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REVIEW

An Update on the Knowledge of Parasite–Vector Interactions of Chagas Disease

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Abstract: This review focusses on the interactions between the etiologic agent of Chagas disease, *Trypanosoma cruzi*, and its triatomine vector. The flagellate mainly colonizes the intestinal tract of the insect. The effect of triatomines on trypanosomes is indicated by susceptibility and refractoriness phenomena that vary according to the combination of the strains. Other effects are apparent in the different regions of the gut. In the stomach, the majority of ingested blood trypomastigotes are killed while the remaining transform to round stages. In the small intestine, these develop into epimastigotes, the main replicative stage. In the rectum, the population density is the highest and is where the infectious stage develops, the metacyclic trypomastigote. In all regions of the gut, starvation and feeding of the triatomine affect *T. cruzi*. In the small intestine and rectum, starvation reduces the population density and more spheromastigotes develop. In the rectum, feeding after short-term starvation induces metacyclogenesis and after long-term starvation the development of specific cells, containing several nuclei, kinetoplasts and flagella. When considering the effects of *T. cruzi* on triatomines, the flagellate seems to be of low pathogenicity. However, during stressful periods, which are normal in natural populations, effects occur often on the behaviour, eg, in readiness to approach the host, the period of time before defecation, dispersal and aggregation. In nymphs, the duration of the different instars and the mortality rates increase, but this seems to be induced by repeated infections or blood quality by the feeding on infected hosts. Starvation resistance is often reduced by infection. Longevity and reproduction of adults is reduced, but only after infection with some strains of *T. cruzi*. Only components of the surface coat of blood trypomastigotes induce an immune reaction. However, this seems to act against gut bacteria and favours the development of *T. cruzi*.

Keywords: triatomines, *Trypanosoma cruzi*, behavior, immunity, fitness, reproduction

Plain Language Summary

This review focusses on the interactions between the etiologic agent of Chagas disease, *Trypanosoma cruzi*, and the vector, triatomines. This disease was once entirely confined to mainly rural Latin America with poor housing conditions, but in the last decades, emigrants brought it to a few other countries, eg, in Europe, but there is no endemic transmission outside the Americas. The flagellate mainly colonizes the intestinal tract of the blood-sucking insect. As in all parasite-vector systems, susceptibility and refractoriness phenomena are determined by the combination of the respective strains. The development in different regions of the gut is influenced by feeding and starvation of the triatomines, affecting not only population densities but also the development of specific stages of the flagellate. Considering the effects of *T. cruzi* on triatomines, the flagellate seems to be of low pathogenicity. However, during stressful periods, which are normal in natural populations, effects occur, often in the behaviour. In pre-adult stages, the nymphs, the duration of the

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Introduction

Chagas disease, also known as American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae). It is the only important tropical disease in which the parasite has at first been detected in the vector, triatomines, and later on the infection of humans.[1–3] In 1975/76, the World Health Organization classified it as one of the "Big Six" of tropical diseases and established a "Special Programme for Research and Training in Tropical Diseases".[4] After 2000, it has been included as one of about 17 "Neglected" diseases in "The Millennium Development Goals for Health – Rising to the Challenges".[5] All of these neglected diseases are of low priority to pharmaceutical companies regarding the development of new drugs and for government officials and public health programs.[6] More than 110 years after the detection of *T. cruzi*, public health measures concerning Chagas disease are still lacking in many endemic areas.[7] In Latin America, urbanization has induced the movement of infected rural populations to the cities. Once entirely confined to Latin America, Chagas disease has been spread widely by infected emigrants in the last decades and is now also present in the United States of America, Canada, many European countries and some African, Eastern Mediterranean and Western Pacific countries.[8] However, vectorial transmission is limited to the Americas. According to actual estimations, 6 to 7 million people worldwide are infected with *T. cruzi* and 75 million people are at risk of infection.[9]

An infection in humans is possible if feces and/or urine of infected vectors gets into contact with the bite wound or other skin breaks or mucous membranes of the eyes or the lips (reviewed by[10]) An oral infection occurs after consumption of infectious meat or juices of fruits or sugar cane contaminated with remnants of triatomines. Transfusion of blood or organ transplantation from infected people is another source as is the transmission from mother to child. After the infection, amastigote stages of *T. cruzi* multiply intracellularly and before rupturing the host cell, they transform into nonreplicative blood trypomastigotes that infect new host cells or enter the blood capillaries and circulate in the blood. In the course of the disease two phases occur, the initial acute phase and the chronic phase (reviewed by[10]). In the acute phase, nonspecific symptoms develop, such as fever and a swelling of lymph nodes near the location of the infection. Usually, the parasite can be found in the blood.[11] After about 1–2 months, the disease passes over to the chronic phase beginning with a latent phase, the indeterminate form, lasting from several years to decades with hardly any symptoms.[10] Hardly any parasites are present in the blood.[11] In the final chronic phase, the intracellular development and destruction of cells induces a dysfunction of organs. Large muscular hollow organs – intestinal tract and heart – increase in diameter, a megaorgan syndrome. Especially, pathological effects in the heart may be lethal.[10,12]

Whereas the mammalian host provides cells for the intracellular development and constitutes a very stable environment in the blood offering glucose and a stable temperature, pH, viscosity and osmolality to the blood trypomastigotes, the infection of a vector is a stressful event for *T. cruzi*. In the triatomine, the exclusive extracellular development is restricted to the lumen of the gut and the Malpighian tubules.[13] In the stomach, glucose from the blood is only available for a short period of time, but proteins, amino acids and lipids must be used for metabolism. In addition, the pH becomes increasingly acidic.[14] In this totally different environment, the blood trypomastigotes have to survive and differentiate (reviewed by[15,16]). They transform into spheromastigotes and epimastigotes, the latter multiplying more intensively than spheromastigotes. Finally, metacyclic trypomastigotes develop, possessing a kinetoplast in a more subterminal position than in blood trypomastigotes (reviewed by[17]).

Trypanosoma cruzi: Taxonomy and Strain Peculiarities

Trypanosoma cruzi is a protozoan hemoflagellate that multiplies by longitudinal divisions that result in a clonal genetic structure of the populations. Genetic recombinations are restricted but seem to occur.[18] Initial groupings of the strains were based on isoenzyme patterns, but multilocus sequence typing separates six genetic subgroups (discrete typing units), named TcI-VI, plus two associated genotypes of bat trypanosomes.[19] This grouping does not correlate with

biological characteristics, eg, multiplication rates during in vitro cultivation or in the vector or mammalian hosts, and also virulence and pathogenicity for mammals and the development of infectious stages in the vector (reviewed by[17]). In Bolivia and Brazil, strains of all or nearly all typing units are present, but not all in other countries. A mixture of strains/clones can occur in the mammalian hosts or vectors (eg,[20–22]). Analysing the geographical and host origin of discrete typing units of over 6000 strains, TcI is widely distributed geographically and predominates in sylvatic and domestic cycles, strains of TcV and TcVI in the latter.[23] The connection of some discrete typing units with humans and the domestic cycle or with wild mammals and sylvatic vectors cannot be generalized because presumably human migration and the change of many mammals between sylvatic and domestic habitats transfer strains to new regions. Wild marsupials often enter houses and are optimal reservoir hosts.[24] After experimental infection, stages of *T. cruzi* that usually occur only in the vector develop in the anal scent glands of marsupials.[25] During evolution, sylvatic mammals seem to be the primary hosts of *T. cruzi*, transmitting the parasite orally by carnivorous behaviour. Millions of years later, hematophagous triatomines evolved, and the dixenous life cycle of *T. cruzi* developed.[25] Thereby, stages occurring in the scent glands of marsupials develop in the triatomines.

Triatomines

Distribution of Triatomines

Triatomines are the biggest blood-sucking insects and are predominantly found on the American continent from latitude 42° N to 46° S, a region between the Great Lakes of North America and Argentina.[26] A few species are present in Asia and Oceania, without a transmission of *T. cruzi*.[27,28] *Triatoma rubrofasciata* (De Geer, 1773) is associated with rats in many tropical and subtropical harbours, also outside the Americas.[29] The majority of the 150 recognized species live in sylvatic areas and are vectors in the sylvatic transmission cycle of *T. cruzi*.[28,29] Other species live peridomestically and feed on domestic animals, eg, chicken and guinea pigs, but also invade houses.[30,31] Only some species are strictly adapted to houses and main vectors in the domestic cycle, eg, *Triatoma infestans* (Klug, 1834), *Rhodnius prolixus* Stål, 1859, *Panstrongylus megistus* (Burmeister, 1835) and *Triatoma dimidiata* (Latreille, 1811).[30,31]

Digestion and Excretion of Triatomines

All post-embryonic stages of all species of triatomines are obligatorily hematophagous, attacking all warm-blooded animals and ingesting about 6–12 times their own body weight.[31] Such full engorgement or several smaller volumes of blood are required for the development to the next of the five pre-adult instars and oogenesis in females.[31] In addition, mutualistic symbionts are required, at least during final nymphal instars and for oogenesis.[32,33] So far, in only three species of triatomines the mutualistic symbionts have been identified, all Actinomycetales.[32] After a full engorgement, triatomines can starve for up to 1 year, depending on the developmental stage.[31] In particular, temperature and carbon dioxide in exhaled air attract triatomines, but also compounds simulating the human skin.[34,35] Having approached the vertebrate host, they push the rostrum that protects the mouthparts onto the skin and the specifically interlocked maxillae penetrate the tissue by rapidly moving back and forth.[36] The blood passes through the first region of the midgut, the cardia, and is stored in the following region of the midgut, the distensible stomach. There it remains mainly undigested, but ions and water are withdrawn, sugars resorbed and erythrocytes lysed.[31] A most recent investigation indicates digestion processes in the stomach,[37] but presumably serum proteins and not hemoglobin are digested. After the withdrawal of compounds without nutritional value, the concentrated blood possesses a jelly-like consistency, but after ingestion of blood from guinea pigs by *R. prolixus* the hemoglobin crystallizes.[38,39] Small portions of blood are passed to the final region of the midgut, the small intestine, where the cells of the intestinal tract secrete extracellular membrane layers containing different glycoproteins and separating the intestinal content from the microvillar border of the cells.[40–42] There digestion of hemoglobin starts immediately,[14,43–46] generating amino acids, including the amino acid tyrosine, but also free iron and heme that are toxic to triatomines.[47,48] Whereas iron is absorbed and excreted, tyrosine and heme are metabolized, the latter to avoid the production of reactive oxygen species.[47,48] Remnants of digestion are stored in the rectum before defecation. The rectal content contains different metabolites, eg, peptides, amino acids, fatty acids, steroids, glycerolipids, nucleotides and sugars.[49]

The increase in the size of the abdomen during blood ingestion is monitored by stretch receptors and induces the

secretion of diuretic hormones and the activity of the most effective excretory system in the animal kingdom (reviewed by[31]). Within 24 hours after blood ingestion, the excretion by the Malpighian tubules decreases the bug's body weight by about 40%. Since the tubules end at the border to the rectum, the first urine sweeps out the dark remnants of digestion. Later yellow-white crystals follow and then new remnants of digestion (reviewed by[50]). During these phases of excretion, pH, osmolality and the concentrations of the different ions change rapidly.[51]

Immune System of Triatomines

As in other animals, the immune system of triatomines consists of cellular and humoral components that respond to pathogens and parasites,[52–54] also including behavioural fever responses.[55] Cellular components have only been found in the hemolymph and not in any section of the intestine. If viruses, bacteria, fungi and parasites gain access to the intestine, the humoral immunity is activated via the immune deficiency (IMD), Toll and JAK-STAT pathways[52,56] that induce the production of different antimicrobial peptides (eg, lysozymes, defensins, prolixin, prophenoloxidase cascade), unidentified bacteriolytic compounds and antimicrobial molecules (eg, nitric oxide).[57–59] The importance of the antimicrobial peptides is reflected by the high number of genes. In *Triatoma pallidipennis* Stål, 1872, 12 different genes encode for three defensins.[60] Presumably as a possibility to reduce infections, in fifth instar nymphs of *T. infestans* antibacterial activities increase after feeding in the stomach and the small intestine, the latter possessing a much lower activity than the stomach,[61] corresponding to the expression levels of genes encoding defensin and lysozyme[62,63] and the prophenoloxidase activities in the Mexican *T. pallidipennis*.[64]

Development of *Trypanosoma cruzi* in the Vector – Effects of the Vector

The combination of the respective strain of *T. cruzi* and the species of the triatomine result in refractoriness or susceptibility phenomena of the vector.[30] In many parasite-vector systems, eg, *Plasmodium* and mosquitoes, an infection induces anti-parasitic responses of the vector, affecting the success of the parasite for the establishment and the intensity of the parasitation.[31] These aspects have only been considered in a low number of triatomine species using a low number of strains of *T. cruzi* (reviewed by[17,30]).

Development of *T. cruzi* in the Stomach

During the initial period immediately after blood ingestion, different compounds from the triatomine are present in or secreted into the stomach. Agglutinins, hemolysins and anticoagulatory and antimicrobial compounds originate from the saliva of the triatomines and the wall of the stomach.[52,65] Bacteria are also present in the saliva of triatomines[66] and presumably ingested together with the blood and added to the intestinal bacteria. Effects on the trypanosomes have only been investigated for the salivary gland-secreted antimicrobial peptide trialysin, produced by *T. infestans*, which lyses blood trypomastigotes[67] and is apparently neutralized by *T. cruzi* epimastigotes.[68] Agglutinins and hemolysins seem to determine the initial establishment of epimastigotes of *T. cruzi* in the vector (summarized by[69]) but blood trypomastigotes must be included in such investigations. None of these factors has been connected to the specific developmental steps of trypanosomes in the stomach.

According to the first detailed study of the morphological transformations of *T. cruzi* in the stomach of *P. megistus*, in the first days post-infection (pi) blood trypomastigotes and some intermediate forms occur. Later on, rounded parasites without changes indicating multiplications are present.[70] Round and pear-shaped forms, aggregating a few days later, also develop in *T. infestans* and *R. prolixus* (eg,[71,72]). Finally, transitions to epimastigotes develop, elongated spheromastigotes.

In *R. prolixus*, the number of blood trypomastigotes of three strains/clones is strongly reduced within the first 24 hours pi,[73,74] and after 96 hours pi no parasites are found.[74] According to qPCR, then only a few dozen parasites are present.[73,74] The high mortality rate of blood trypomastigotes seems to be caused by compounds secreted into the stomach after blood feeding as indicated by in vitro incubations of blood trypomastigotes with extracts of the stomachs of either unfed or recent blood-fed *R. prolixus*.[74]

The stomach is not totally hostile to epimastigotes. If after the molt the residues of the blood are passed to the small intestine, the colour of the intestinal contents becomes brownish and epimastigotes are present, presumably by a reflux from the small intestine. These epimastigotes survive or are killed after feeding on mice or hens, respectively.[75]

Development of *T. cruzi* in the Small Intestine

After ingestion of infectious blood, epimastigotes develop only in the small intestine and rectum. There, spheromastigotes and especially epimastigotes multiply enormously.[76]

One week after an initial uptake of 8000 to 10,000 blood trypomastigotes/second instar nymph of *T. infestans*, there are about 30,000 parasites per small intestine. Using starvation periods of three or four weeks before blood-feeding the following instars and dissection of cohorts in weekly intervals, the number of trypanosomes is always slightly reduced before feeding, but increasing in each successive nymphal instar, up to a 20-fold higher population density at 12 weeks pi.[76]

The flagellates are rarely in direct contact with the microvillar border of the cells of the intestinal wall, and more often they are present near the perimicrovillar membranes, which develop after blood ingestion and are reduced during starvation (reviewed by[13]). However, the development of *T. cruzi* is reduced by changing hormonal concentrations or feeding antibodies against perimicrovillar membranes.[77–79] Presumably epimastigotes attach to the perimicrovillar membranes through different compounds, including cruzipain, heparin-binding molecules, cysteine peptidases and glycoinositol phospholipids (eg,[80–83]). These membranes also contain many different glycoproteins, suggested to interact with the flagellate.[42]

Development of *T. cruzi* in the Rectum

The rectum contains the highest density of trypanosomes, in *T. infestans* about three times more than in the small intestine, although the volume and surface area are much smaller in the rectum.[76] Presumably, one reason for the difference in population densities is the possibility of optimal attachment, and about 60% of the rectal trypanosome population is attached to the rectal cuticle. A small hydrophobic region on the flagellum of epimastigotes seems to bind to the wax layer that covers the whole rectal cuticle (reviewed by[13]). In transmission electron microscopy flagella are enlarged in the contact region and possess hemidesmosome-like material beneath the plasma membrane.[84] Also, surface mucins of *T. cruzi* seem to be involved in the attachment, but Gp35/50 kDa mucins cover the whole body of the epimastigotes, not only the small hydrophobic region.[85]

Attachment is important for metacyclogenesis,[86] presumably for at least one mode of metacyclogenesis, the unequal cell division of the epimastigotes, resulting in one daughter epimastigote and one daughter metacyclic trypomastigote, the latter possessing no properties for attachment by the short free flagellum and the thicker surface coat. Metacyclic trypomastigotes also develop from unattached long and short epimastigotes and spheromastigotes, as indicated by the translocation of the kinetoplast.[76] Metacyclic trypomastigotes also occur in the small intestine, but mainly in the rectum. They possess a kinetoplast in a more subterminal position than in blood trypomastigotes.[17] At two weeks pi 25% of the entire rectal population are trypomastigotes and from 10 weeks pi onwards it increases to 50%.[76] In addition, after maintenance of infected *R. prolixus* at 26, 28 and 30°C, more metacyclic trypomastigotes are present at the latter temperature than at other temperatures.[87]

A clear effect of the vector on metacyclogenesis in the rectum is evident after blood ingestion by short-term starved nymphs. Within four hours, the proportion of slender intermediate stages increases significantly from <7% to 10%, but only of these intermediates between unattached epimastigotes and trypomastigotes. Although pH, osmolarity and ion concentrations change drastically, the inducing factors are hemolymph proteins of about 17 kDa that pass into the rectum presumably via the Malpighian tubules at the beginning of diuresis.[13,51,86] Another feeding-induced peculiar effect is evident after a long starvation period: One day after feeding, "giant cells" are present, ie, multiple cell division stages, containing several nuclei, kinetoplasts and flagella. Up to three days after blood-feeding, these "giant cells" represent on average 30 to 50% of the total parasite population. However, between 5 and 10 days after feeding they disappear, correlated with a strong increase in the rectal population density.[88]

In addition to feeding, starvation of the vector affects the rectal population. During starvation, the number of dead flagellates increases, but even then, all recta are colonized, sometimes restricted to the rectal pads (reviewed by[13]). Not only do population densities change but also the percentages of the different stages.[89] At 20 days after blood-feeding, 2% and 1% of the population are either spheromastigotes or drop-like forms, ie intermediates between either sphero-, epi- or trypomastigotes, respectively. The percentage of spheromastigotes increases to about 20% after an additional 40 and 70 days.

Effects of *T. cruzi* on Triatomines

Since trypanosomes are classified as parasites, an infection with *T. cruzi* without any effect is improbable, but strong effects only occur regularly in infections of triatomines with other species of trypanosomatids (reviewed by[90]). We classify *T. cruzi* as weak pathogenic or subpathogenic, ie, pathologic effects only develop under adverse conditions

by the presence of stressors, eg, starvation and missing mutualistic symbionts (reviewed by[91]). However, neither *T. cruzi* nor the vector are a homogenous group of organisms, and effects or missing effects should not be generalized for all *T. cruzi*-vector systems. The importance of the *T. cruzi*-strain is indicated by infections with different *T. cruzi*-strains which differently affect the same vector.[92,93]

According to many investigations, *T. cruzi* affects the behaviour and the development of nymphs and adults, their physiology and life history traits (reviewed by[16,91,94–96]). However, investigations in the laboratory are affected by the choice of the system, the mode of maintenance of the triatomines and the supply with mutualistic symbionts. In addition, a very important aspect is the source of blood. The optimal system consists of colonies of triatomines which possess a nymphal mortality rate of <10%. The strains of *T. cruzi* should originate from the respective strain/species of triatomines, belong to different typing units and should be maintained by cyclical passages between vector and mammalian host or be stored frozen. Permanent passages between mice or in vitro cultivation should be avoided (Schaub unpublished). The colonies of triatomines should be maintained under optimal temperature, humidity and illumination conditions, choosing group sizes that exclude crowding stress.[97] The presence of mutualistic symbionts in the nymphs is crucial and needs to be arranged. The development of the symbionts in the different regions of the intestine is regulated by the triatomines, resulting in high population densities in the stomach and very low numbers in the small intestine and rectum.[98] When beginning with experimental groups of first instar nymphs, an addition of some males to the cohort provides the nymphs with the opportunity to acquire symbionts. In vitro culture-derived mutualistic symbionts can be given to experimental groups of nymphs after blood-feeding, but only in those species of triatomines, for which the mutualistic symbionts are known.[32] Without the identification of the mutualistic symbiont, rectal bacteria originating from adult triatomines captured in the field can be cultured on agar plates. The supply of the experimental groups with a mixture of all slow-growing actinomycetales avoids investigations of aposymbiotic bugs (Schaub unpublished). In aposymbiotic nymphs, a feeding on live hens and mice enables normal nymphal development. Artificial feeding devices using defibrinated or citrated blood of cows, sheep or humans usually affect the development of nymphs and adults (Schaub unpublished). Only the use of defibrinated blood of pigs is optimal[32] and equivalent to a feeding on live hosts. Also, in other laboratory investigations, the blood source affects the development of triatomines (eg,[99–101]).

Effects of *T. cruzi* on the Behaviour of Triatomines

In the majority of the wide range of behaviours of triatomines (reviewed by[102]) the effects of the flagellate have not been investigated. Considering the attraction of triatomines by the host, blood-feeding and subsequent defecation results differ. No effects of *T. cruzi* are evident after experimental infection of *R. prolixus* and *Triatoma rubrovaria* (Blanchard, 1843): infected and uninfected individuals probe similarly frequently, require identical periods of times for feeding on live hosts, ingest similar volumes of blood and begin to defecate after identical periods of times.[103,104]

However, using second instar nymphs of *Triatoma longipennis* Usinger, 1939 and *T. pallidipennis* that were fed after the infection on uninfected mice, only third instar nymphs but not the following nymphal instars react more rapidly to human odor than uninfected nymphs.[35] However, third instar nymphs are also more active than fifth instar nymphs indicating perhaps a more advanced physiological state of starvation. Also, long-term infected fifth instar nymphs of an indigenous species of Chile, *Mepraia spinolai* (Porter, 1934), starved for seven weeks after molting, orient themselves to the vertebrate host twice as fast as uninfected nymphs.[105] In these infected nymphs, the number of probings is increased, and they begin to defecate earlier than uninfected bugs. The latter also occurs in fifth instar nymphs of *T. infestans*.[106] Naturally infected *R. prolixus* nymphs feed less frequently than uninfected nymphs. Perhaps, the earlier defecation, the lower frequency of feeding and the lower volumes of ingested blood are induced by starvation: After long periods of starvation of 5–6 months, uninfected *R. prolixus* ingest lower volumes of blood but defecate earlier than bugs fasted for 2–3 months.[103]

An earlier defecation increases the transmission risk, but defecation behaviour differs between species and developmental stages, eg, in *Triatoma rubida* (Uhler, 1894).[107] The earlier approach to the host seems to be without an effect on the transmission rate considering the usual single feeding per nymphal instar. It also remains to be investigated whether or not the earlier approach is an

effect of the study design. If *T. cruzi* and the vector compete for nutritional resources, then infected nymphs may possess a more advanced state of starvation in comparison to uninfected nymphs of the identical age. The hypothesis that the behavioral changes observed can be related to a reduction in essential components of the blood is supported by a field study, in which infected *M. spinolai* possess a lower standardized body mass index than uninfected ones, and in which more *T. cruzi*-infected bugs are captured within the first hour of exposition of humans as hosts.[108]

There are several investigations considering effects on locomotion. A competition for nutritional resources seems to have reduced the locomotion of infected fifth instars of *R. prolixus* by 20% as compared to uninfected individuals.[109] About 8–12 days after infection of adult *Rhodnius pallescens* Barber, 1932 using a mixture of epimastigotes and blood, the infected females fly faster than infected males in a flight mill.[110] Although *T. cruzi*-infected males and females collected in the field have larger wings than uninfected individuals,[111] the dispersal capabilities of females of *T. dimidiata* are increased by an infection, but similar in *T. cruzi*-infected and uninfected males.[112] One possibility is that females include nutrients in the eggs that are deposited resulting in a lower weight and nutritional status of infected females than of infected males. In *T. infestans*, especially starvation increases flight initiation.[113] In the laboratory, levels of negative geotaxis and aggregation are higher in both female and male adults of *T. infestans* infected with *T. cruzi*.[114] Comparing the ecological niches of seven Mexican triatomine species, the ecological niche used by *T. cruzi*-infected populations is often reduced in comparison to uninfected populations, perhaps caused by an effect of *T. cruzi* on insect fitness.[115]

Effects of *T. cruzi* on Nymphs of Triatomines

Considering the effects of *T. cruzi* on nymphs, the results differ. The developmental time of individually maintained first instar nymphs increases fivefold and *T. cruzi* retards also the development of older infected nymphs of *T. infestans*.[116] Retardations are also evident in *M. spinolai*, after blood-feeding on *T. cruzi*-infected mice, and nymphs are significantly lighter than controls. In addition, the mortality rate of fourth and fifth instar nymphs of *M. spinolai* is increased compared to uninfected nymphs.[117,118] However, after blood-feeding on *T. cruzi*-infected mice, the period of time until molting of first instar nymphs of *Triatoma brasiliensis* Neiva, 1911 is increased, but development and mortality rates of older nymphs are unaffected.[119] The difference between both systems is a feeding of the older instar nymphs of *M. spinolai* on infected mice and of *T. brasiliensis* on uninfected mice. Perhaps repeated infections strongly increase the population density of the flagellate in the vector or *T. cruzi* affects the nutritive value and/or on the concentration of essential unknown compounds of the blood of infected mice. This can be compensated by either increasing the number of blood-feeds and/or the volume of blood ingested: Infected nymphs of *T. rubrovaria*, ingest significantly more blood than uninfected nymphs.[104] The indication to the population density or the quality of blood is supported by the normal development of nymphs of *T. infestans* after infection via mice and subsequent feedings on uninfected mice or hens.[120–122] The mortality rates of these nymphs of *T. infestans* are in the normal range and also the nymphal development of *P. megistus* and *T. brasiliensis*.[119,123] Temperature modifies the effects: after a single feeding of second instar nymphs of *R. prolixus* via an artificial feeder with a mixture of human blood and epimastigotes and a maintenance between 21 and 30°C, the molts of infected second instar nymphs are strongly delayed by 6–11 days.[124] Whereas, at 90 days pi, the starvation induced mortality rate is high and similar in uninfected and infected groups maintained at 21 and 30°C, it is significantly increased by the infection at 24 and 27°C, temperatures at which *R. prolixus* is found in the wild.[100,124]

Not only do temperatures vary in the field but also the availabilities of hosts. During monitorings in the field, high percentages of starved individuals are common.[125,126] The data regarding whether or not the starvation capacity is affected by the infection differ. The period of time before the death of either third and fourth instar nymphs or adults of *M. spinolai* from the field is unaffected by the infection after feeding two times on uninfected mice before starvation.[127] However, after an infection of fifth instar nymphs of *T. pallidipennis*, the starvation capacity is reduced.[64] In experimental infections of first instar nymphs of *T. infestans*, followed by either one, two or three additional uninfected blood feedings to the subsequent nymphal instars on hens, the mean periods of survival of the resulting fourth and fifth instar nymphs are, respectively, 14 and 17% significantly shorter than those of uninfected nymphs.[128] Death is not caused by

a depletion of hemoglobin because more infected than uninfected nymphs contain the brown hemoglobin remnants of the blood in their small intestines. Therefore, *T. cruzi* and its vector seem to compete for essential metabolites whose depletion results in death. Since many trypanosomes die from starved nymphs, an accumulation of toxic products by *T. cruzi* seems unlikely.[13]

Effects of *T. cruzi* on Adults of Triatomines

Similar to nymphs, investigations of adults also show contradictory results considering fecundity and longevity. In *M. spinolai* fed on *T. cruzi*-infected mice, the weight of the gonads and the body size is reduced in comparison to females fed on uninfected mice,[117] perhaps due to differences in the population densities or the quality of blood. This is also possible in four groupings of infected and uninfected females and males, in which uninfected females produced more and heavier eggs independently of the infection status of the males.[129] In *T. infestans* fed on hens, the infection seems to reduce slightly both the egg-laying rate during the first weeks of oviposition and the hatching rate.[130] After an in vitro infection of second instar nymphs of *R. prolixus* with epimastigotes, the period of time before the first egg laying is similar in infected and uninfected females.[131] Comparing a maintenance at 25°C and 30°C, the latter decreases the fecundity of infected females in the first reproductive cycle and significantly fewer nymphs hatch from eggs laid in the third reproductive cycle.[131] Also, infected couples of *P. megistus*, infected in the first instar on mice and fed in the following instars on defibrinated sheep blood, produce less eggs, fertile eggs and resulting nymphs.[123] Similar effects are evident after infection of fifth instar nymphs of *T. pallidipennis* on mice and a subsequent feeding on uninfected mice.[93] In *T. infestans* spermatogenesis is similar in uninfected and infected males.[132] The importance of the respective strain of *T. cruzi* is highlighted after an infection of Colombian *R. prolixus* fifth instar nymphs with five different Colombian TcI strains and subsequent feedings on hens.[92] In a comparison to uninfected adults, some *T. cruzi* strains significantly reduce survival while others have no effect.[92] The reproduction is also reduced by one of these strains.[133] Also, in *Triatoma* sp. and *R. prolixus,* the longevity of *T. cruzi*-infected adults is reduced,[134,135] in the latter even more after feeding on *T. cruzi*-infected guinea pigs compared to groups fed on uninfected guinea pigs. After feeding on uninfected hosts, no effects of *T. cruzi* infection are evident on the mean lifespan of both adult males and females of *T. brasiliensis* and *T. dimidiata*, as well as the hatching rate of eggs, the period of time before oviposition, the number of ovipositions, and both the total number of eggs laid and number of fertile eggs.[119,136]

Effects of *T. cruzi* on Immunity Responses of Triatomines

In addition to access of bacteria, ingestion of *T. cruzi* induces an immune response, not only in the intestine but also synergistically in the hemolymph and other organs (summarized by[90]). A methodological problem is the use of the whole intestinal tract because the immune reactions in different regions differ strongly (see above). In addition, investigations using a mixture of epimastigotes and blood do not reflect the natural conditions. Epimastigotes are only ingested during coprophagy and then after blood ingestion, avoiding contact of fecal material with the blood.[16] Describing the long-term effects of *T. cruzi* on the immunity, such investigations can be considered. The importance of the respective stage is indicated by the comparison of infections with epimastigotes and blood trypomastigotes.[137] The surface of the latter is highly organized and contains lipid-driven domains with different protein compositions (eg,[138]). Shedding of the surface coat of blood trypomastigotes can be induced by strong centrifugation forces and incubation in a protein-free buffer,[139] as well as during a forced passage through a fine (ie, high gauge) syringe needle, increasing the shearing forces. After feeding fifth instar nymphs of *T. infestans* on *T. cruzi*-infected rats or a mixture of complement-inactivated rat blood with epimastigotes, the separated surface coat of the blood trypomastigotes and the resulting "naked" trypomastigotes, up to 5 days after feeding, antibacterial activity is significantly increased in the small intestine of nymphs that ingested either separated surface coats or intact blood trypomastigotes, but not "naked" trypomastigotes or in vitro culture-derived epimastigotes.[137]

Several investigations report the effects of *T. cruzi* on antimicrobial peptides. Using the whole intestine of *R. prolixus*, at 7 and 14 days pi with blood trypomastigotes, the expression of a gene of the most intestinally active lysozyme is increased >20-fold.[140] At 20 days pi with epimastigotes, in the small intestine of *T. brasiliensis*, the expression of the gene encoding a defensin is nearly 10-fold higher than in uninfected nymphs.[141] In the stomach of *T. infestans* at 24 hours pi on infected mice, the

expression of genes encoding lysozyme, cathepsin D, a nitrophorin-like protein and a putative 14 kDa protein are all significantly upregulated, while the gene encoding thioredoxin reductase is downregulated. Expression of genes encoding infestin, lipocalins, and defensins are unchanged.[142] The activity of cathepsin D is higher in the small intestine of *R. prolixus* at 1 day and 3 days pi with epimastigotes,[143] and a synergistic activity with lysozyme in the degradation of intestinal bacteria is discussed.[14] Expression of the gene that encodes a cysteine protease inhibitor and perhaps acting against the enzyme of *T. cruzi* is significantly higher than in uninfected adults of *T. infestans*.[144]

Not only is the production of antimicrobial peptides and enzymes induced but also that of other antimicrobial compounds. Investigating one of these, nitric oxides, the concentrations cannot be determined directly, only via its metabolites, nitrite and nitrate. In *R. prolixus*, at 1 and 2 days pi concentrations of nitrite are higher than in uninfected blood-fed controls in both midgut regions.[145] However, it is also increased in the stomach at 2 weeks pi, although no parasites are present there, and in the rectum, even before the parasites have passed to this region.[145] Compared to uninfected blood-fed controls, the expression of the gene encoding nitric oxide synthase increases in the stomach at 1 day or 2 days pi when the development of *T. cruzi* is confined to this region. In the stomach of *T. pallidipennis*, the activities of prophenoloxidases are significantly higher in infected fifth instar nymphs at 28 days pi, while those of phenoloxidases are significantly lower at 9 days pi.[146] In this system, the increase in prophenoloxidase activity is evident at 15 days pi and at 20°C, but not at 30°C and 34°C.[64]

The initial induction of these immune reactions seems to be without adverse effects on *T. cruzi*. However, after knockdown of antibacterial proteins, more bacteria are present in the stomach and the number of trypanosomes is significantly lower than in controls without a silencing of the antimicrobial proteins.[147–149] Therefore, a short-term upregulation of immune proteins by blood trypomastigotes suppresses the development of the bacteria.[147–151] However, in long-term infections, *T. cruzi* seems to induce an immune suppression in the intestine. After feeding third instar nymphs with a mixture of blood trypomastigotes and different microorganisms, high numbers of fungi and bacteria are present only in *T. cruzi*-infected fifth instar nymphs, but not in uninfected controls.[98]

Conclusions and Open Questions

There are many reports on the effects of the vector on *T. cruzi* and vice versa. However, in some of them the effects seem to be caused by infections with attenuated strains of *T. cruzi* or by a sub-optimal maintenance of triatomines. In optimal systems both components originate from the same locality. Thereby, the virulence of different strains of *T. cruzi* can be compared.[152] In optimal colonies of the triatomines, the access to mutualistic symbionts is supported. However, these symbionts must be identified in the majority of species of triatomines and also the function of the microbiota.[153]

Focussing on the development of *T. cruzi* in the different regions of the triatomine intestine, some interesting questions still require further investigation. Regarding the development of *T. cruzi* in the stomach, the use of optimal parasite-vector systems might clarify whether or not the initial death of the majority of blood trypomastigotes can be generalized for all species of triatomines and all lineages of *T. cruzi*. With regard to the population in the rectum, the molecules of the hydrophobic attachment zone of the flagellum of epimastigotes remain to be identified. Metacyclogenesis is another fascinating phenomenon investigated in detail using in vitro cultures (eg,[154]). However, determination of the concentrations of oxygen and free amino acids and the pattern of lipids and proteins/peptides is required to compare them with the factors inducing metacyclogenesis in vitro. Investigations of the effects of *T. cruzi* on the vector must avoid stress conditions and use blood trypomastigotes. Considering the interesting *T. cruzi*-induced manipulation of the microbiome of triatomines by the induction of immune reactions, different lineages of *T. cruzi* should be used and the population densities of the respective bacteria should be determined, considering also the mutualistic symbiont of the respective species. The available genomic and proteomic data of triatomines, symbionts and *T. cruzi*[46,155–159] can be connected to gain insight into the molecular base of the interactions and to find new potential targets for vector control.

Abbreviations

pi, post infection.

Ethics Approval and Informed Consent

The author reports no relevance.

Consent for Publication

The author reports no relevance.

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Authors' Information

I am engaged in investigations of *T. cruzi* and triatomines since 1978. I apologize if my emphasis on using optimal systems in such investigations seems to be too restrictive to colleagues with whom I have shared so many discussions, especially in Brazil. However, only then, a transfer to natural systems is possible. Administrative regulations against the feeding of triatomines on living animals and against the infection of laboratory mammals with *T. cruzi* reduce the quality of such investigations and the chance to attack the etiologic agent of this "neglected" disease.

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