However, not all of these are equally accessible. Vector control is the obvious main link of the lifecycle that could be a target for interventions, while ensuring that foodborne transmission does not occur involves hygiene in food handling and preparation, and ensuring that food is stored such that it is inaccessible to either vectors or reservoir hosts. Health education is of paramount importance, and using a HACCP-based system in order to determine where mitigation should be targeted and where there may be weak points in the cycle of infection. In the opinion of the authors, the removal measures for the control of *T. cruzi* in fruit juices by CCFH from their mandate in 2014 was ill advised. It is well known that "artisan" food products are often sought by tourists and visitors, whilst also perhaps being a mainstay of local diets. Although local or perhaps cross-border trade of potentially contaminated products is probably more likely than international trade, it is not impossible, and exclusion of this parasite from the CCFH guidelines may be considered to diminish the perceived importance of this foodborne parasite. Furthermore, the remit of CCFH should not only be concerned with products that are traded internationally.

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Chapter 9
Future Challenges and Final Remarks

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9.1 A New Epidemiological Reality

Important epidemiological changes have occurred worldwide in the past decades. One of the most important is the large migrations from rural areas to urban centers. Nowadays only 21 % of the population of Latin America resides in rural areas. These migration processes have also resulted in changes in the classical rural housing of adobe walls and thatched roofs; new houses are usually made with a zinc roof and walls of waste materials or cement. These changes have helped to minimize the transmission of Chagas disease (ChD) in children.

However, the slums surrounding the cities have grown very fast, causing a significant ecological imbalance. This has resulted in a decrease in wildlife populations on the periphery of urban centers, forcing invasions by triatomines that are of secondary transmission importance for *T. cruzi* (Pifano 1960, 1986; Urdaneta-Morales 2014) due to their low vectorial capacity because of their delayed defecation reflex (*Panstrongylus geniculatus*, *P. megistus*, *Triatoma tibiamaculata, T. rubrofasciata*). However, due to the absence of wildlife to prey upon, these triatomines have ended up as becoming domiciled in houses, thereby posing a transmission risk (Schofield et al. 1999; Reyes-Lugo and Rodríguez-Acosta 2000) - in particular by the foodborne route. At the same time, new settlements in the Amazon region, mainly associated with agricultural activities, have facilitated triatomine-man contact. This is particularly the case for different fruit bunches from palms, such as “açai”, that are

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usually consumed and are frequently colonized by *T. cruzi*-infected triatomines. These cultural and epidemiological factors have provided conditions for the sustained increase in oral transmission of ChD in the Brazilian Amazon area (Valente et al. 1999, 2009; Pinto et al. 2008; Coura et al. 2002).

In addition to migration to the cities within each country in Latin America, over the same period of time the emigration of citizens from endemic countries to non-endemic countries has increased; it has been estimated that approximately 400,000 people infected with *T. cruzi* may have taken up residence in the United States and several European and Asian countries (Gascón et al. 2010). These migrations of human populations have blurred the traditional epidemiology of ChD; it is no longer a condition that should be associated just with rural areas of Latin America, but is now also an urban condition of both endemic and non-endemic countries at other latitudes.

While the traditional transmission routes, such as cutaneous, transfusional, congenital, transplants and laboratory accidents have been declining, oral transmission has been acquiring greater relevance, most notably in the Brazilian Amazon area. Other countries from which oral transmission has been demonstrated, Venezuela, Colombia, Bolivia and French Guyana, have joined the list. In these countries, conditions for the environmental imbalance in urban and rural centers have been created, promoting the invasion and subsequent domiciliation of different triatomine species, where *Panstrongylus geniculatus* has the broadest regional distribution and is the species most often incriminated in oral outbreaks (Alarcón de Noya et al. 2015). In Caracas, where the slums are being colonized by this species, it has been observed that oral transmission is of considerable concern regarding the future situation. In these neighborhoods, large numbers of people are concentrated and exposed daily to the possibility of food being contaminated infected with triatomines within a successful peridomiciliary cycle, due to the close contact with rats and dogs that act as urban reservoirs.

The population size of expansive slums in most big cities thwarts the ready implementation of measures for controlling this metaxenic infection using only intradomiciliary pesticides, and additional measures, as described in this book, need to be considered. In Rio de Janeiro, for example, with a population of 11.8 million inhabitants and 968 favelas (Ferreira 2009) with an estimated population of 1.7 million people, the invasion of triatomines (*Panstrongylus megistus*, *Triatoma tibiamaculata* and *T. rubrofasciata*) is of major concern. There are additional warning signs about changes in the epidemiology of *T. cruzi* in Latin America and lead us to suspect that we are barely detecting only the tip of the iceberg of a new epidemiological reality. This suspicion is reinforced by the fact that 41.2 % of acute cases of ChD in Brazil are orally transmitted and are of urban origin (Pinto et al. 2008), and that six out of the ten oral outbreaks that have occurred in Venezuela are also of urban origin (Alarcón de Noya et al. 2015). Additionally, the implementation of comprehensive school-feeding programs in some countries, such as Venezuela, while of good intention and with nutritional and socio-economic benefit, pose new risks; in fact two of the largest oral outbreaks occurred in school canteens (Alarcón de Noya et al. 2015).

These events stand out not only due to the large number of people affected, but also because the vast majority of cases are children.

Although the occurrence of congenital transplant, or blood transfusion cases as the result of the migration of people with ChD to first world countries has been significant (Gascón et al. 2010), oral transmission has not yet been documented in these countries. However, the introduction of triatomines to Southeast Asia is, and should be, a worry to health authorities; this is the case for *Triatoma rubrofasciata* in Southeast Asia and *Triatoma sanguisuga*, a species brought to India (Schofield et al. 2009). The possible arrival of rats infected with *T. cruzi* in cargo ships, could serve as a gateway for this parasitosis into the Old World.

A further aggravating circumstance that must be borne in mind is that it has recently been demonstrated that the bed bug, *Cimex lectularius,* is an excellent biological vector for *T. cruzi* and able to transmit the infection either by the skin or by the oral routes (Salazar et al. 2015). The worldwide distribution of this arthropod, and the known difficulties in its control, could be of importance regarding their participation in the oral transmission of *T. cruzi* in both endemic and non-endemic countries.

Taken together, these new biological and epidemiological elements predict a more complex and unpredictable future control of ChD, including by oral transmission, which remains often under-estimated by global organizations concerned with food safety. As a disease that is linked to poverty, if progress is not made in the economic, social, and cultural development of inhabitants from urban areas, particularly slum dwellers, there is a strong potential that it will be very difficult to manage. Special emphasis must be placed on schools and other public and private institutions with canteens, where it is necessary to insist on improving food hygiene; this requires not only the regular hygiene measures with which many are familiar, but also protecting kitchens with mosquito nets to prevent the entry and domiciliation of triatomines. As a foodborne disease, the largest prevention support should be based on a HACCP approach focus on the sanitary control of food processing, transport, and distribution to ensure that contact between food and food handlers with triatomines or their droppings is avoided. Obviously, a suitable vector control considerably decreases the possibility of contact.

9.2 A Challenge for the Pharmaceutical Industry

The low cure rate for ChD observed with benznidazole in some countries, such as the case of Venezuela, along with the side-effects of treatment, means that the pharmaceutical industry should be motivated to focus on the development of new drugs for this endemic disease. Early diagnosis and prompt treatment of infected individuals is crucial, particularly when transmission is foodborne as in this grave form of infection there is rapid spread in the body after the efficient penetration and multiplication in the stomach mucosa.

9.3 Education, Communication, and Information Dissemination Are Key

In order to prevent contamination of food and beverages, educational initiatives are essential to ensure that clear and accurate knowledge is spread about the various routes of transmission of ChD. Information should be disseminated at the various different levels of society through education in schools, families, communities, and the health sector. This Brief can perhaps be considered as one small component of information dissemination.

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