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Environment, interactions between *Trypanosoma cruzi* and its host, and health

Meio-ambiente, interações entre *Trypanosoma cruzi* e seu hospedeiro e saúde humana

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Abstract

An epidemiological chain involving Trypanosoma cruzi *is discussed at the environmental level, and in terms of fine molecular interactions in invertebrate and vertebrate hosts dwelling in different ecosystems. This protozoan has a complex, genetically controlled plasticity, which confers adaptation to approximately 40 blood-sucking triatomine species and to over 1,000 mammalian species, fulfilling diverse metabolic requirements in its complex life-cycle. The* Tr. cruzi *infections are deeply embedded in countless ecotypes, where they are difficult to defeat using the control methods that are currently available. Many more field and laboratory studies are required to obtain data and information that may be used for the control and prevention of* Tr. cruzi *infections and their various disease manifestations. Emphasis should be placed on those sensitive interactions at cellular and environmental levels that could become selected targets for disease prevention. In the short term, new technologies for social mobilization should be used by people and organizations working for justice and equality through health information and promotion. A mass media directed program could deliver education, information and communication to protect the inhabitants at risk of contracting* Tr. cruzi *infections.*

Trypanosoma cruzi*; Chagas Disease; Host-Parasite Interactions; Environment*

The ancient game

To understand the relationships amongst living organisms with extensive epidemiological chains, affecting both health and species survival, requires that we begin when life began, in salt water [1,2]. During the early Proterozoic era (the pre-Cambrian period) approximately 4.5 billion years ago, primitive life forms such as eubacteria and archeabacteria emerged from the organic soup on the earth's crust. These pro-karion microorganisms incorporated organic constituents (proteins, nucleic acids, lipids and carbohydrates) which gave rise to life. In the presence of these ingredients, microorganisms initiated the synthesis of ATP (adenosine-triphosphate), universally used for energy storage [2,3].

Every miniscule organism on earth may come in to contact with every other, given sufficient time [4]. This "game" resulted in a revolution approximately 1.5 billion years ago. The approximation of archea and eubacteria led eventually to their association. Subsequently, a biochemical event probably determined cooperation and exchange of nutrients among these organisms. Extreme solidarity became symbiosis, resulting in the formation of undulipodia. This ancient protozoan retained its flagellum, which emerged from the mitochondrion of an archea bacteria spirochete. Other organelles from eubacteria completed the biochemical machinery of this eukarion, a unicellular living microorganism which

contained membrane-wrapped DNA, in a package-type nucleus.

At present, morphological evidence at the ultra structural and molecular levels are consistent with this pathway of primitive life [2].

An occurrence 670 million years ago resulted in ancestral eu-karion undulipodia giving rise to primitive trypanosomes, which were promptly recognized in fish. The flagellate protozoa *Trypanosoma gray*, which occurs in crocodiles, is related to mammal trypanosomes [5]. It was, therefore, assumed that reptiles and batrachians were primitive vehicles that brought these flagellates onto solid ground. The presence of trypanosomatids in the blood of aquatic invertebrates and vertebrates favored secondary acquisition of whole microorganisms by a host during the Phanerozoic period, 570 million years ago [4]. These interactions among the trypanosomes and cold-blooded animals require further study. The construction and remodeling of the undulipodia served to fabricate metazoans. These occurrences explain multicellular organization, which depends on relationships at molecular and physiological levels. The concepts in this review paper show that life is a continuous process and constantly changing.

The protozoa that belong to the Class Zoomastigophorea (Eukaryota, Excavata, Euglenozoa) include the Kinetoplastid flagellates of the Trypanosomatidae family and have a major impact on public health and veterinarian medicine. Phylogenetic analyses have placed the protozoan *Tr. gray,* that can be found in the blood of crocodiles and possibly date to around 480 million years ago, at the root of the kinetoplastids, next to bodonids (*Bodo saltans*). These kinetoplastids include *Tr. cruzi*, the agent of Chagas disease in the Americas, *Tr. brucei*, the agent responsible for sleeping sickness in Africa, and the *Leishmania* species that infect mammals and produce different forms of diseases worldwide [3]. The life-cycles of these trypanosomatids account for their major division into Salivarian and Stercorarian branches, completing the infective metacyclic stages, respectively, in the salivary gland and in the hind gut of invertebrate vectors. This feature introduces important diversifications in the mode of infection transmission to mammals.

Vascular plants and the emergence of invertebrate-vectors

During the Paleozoic and Silurian periods, around 434 million years ago, drastic changes in the mixture of atmospheric gases led to an increase in the oxygen diffusion coefficient in the environment and a constant change in the temperature. In this environment macroscopic plants grew, with roots for uptake from soils and vascular systems for distribution through the plant; in turn, these plants became a major source of food for animals. Those changes made grounds for co-evolution of Annelida-Molusca, which are close relatives of Arthropods, and of vascular plants. Paleontological data indicate that the invasion of the terrestrial environment by vascular plants, arthropods and higher vertebrates occurred relatively late, after the invertebrate phyla were well established in marine environments. Among the vascular plants, the pteridophytes relied upon spores for dispersal. Among animals, complete adaptation to plant-sucking resulted from the development of mouth-parts with a pump connected to the proboscis [5]. Experiments using pteridophytes and living arthropods indicate that some spores remain viable after passing through the gut and hence it is believed that this feeding habit may have also been advantageous to some early plants for propagule dispersion [6]. It has been proposed that many occurrences provided the grounds for diversification into over a million species of arthropods in the Class Insecta: Hemiptera that presently inhabit the earth [7]. This reasoning can help to explain the happenings during the Devonian epoch, 360 million years ago, where accounts of wide scale exchange of organelles, messengers and moieties have been reported among early species of life [2,8]. Numerous representatives of the Class Insecta may have become second stage vehicles for the delivery of macromolecules which were widely exchanged between species. It appears that these insects became dependent on vascular plant carbohydrates that were sucked in by its mouth parts, leading to the accumulation of large energy surpluses. These surpluses could be readily used for subsequent transportation and delivery of whole microorganisms or their spare-parts during further interactions with newcomers.

Efficient oxygen transportation system in warm-blooded animals

During early life, the plant and animal kingdoms branched into two different energy-producing systems. Photosynthetic chloroplasts in green plants captured energy directly from the solar system, and mitochondrion cytochrome in extant microorganisms used anaerobic metabolism which enabled them to survive during a time when oxygen on the earth's surface was scarce [2,8]. Following modifications in the gas composition of the atmosphere, a respiratory chain de-

veloped in vertebrate animals that carry ionized Fe^{2+} in the hemoglobin molecule. A constant flux of hemoglobin-rich red blood cells made it possible for hemin-bound Fe^{2+} to become an important link in a metabolic pathway which is closely related to species fertility and reproduction [9,10]. In vertebrate animals, an increase in oxygen led to complex biochemical pathways using a high consumption of glucose and energy production, resulting from a reaction requiring ATPases, enzymes that hydrolyse ATP into Adenosine Difosfato (ADP) with the release of inorganic phosphorus [11]. These energy-producing metabolic pathways generated efficient species mobilization, growth and reproduction throughout the kingdom. This biochemical pathway was promptly acquired by marsupials, which are the most ancient mammals on earth, being present since the Permian era, 245 million years ago [3]. Thereafter, the circulation of blood increased considerably, with numerous species of small mammals appearing on earth during the Triassic and Mesozoic periods, 208 million years ago. It took a long time until an efficient hemoglobin system for oxygen capture and transportation appeared in the triatomines, during the Cretaceous period, 100 million years ago. At present, there are approximately 14,000 species of insects, which depend on ionized iron [Fe^{2+}] bound to a heme protein, in the core of the hemoglobin molecule, to complete their complex life-cycles. There follows a short account of these insects in the triatomine subfamily that suck blood from a wide variety of vertebrate animals and initiate a broad chain of transmission of blood-born trypanosomes.

The coevolution of Trypanosomes and Triatomines

The adaptation of trypanosomes to the intestine of triatomines created grounds for species survival, growth of *Tr. cruzi* forms and differentiation of infective metacyclic trypomastigotes. The coevolution of trypanosomes and triatomines appeared to result from their gradual adaptation to invertebrate and vertebrate hosts, possibly between 99.8 and 93.5 million years ago. Although there are no secure recordings of such putative adaptation steps, there are closely related accounts among lizards and insect-vectors in Baja California. In the absence of hot-blooded animals in that ecosystem, infections of lizards that ingested triatomines contaminated with *Tr. cruzi,* as well as the complete life-cycle of the parasite, can be observed [3]. These observations are of practical importance, since it appears that reptiles were ancient reservoirs of *Tr. cruzi* populations, now infecting man and domesticated mammals. Some insects deciphered that hematophagy helped their development and growth, hence small animals that populated the American Continent became providers of blood for these predators.

Triatomine and *Tr. cruz*i coevolution is generally considered to have been amazingly successful. It was shown that following blood ingestion, the flagellate protozoa remains in the insect's fore-gut for a few days. There, the ingested blood trypomastigotes transform into epimastigotes, which multiply by binary fission and colonize the gut. The insect's hind-gut epimastigotes transform into non-dividing metacyclic tripomastigotes that pass with excreta. Amastigotes may usually be found in the mid-gut of insects subjected to prolonged fasting [12]. The development of the parasitic forms in the triatomine's gut depends on interactions between the parasite and the intestine lining mucosal cells as the epimastigotes forms adhere to a peri-microvillar membrane. This membrane protects the mucosal cells against trauma by pathogens, and by toxins and chemical compounds [13,14,15]. The partial permeability of the peri-microvillar membrane to macromolecules regulates the flow of nutrients to mucosal cells at different compartments of the insect's gut. Also, it has been considered that 10 days after *T. infestans* and *Rhodnius prolixus* feeding, the peri-microvillar membrane becomes a selective physiologic barrier for enzyme regulation of absorption and digestion of blood nutrients [16,17]. The full development of peri-microvillar membrane lining mucosal cells of triatomines depends on abdominal distension after a full blood meal, provoking neurosecretion of a prothoracicotrophic hormone [18]. This hormone acts upon prothoracic glands producers of molt-inducing ecdisone [19]. Also, hydrophobic proteins and carbohydrate residues that bind the *Tr. cruzi* to peri-microvillar membrane lining mucosal cells have been identified [19]. The study showed significant differences among trypomastigote and amastigote membrane bound molecules such as lipids, carbohydrates and proteins. Actually, the *Tr. cruzi* genome sequencing revealed an increasing plethora of genes expressing metaloproteases, mucin and transialidase associated proteins related to the parasite evasion of the insect's innate and acquired immunities [20]. Furthermore, it was shown that some insect's gut enzymes are essential for the parasite development. Serine-, cystein- and aspartic-proteases, carboxi-peptidases and aminopeptidases in the insect's gut are important for blood digestion and for absorption of its by-products [21]. However, the

triatomines' digestion appears to be trypsin-free and catepsin-dependent, which favor adaptation to its acidic intestine environment. Also, lectins, hemolytic factors and α-D-globin peptides influence the development of the parasite in the insect's gut [13,21]. In addition, high levels of superoxide and the presence of a cationic peptide named defensin prevent the parasite invasion in the insect's heme lymph. Lately, it has been shown that the inoculation of *Tr. cruzi* in the *R. prolixus* heme lymph led to production of immune-related molecules such as nitrophorin, iron-related transferins and a prophenoloxidase activating protease [22].

Interactions of *Trypanosoma cruzi* with vertebrate hosts

The Stercorarian protozoan *Tr. cruzi* has a complex life-cycle. It undergoes extracellular multiplication in the invertebrate host, but grows by obligate intracellular multiplication cycles in vertebrate hosts. The metacyclic trypomastigotes enter the human body through triatomine-made skin abrasions at the point of the insect bite. It appears that insectivorous mammals used to get the *Tr. cruzi* infections *per os*. Regardless of the route of entry, the parasite interacts with skin histiocytes or with intestinal mucosal mononuclear cells, and macrophages occur immediately. After cycles of multiplication into the phagocytes, the *Tr. cruzi* trypomastigotes are released into blood circulation and the infection spreads to the body tissues. The parasitic forms can enter any cell type in the body, except the neurons [3].

The entry of *Tr. cruzi* forms into host cells causes a wide variety of interactions with surface membrane glycoproteins, hydrolytic enzymes and diverse signaling metabolic pathways of cell growth and differentiation [10,23]. Ancient biochemical signaling pathways associating cell growth and differentiation were found to be similar in the protozoa and in its vertebrate host cell. It was also shown that some strategic features of parasite-host cell invasion appear to be unique to this infectious process. Earlier studies had shown that invasion of the host cell by *Tr. cruzi* had a feature similar to that involving bacterium and a phagocyte, in which actin cytoskeleton mobilization and emission of pseudopodia led to engulfment of the microorganism by the host cell [24]. Other experiments showed that the *Tr. cruzi* invasion augments significantly when host cells are treated with a specific actin inhibitor [25,26]. Furthermore, it was shown that the treatment of cells with cytochalasin did not prevent the *T. cruzi* invasion, whereas the entry of the intracellular Salmonella was readily blocked by an actin inhibitor [24]. This finding is in keeping with electron microscopy images, where there is a lack of pseudopodia at the site of the *Tr. cruzi* invasion in the plasma membrane of the host cell [24].

It appears that the signaling pathways initiated upon parasite contact with the host cell are critical checkpoints in the invasion process [25,26]. The invasion occurs after recruitment of vacuoles beneath the plasma membrane level, which invaginates to increase the ratio of fusion with lysosomes. It has been said that lysosomal fusion is essential for the retention of *Tr. cruzi* inside host cells [24]. Biochemical events related to parasite invasion include increasing concentration of Ca^{2+} intracellular levels prior to lysosome fusion, which is triggered by parasite-induced stress at the plasma membrane level [25,26,27]. Also, increasing levels of cAMP (cyclic adenosine monophosphate) play an important role in the internalization of the parasite into the host cell and adenyl cyclase inhibitors reduce the rate of invasion [24]. In contrast, increasing intracellular levels of cAMP and Ca^{2+} have been associated with exocytosis in many cell types [24].

In summary, the parasite invasion of a host cell evokes plasma membrane vesicle transportation, requiring blockage of the cytoskeleton actin barrier prior to fusion with the lysosomes. After internalization, the length of permanence of the parasitic form in the parasitophorous vacuole appears to be short; the infections may be controlled by innate and acquired immune response mechanisms which are efficient in eliminating the parasites. On the other hand, the virulent trypomastigotes readily disrupt the lysosome-like vacuole, and the parasitic forms are set free for replication in the cytosol of the phagocytes [24]. Some proteins that have been associated with iron transport [10] may well be essential for parasite replication within macrophages and non-phagocytic cells. For *Tr. cruzi* molecular interplays of the host cell associated with parasite location, invasion and retention, the reader should consult a recent review [24].

The escape of parasites into the host cell cytoplasm may occur during S phase, in which a stress-induced burst of oxygen leads to an increase in glucose consumption and energy production, thus triggering signaling pathways for parasite and host cell growth and differentiation. *Tr. cruzi* infections of monocytes, macrophages and tissue histiocytes appear to be self-limiting, possibly because the parasite may not tolerate the acidic environment of parasitophorous vacuoles or because parasite overload kills the host cell. However, under a moderate parasitic load, over a short period of time, the dividing amas-

tigotes in the cytoplasm of muscle cells appear to undergo a full cell cycle, whereby free-swimming trypomastigotes are released and reach the blood stream to infect other cells in the body. For instance, after reaching muscle cells, dividing amastigotes appear to be out of the reach of immune system factors. Natural infections in humans and experimental chronic infections in laboratory animals show small, non-dividing *Tr. cruzi* amastigote forms to be dormant, due to the lack of tissue reactions in their surroundings. This parasite form in healthy muscle tissue is herein called "*hypnomastigont*". The finding of muscle cell "*hypnomastigont*" nests (http://www.ecb.epm.br/~renato/nest.JPG) may explain the long lasting chronic infections in patients showing fully mature specific immune responses.

"*The* Trypanosoma cruzi *perpetuation would hardly be possible without the renewing forces of sexual reproduction*" [28] (p. 64). In spite of Chagas' [28] opinion, *Tr. cruzi* sexual reproduction was not considered an option for explaining the genetic diversity of *Tr. cruzi* populations that circulate in invertebrate and vertebrate hosts. More recently, some studies have shown that trypanosomes isolated from a single Chagas patient are made up of diversifying populations [3]. It was expected that each *Tr. cruzi* was a clone, giving rise to a homogeneous parasite population. However, it has been shown that such *Tr. cruzi* populations show enormous plasticity, and are potentially able to transpose their own lineages. A direct explanation to the above observations was given by Gaunt and co-workers [29], showing that haploid and aneuploid *Tr. cruzi* forms originate from sexual reproduction. In this regard, Devera et al. [30] propose the designation "cruzi complex", which encloses the entire potential of parasite population diversity. It appears that sexual reproduction generates the enormous genetic diversity that has been observed in *Tr. cruzi* isolates from humans, as well as from wild invertebrate and vertebrate animal reservoirs.

In recent years, a highly intimate interaction between intracellular *Tr. cruzi* forms and the host cell has been brought onto the scientific stage by the work performed at our laboratory [31,32,33,34]. These authors have described the horizontal transfer of *Tr. cruzi* mitochondrial minicircle DNA to the genomes of mammals and birds. The minicircle integrations within LINE-1 retrotransposon appeared to create the potential for foreign DNA mobility within the host genome via the machinery associated with that element. On one occasion, the minicircle sequence integrated into a LINE-1 retrotransposon, and subsequently relocated to another genomic location in association with the parasitic DNA. As a consequence of the translocation, the p15 locus was altered, resulting in elimination of p15 mRNA [33]. This phenomenon produced gene knock-out, which is a molecular pathology stemming from mobilization of a kDNA-LINE-1 mutation. It appears that the mutation-made genomic modification and subsequent transcript variation is consistent with the hypothesis that genotype induced phenotype alterations might be a causal component of parasite-independent, autoimmune-driven lesions in Chagas disease.

The above description detailing fine interactions among parasitic forms and host cells is elaborated in this review article, as we aim to show the importance of environmental factors at the molecular level, which can cause severe effects on human health.

The triatomines and mammal reservoirs

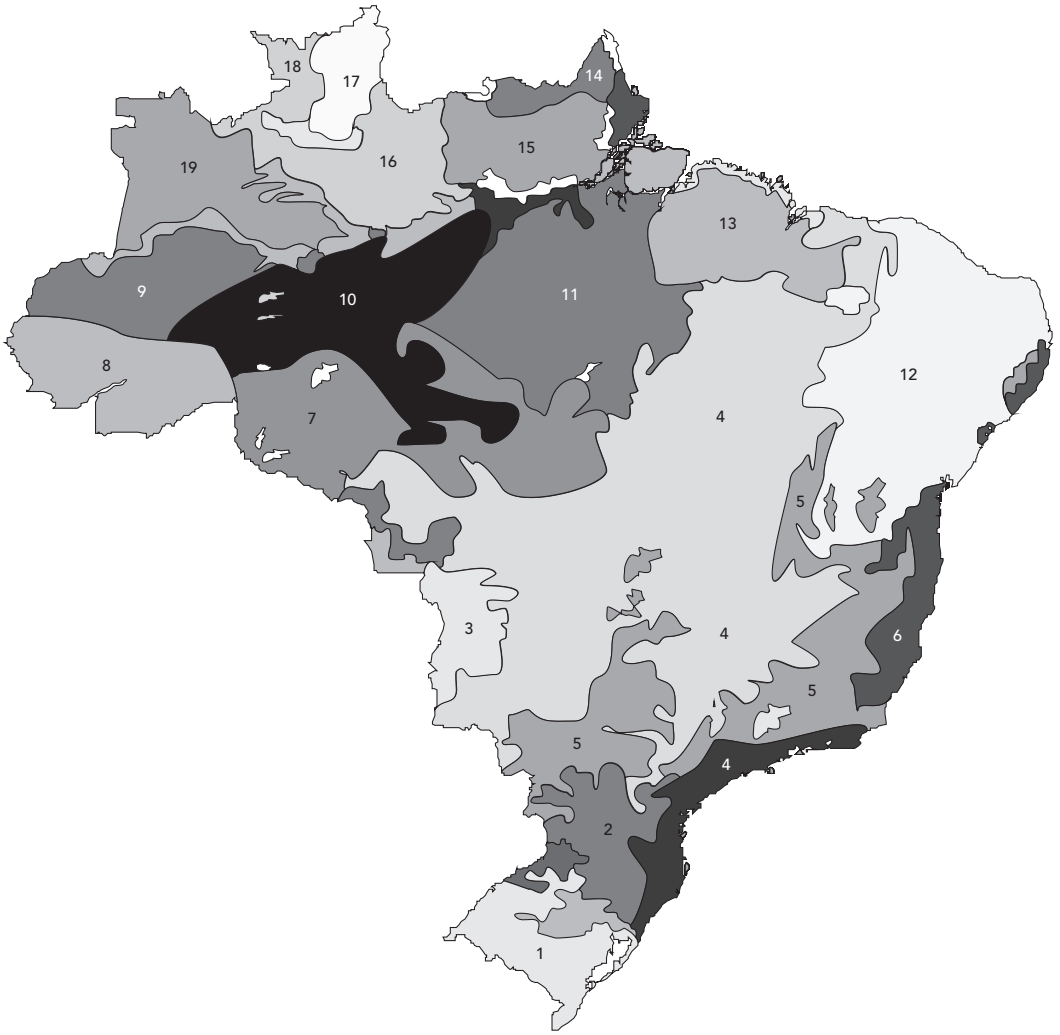
The triatomines of the Reduviidae family include those strictly hematophagous insects belonging to the subfamily Triatominae, that became readily adapted to different types of ecosystems [35]. The broad diversity among triatomine vectors of *Tr. cruzi* infections results from sexual reproduction [35]. The eggs hatch nymphs of first instars, which reach fifth instars after four molts and then become adult. Each triatomine stage is strictly hematophagous. A full blood meal is required for molting; however, when a nymph reaches the adult stage it requires multiple blood meals for copulation and oviposition. This feature of the insect life-cycle, requiring constant feeding on different prey, appears to have broad epidemiological importance, as it is a multiplication factor for transmission of infections.

One aspect deserving specific attention is the divergence that took place during the Cretaceous period in the early Mesozoic era, introducing modifications in existing triatomine tribes of some major ecosystems, which meant that the fulfillment of feeding patterns required for completion of the insects' life-cycles became possible. The Rhodniini tribe dwells in the humid tropical broad-leaf forest, and is mainly adapted to life in palm trees. On the other hand, the Triatomini tribe became adapted to rock crevices, tree burrows, under tree barks, and in burrows in the ground, which serve as dwellings for small mammals in the dry ecosystems of the cerrado (or savannah) and caatinga. Table 1 shows the main species of triatomines inhabiting major ecosystems. The diversity of triatomines adapted to dry climate ecosystems is observed alongside the large range of mammals that serve as blood sources for the insect

vectors co-inhabiting animal dwellings. These reservoir hosts, upon whom triatomines prey, participate in the transmission cycle of *Tr. cruzi* and belong to the Classes, Marsupialia, Edentata, Chiroptera, Carnivora, Arthiodactyla, Rodentia and Primata. Over 1,150 wildlife mammalian species belonging to these seven classes are potential reservoirs of *Tr. cruzi* [3]. Additionally, a broad diversity of insect-vectors spread the *Tr. cruzi* infections to those mammals dwelling in 19 defined ecosystems (Figure 1) [36]. Although field studies, aiming at the discovery of wild life triatomines, have been designed on the basis of the political division of the Brazilian Federation, it is expected that future studies will focus on specific features of the ecosystems, determining

Figure 1

Main ecosystems in Brazil.



(1) Savannah; (2) Araucaria forest; (3) Mato Grosso Pantanal swamp; (4) *Cerrado* (savannah); (5) Inland Atlantic forest; (6) Coastal Atlantic forest; (7) Flooded grasslands; (8) Southeast Amazon forest; (9) Rondonia and Mato Grosso rain forest; (10) Choco-Darien humid Forests; (11) Tapajós/Xingu humid rain-forest; (12) *Caatinga* (Scrub Forest); (13) Tocantins humid rain-forest; (14) Guiana humid rain-forest; (15) Amapá humid rain-forest; (16) Uarama humid forest; (17) Guyana savannah; (18) Guiana humid forest; (19) Juruá/Negro humid forest.

Source: Dinerstein et al. [36].

species selection pressure and disease transmission. Pressures imposed by climate, vegetation and fauna are main actors in the huge *Tr. cruzi* epidemiological chain, which is independent of political divisions of the national territory. The field study approach by ecosystems appears to serve better future strategies for control and prevention of disease transmission. In this respect, many more field studies are required to elucidate triatomine distribution in major ecosystems. Studies aiming at prevention of insect-borne disease should consider as top priority environmental public health problems. Policy-makers should build up County, State and Central Federation consortia to deal with disease prevention activities.

A wide range of factors in the major ecosystems pose a real, every day threat for the spread of the epidemic and these should to be dealt with on a permanent basis. Meanwhile, various wild species of animals interact with elements participating in the *Tr. cruzi* epidemiologic chain and require original solutions to create novel strategies for control of infections transmitted to the human population. It is impossible to eradicate many actors belonging to diverse phylum in the kingdom from nature, which have been interacting in the various ecosystems for several million years, therefore making the prevention of Chagas disease a very difficult task. This concern leads us to suppose that interactions within such a complex epidemiological chain require proper use of the land and preservation of ecosystems for securing human health. The anthrop predation that causes outbreaks of acute *Tr. cruzi* infections in human populations is described in the following section.

Emerging Chagas disease

The American trypanosomiasis is an ancient zoonosis that emerged approximately 95 million years ago [3]. Since that time, the triatomine vectors that had developed hematophagy could sustain the symbiotic *Tr. cruzi* in their gut. It is important to note that the insect and protozoan were brought together due to a metabolic necessity, the association with Fe^{2+} needed for fertility, reproduction and completion of their life cycles. Nowadays, possibly as a consequence of these interactions, insect vectors and mammal hosts sympatrically occupy vast areas of South America.

The introduction of *Homo sapiens* in the enzootic areas may have occurred upon arrival of Polynesians on the Continent about 50,000 years ago [37]. An early sylvatic cycle, which used to maintain the *Tr. cruzi* infections among mammal reservoirs, was readily introduced into Amerindians. By that time, it appears that recrudescence of epidemics took place after small mammals were domesticated and the insect-vectors initiated colonization of human dwellings, about 9,000 years ago [38,39,40]. The readiness with which pathways made by insect-vectors carrying the *Tr. cruzi* infections were reconstructed when the new settlers arrived on the American continent and acquired Chagas disease should be noted. These reconstructions appear to be essential for understanding this major zoonanthroponosis, which is now considered an important public health problem. At present, new studies about *Tr. cruzi* infections in different ecosystems are fundamental for obtaining sound information about their many camouflage features, hidden within wild and human populations [41, 42]

In this review, we consider insecticide spraying, leading to subsequent dislodgment of *Triatoma infestans* from human huts in the Brazilian dry ecosystems of the cerrado and caatinga, to be a very important activity, as it resulted in a spectacular decrease in the ratios of vector-transmitted *Tr. cruzi* infections in these human populations.

However, data showing the distribution of triatomines in these dry ecosystems reveal at least six other main species carry *Tr. cruzi* infections and, therefore they are potentially infective to human populations [35]. On the other hand, each of the 19 main ecosystems, composing the Brazilian landscape, harbor triatomine species contaminated with *Tr. cruzi*. These species include *Panstrongylus megistus* adapted to humid ecosystems, which is the main transmitter of the infections in the moist Atlantic forest and where this branches into many different ecosystems along streams and swamps. The importance of *P. megistus*, a major vector transmitting the *Tr. cruzi* infections, has been recognized since the year 1909 when Chagas discovered the flagellate in the insect's gut in [43]. The ubiquitous habits of *P. megistus,* characterized by its adaptability to peridomicile and sylvatic life, where it is capable of obtaining blood from different animal hosts, as well as from humans, make this triatomine an important target for future field studies aimed at the prevention of transmission of *Tr. cruzi* infections in the humid ecosystems where this species dwells (Table 1).

Other species thriving in the humid Atlantic tropical forest are equally important: *T. tibiamaculata*, *T. vitticeps*, *P. geniculatus* and *P. lignarius*. Frequently, these triatomines have been captured in a variety of palm tree species of various ecosystems [35]. Each of these species has been associ-

Table 1

Feeding habits and ecotopes of main triatomine species transmitting *Trypanosoma cruzi* infections in ecosystems located in Brazil.

Triatomine	Climate/Ecosystem	Ecotopes	Feeding habits
Triatoma			
T. infestans	Dry/*Cerrado, Caatinga* (scrub forest) and savannah	Artificial: intra- and peri-domicile; sylvatic: mammal dwellings on rocks and in the ground.	Synantrophic but highly antropophylic
T. tibiamaculata	Moist/Atlantic coastal forest	Sylvatic: palm trees in proximity to mammal dwellings; artificial: peri-domicile.	Synantrophic
T. viticipes	Moist/Atlantic coastal forest	Same as *T. tibiamaculata*	Synantrophic
T. sordida	Dry/*Cerrado*	Sylvatic: burrows, and under tree bark; artificial: peri- and intra-domicile	Synantrophic
T. braziliensis	Dry/*Caatinga*	Sylvatic: on rocks close to rodents' dwellings; artificial: peri- and intra-domicile.	Synantrophic
T. pseudomaculata	Dry/*Caatinga*	Sylvatic: birds' nests; Natural: intra- and peri-domicile	Ornitophylic; occasionally anthropohylic
T. rubrovaria	Dry/Savannah flat land	Natural: intradomicile	Anthropophylic
Panstrongylus			
P. megistus	Moist/Atlantic coast and in broad leaf areas within any ecosystem.	Sylvatic: on rocks, wood bark and on the ground. Artificial: peri- and intra-domicile	Synantrophic and highly antropophylic
P. lutzi	Dry/*Cerrado* and *caatinga*	Sylvatic: dwellings of armadillos; artificial: peri- and intra-domicile	Synantrophic
P. geniculatus	Humid/Ubiquitous	Sylvatic: palm trees	Ornitophylic, occasionally antropophylic
P. lignarius	Humid/Broad leaf forest	Sylvatic: palm trees	Ornitophylic
Rhodnius			
R. neglectus	Ubiquitous	Sylvatic: palm trees; Artificial: peri-domicile	Ornitophylic, synantrophic
R. nasutus	Ubiquitous	Sylvatic: palm trees; Artificial: peri-domicile	Ornitophylic, antropophylic
R. pictipes	Humid/Broad leaf forest	Sylvatic: palm trees; Artificial: peri- and intra-domicile	Synantrophic, antropophylic
R. robustus	Humid/Broad leaf forest	Sylvatic: palm trees	Synantrophic
R. brethesi	Humid/Broad leaf forest	Sylvatic: palm trees	Synantrophic

Note: the data compiled from previous publications (Coura et al. [42]; Teixeira et al. [41]; Diotaiuti [35]) show that triatomines are considered to be important in the transmission of *Tr. cruzi* infections in Brazil.

ated with transmission of the *Tr. cruzi* infections to the human population. An outbreak of acute Chagas disease in a resource area of the Atlantic coast [44], comprising the county of Navegantes, in the State of Santa Catarina, Brazil, was readily identified and immediately broadcasted, but generated great public concern. This epidemic was apparently limited to less than 30 people and direct sampling of their blood confirmed the diagnosis of acute *Tr. cruzi* infections. Two patients died. However, the public would be shocked if they knew that for each acute case there are around 100 infections that go unperceived. The epidemiologic study in the area where these cases were identified showed a direct correlation between the ingestion of sugar-cane juice and the infections. Palm trees were found at a particular spot where sick people had drank this juice. The search for triatomines in the palm clefts revealed *T. tibiamaculata* that harbored the *Tr. cruzi* forms in their guts [45]. It was assumed that the triatomines with *Tr. cruzi* in their intestinal contents

were attracted by light and, then, they may have contaminated the sugar-cane mill. Other case studies have implicated different species of triatomines, thriving in different ecosystems, with *Tr. cruzi* contamination of food ingested by people during barbecues [46,47].

Nowadays, *Tr. cruzi* infections occupy every ecosystem where suitable insect-vectors have been captured and identified. Regardless of current knowledge, early maps showing distribution of human *Tr. cruzi* infections in Brazilian territory did not show human Chagas disease in the major humid broad-leaf tropical forest. Nevertheless, many outbreaks of acute Chagas disease arising in the Amazon Basin over the last three decades have been reported [47,48]. These observations meant that frequent outbreaks of the *Tr. cruzi* infections in the Amazon have been brought to the public's attention. It has been hypothesized, therefore, that severe anthropic modifications introduced in the Amazon basin, may correlate with outbreaks of acute *Tr. cruzi* infections in that major ecosystem, with expected dramatic changes in ratios of disease prevalence in the region [41,42,47,48]. This hypothesis was examined in a study area in the county of Paço do Lumiar, in the State of Maranhão [41]. This case study is presented below.

Trophic network and the cycle of transmission of *Trypanosoma cruzi* from palm trees in the Amazon

The tropical moist broadleaf forests of the Amazon Basin are increasingly subjected to anthrop modifications. Although blocks of original habitats are relatively intact, some ecoregions have been converted or degraded, and elements of their biodiversity have been eroded. Correspondingly, population numbers of wildlife species have decreased where human settlers have established land colonization and villages, which are now considered threats to native species and communities. We hypothesize that human predatory economic activity in a defined ecoregion of the major tropical broad-leaf moist forest poses a risk for transmission of *Tr. cruzi* infections. A field study was conducted to assess seropositivity for these infections in the human population of Paço do Lumiar County and to search for triatomines that harbor these infections in palm trees [41].

An indirect immunofluorescence test was used to search for anti-*Tr. cruzi* antibodies in human blood, and positive results were found to increase in younger sections of the populations studied, where recent transmission and acute infections were found in 0.18% of children (46 in total) below the age of 10. Seroprevalence of *Tr. cruzi* infections in the Paço do Lumiar County human population was in the absence of hematophagous insects or their vestiges in the dwellings. A search for triatomines was carried out in the ecosystem surrounding the households where the population was infected or continues to be at risk of contracting the infections. This approach counted on the local population for the capture of triatomines in their houses, which were kept in plastic containers. These containers were delivered to the laboratory for microscopic search of metacyclic trypomastigotes in the insect's hind gut. In addition, palm trees in the backyards of households in five villages were cut down and dissected [41].

Using this householder-assisted surveillance and capture method, 36 *R. pictipes* and 16 *R. neglectus* were obtained. On the basis of this information, it was concluded that triatomine excreta and molted skins in these houses were either not reported by the inhabitants or detected by field workers. Also, different developmental stages of 133 triatomines were captured in clefts of palm frond-sheets carefully dissected in backyards in five villages. However, the remains of animal species were found in their nests in palm clefts where triatomine bugs rest and prey. Marsupials and birds were easily detected on palm fronds and crowns. Furthermore, we identified molds and captured and identified different species of various taxa of invertebrate and vertebrate animals upon dissection of 23 palm trees. Molds were found in stipes, fronds, and crowns, and insects in roots, stipes, inflorescence, fruits, fronds, crowns and leaves. The clefts formed by frond sheets were rich in Amphibia, Arachnida, and Hemiptera. Triatomines were detected at the bottom of clefts where marsupials build their nests. Bird nests were found in the fronds and crowns where abundant species of insects were available for predation.

Also, ten marsupials were captured in their nests in palm trees throughout the neighborhood. These marsupials presented few blood flagellates, which could not be demonstrated by direct microscope examination. However, the metacyclic flagellates were recovered from nine out of ten marsupials subjected to xenodiagnoses and hemocultures. Furthermore, these flagellates were expanded in liver infusion tryptose medium (LIT) cultures aiming at further isolating characterization. Phenotype and genotype molecular markers were used to demonstrate whether these isolates are virulent *Tr. cruzi*. In the first group, antibodies in serum of chronic Chagas disease patients reacted with antigens on culture forms of archetype *Tr. cruzi* Berenice

stock and, also, with isolates DM1, DM2, and DM3 from *Didelphis marsupialis* and with *Rp1* from *R. prolixus*. In the second, genotype kDNA and nuclear DNA markers were used to characterize these wild flagellates. PCR amplification of DNA from each of these isolates showed patterns that were similar in standard virulent *Tr. cruzi* and in test isolates [3]. The molecular characterization was further confirmed by *in situ* hybridization of wild *Tr. cruzi* isolates with a probe derived from *Tr. cruzi* Berenice [3].

It was shown that trophic networks comprising six different levels sustain the cycle of transmissions of *Tr. cruzi* in Babassu trees located in the backyards of households in five villages in Paço do Lumiar County, Maranhão State, Brazil. Developmental stages of *R. pictipes, R. neglectus* and *P. lignarius* were captured in palm trees (68% *Tr. cruzi* infected) and in houses (28% *Tr. cruzi* infected). These triatomines feed on birds, marsupials, rodents, dogs and horses. However, 6.8% of *R. pictipes* captured inside households had fed on human blood. Immunologic, genetic and molecular biology assays disclosed that the flagellates infecting reservoir hosts and humans are indeed virulent *Tr. cruzi* that could be associated with a growing prevalence of the infections in the young human population. The data show the importance of an intact trophic network to keep the *Tr. cruzi* transmitters in their sylvatic environment. Anthropic predation of fauna, with resulting scarcity of wild animals to feed upon, is considered a primary cause of the spread of *Tr. cruzi* infections to the human population.

Discussion and conclusions

Existing evidence suggests that about 670 million years ago an ancestral undulipodia gave rise to primitive trypanosomes, which were promptly recognized in fishes inhabiting the oceans ever since. These are considered to be the closest relatives of the trypanosomes. It therefore appears that amphibians, not mammals, are ancient reservoirs of Trypanosomatids that gave rise to *Tr. cruzi*. We believe that life flows and changes continuously and, therefore, the trypanosome evolution feature, which links *Tr. cruzi* relatives to early reptiles and amphibians, should be carefully considered by scholars, before propagating eradication of *Tr. cruzi* infections and Chagas disease.

During the cretaceous period, around 100 million years ago, insects in the subfamily triatomine, sucking up blood from a broad variety of vertebrate animals, founded the basis for a broad chain of transmission of trypanosomes. At the present time, the blood-borne *Tr. cruzi* infections are transmitted by triatomine vectors to around 1,150 mammal species belonging to seven major classes. Therefore, the complexity of the *Tr. cruzi* life-cycle involving at least 40 species of triatomines and over 1,000 mammal hosts, inhabiting 19 major ecosystems, supports the assertion that presently available strategies for preventing the *Tr. cruzi* infections may not be sufficient to protect the human population. We believe that the described gamut of actors, playing important roles in a multi-factorial chain of events related to transmission of *Tr. cruzi* infections in different ecosystems, requires further studies in laboratories and in the field, to find novel strategies for its prevention and control.

The different levels of interactions of *Tr. cruzi* in invertebrate and vertebrate hosts require much research at the parasitological, genetic, molecular biochemistry, immunology and pathology levels. Future research and development should unravel intricate features of parasite-host cell 'cross-talking' that leads to either a long-lasting symbiotic relationship with no harm to the host, or to an autoimmune type disease causing several biochemical and molecular disturbances at physiological and pathological levels, leading to clinical manifestations and Chagas disease. It should be emphasized that the hierarchy distinction among laboratory and field research work is solely defined by quality that yields a real contribution to knowledge. Such epistemological definition is essential, because a broad variety of questions have been brought into play by an enormously complex epidemiologic chain of events related to *Tr. cruzi* infections and Chagas disease. This requires a multidisciplinary approach and hard work to unravel the problem over several years.

Hopefully, many unresolved questions may drive scientific research to generate answers and new tools for the prevention and control of human Chagas disease. Meanwhile, the selfish competition that tends to push laboratory (academic) and field (pragmatic) workers apart should be avoided at all costs. In this respect, the results stemming from a field study that led to the description of a trophic network connecting the risk of contamination of the human population with the *Tr. cruzi* infections emerging from palm trees in the Amazon was described. This required a multidisciplinary approach and close collaboration among laboratory workers and field researchers. An alternative route could be to build up accessible knowledge on organization of space, promotion empowerment and development, aiming at disease prevention and environmental preservation. This knowledge could lead

to the formation of a critical conscience united with political participation, required to achieve social transformation that secures equality and social justice [49].

Concluding remarks

New control strategies need to be devised before success can be obtained in eliminating the species close to human populations in rural areas, as well as the peripheries of towns and cities. We consider the risk factors associated with the possibility of emergence of Chagas disease in a major ecosystem as follows: (a) invasion of an ecosystem by triatomine species which could be easily adapted to feeding upon human blood; (b) deforestation and new population settlements, shifting cultivation, and rapid human colonization of the vectors' natural ecotopes as well as predation of wild fauna, with a subsequent lack of multiple blood sources for the vectors. Therefore, the control of emerging Chagas disease in major ecosystems appears to be an enormous task for the reasons pointed out above, especially those related to its complex, multi-factorial trends associated with vector transmitted *Tr. cruzi* infections.

In this paper, we emphasize that control of vector-transmitted *Tr. cruzi* infections should rely initially on an information, education and communication program, which encourages control measures by the householder. For example, the identification of triatomine in the proximity of the household and its elimination by cleaning and spraying with insecticide, the use of screens, bed nets, and vegetation management with conservation of local fauna, should be encouraged. Also, a program for preventing the human population from close contact with triatomines should be conducted directly in communities, elementary schools, churches and social clubs, reinforced by social marketing and mass media communications. Finally, further studies are also needed. These may not necessarily be similar to those already shown to be partially effective in controlling the vectors of *Tr. cruzi* infections in various ecosystems. It appears that the inhabitants in each of 19 ecosystems in the Brazilian territory should be promptly informed about the modes of transmission of the infections, with emphasis on measures which avoid contact with a broad variety of insect vectors and animal reservoirs, excreta and fresh tissues that may pose daily threats to human health.

Resumo

Uma rede epidemiológica envolvendo o Trypanosoma cruzi *foi discutida nos níveis ambientais e de interações moleculares nos hospedeiros que habitam em 19 diferentes ecossistemas. O protozoário tem uma enorme plasticidade controlada geneticamente que confere sua adaptação a cerca de quarenta espécies de triatomíneos e mais de mil espécies de mamíferos. Essas infecções estão profundamente embutidas em inúmeros ecótopos, onde elas estão inacessíveis aos métodos de controle utilizados. Muito mais estudos de campo e de laboratório são necessários à obtenção de dados e informação pertinentes ao controle e prevenção das infecções pelo* Tr. cruzi *e as várias manifestações da doença. Ênfase deve ser dada àquelas interações que ocorrem nos níveis celulares e ambientais que se poderiam tomar como alvos seletivos para prevenção da doença. Novas tecnologias para mobilização social devem ser disponibilizadas para os que trabalham pela justiça e pela igualdade, mediante informação para a promoção da saúde. Um programa direcionado de educação de massa pode prover informação e comunicação necessárias para proteger os habitantes atualmente expostos ao risco de contrair as infecções pelo* Tr. cruzi.

Trypanosoma cruzi; *Doença de Chagas; Interações Hospedeiro-Parasita; Meio Ambiente*

Contributors

This article is a team-work production.

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