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Effects of the infection with *Trypanosoma cruzi* on the feeding and excretion/defecation patterns of *Triatoma infestans*

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Abstract

Transmission of *Trypanosma cruzi* (Kinetoplastida: Trypanosomatidae) occurs when feces/urine of infected triatomines come into contact with mucous membranes or damaged skin, and this occurs mainly when insects defecate while feeding on the host. Thus, the vector competence of the triatomines is associated with their feeding and excretion/defecation behavior. This work studied for the first time the effect of *T. cruzi* infection on feeding and excretion/defecation patterns of *Triatoma infestans* (Hemiptera: Reduviidae). Uninfected and infected fifth-instar nymphs were fed *ad libitum* and their feeding behavior and defecations were registered during and after feeding. The feeding pattern did not show differences between the experimental groups. However, the infected nymphs began to defecate earlier, defecated in greater quantity and there was a greater proportion of defecating individuals compared to uninfected nymphs. These results show that *T. cruzi* affected the excretion/defecation pattern of *T. infestans* in a way that would increase the probability of contact between infective feces and the mammalian host.

Keywords: *Triatoma infestans*, *Trypanosoma cruzi*, Chagas disease, vector competence

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Introduction

Chagas disease is considered one of the most important human parasitic diseases in America. The disease is caused by the protozoan *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) and its main mode of transmission is through hematophagous insects of the subfamily Triatominae (Hemiptera: Reduviidae). *Triatoma infestans* is a triatomine that is well adapted to the anthropic environment and is the main vector of the disease in the Southern Cone of South America (Schofield *et al.*, 2006). Transmission of *T. cruzi* occurs naturally when feces or urine of infected insects come into contact with mucous membranes or damaged areas of skin, and this occurs mainly when insects excrete/defecate while feeding on the mammalian host. In this way, the vector competence of the triatomines is directly associated, among other factors, with their feeding and excretion/defecation behavior (Lent & Wygodzinsky, 1979). The efficient vectors (i.e. most likely to transmit the parasite) defecate/excrete during or immediately after feeding. Thus, the epidemiologically relevant variables of the defecation processes are the time interval between a bloodmeal and the beginning of defecation, the proportion of insects that defecate and the number of defecations per insect during or close to the end of feeding (Zeledón *et al.*, 1977; Lent & Wygodzinsky, 1979). In addition, the excretion/defecation pattern is determined, in part, by the feeding pattern. The amount of blood ingested was showed to correlate negatively with the time to the first defecation and positively with the number and volume of defecations (Trumper & Gorla, 1991; Crocco & Catalá, 1996; Rodríguez

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et al., 2008). On the other hand, prolonged feeding increases the contact time between the host and the vector, which increases the probability of contact between infective feces and the host (Wood, 1951; Zeledón *et al.*, 1977).

The presence of the parasite inside the vector can modify a wide range of physiological processes of the insect, including those directly related to the entry, development and discharge of the parasite (Libersat *et al.*, 2009). The existence of these modifications as a characteristic of a given parasitic-vector system could be the consequence of different adaptive or non-adaptive scenarios (e.g. adaptive host manipulation) (Poulin, 2010; Heil, 2016). Multiple studies on the interaction between *T. cruzi* and triatomines have been carried out and most of them addressed the possible alterations in the vector from a pathological perspective (Schaub, 2006, 2009; Vallejo *et al.*, 2009). The results of many of these studies led to establish the convention that *T. cruzi* is not pathogenic for their insect vectors, although the term 'sub-pathogenic' was proposed for this species since some effects may occur if there is a stressor present (Schaub, 2009). However, in recent years the effects of *T. cruzi* on survival, post-embryonic development, behavior and different physiological processes in different species of triatomines have been described (Eichler & Schaub, 2002; Botto-Mahan *et al.*, 2006, 2008; Schaub, 2006, 2009; Vallejo *et al.*, 2009; Oliveira *et al.*, 2010; Fellet *et al.*, 2014; Elliot *et al.*, 2015; Marliére *et al.*, 2015).

Many authors have studied the patterns of feeding and defecation/excretion in many species of triatomines (Wood, 1951; Zeledón *et al.*, 1977; Trumper & Gorla, 1991; Crocco & Catalá, 1996; Nattero *et al.*, 2002; Biral dos Santos *et al.*, 2006; Rodríguez *et al.*, 2008; Reisenman *et al.*, 2011; Lobbia *et al.*, 2018). However, considering the mode of transmission of *T. cruzi*, strikingly only two studies investigated these two patterns together in triatomines infected with *T. cruzi*. Takano-Lee & Edman (2002) showed that infected *Rhodnius prolixus* did not differ significantly in the number of feeding attempts, feeding time, time to first fecal drop and number of excreta after feeding with respect to uninfected insects. On the other hand, Botto-Mahan *et al.* (2006) reported that infected *Mepraia spinolai* showed a higher rate of feeding attempts and a higher speed in the detection of the host and in the production of the first fecal drop than the uninfected insects. The authors interpreted these results mainly as a consequence of fasting caused by competition for nutrients from the blood ingested between the parasite and the vector.

The objective of this study was to determine for the first time the effect of *T. cruzi* infection on feeding and excretion/defecation patterns of *T. infestans*. The hypothesis was that infection with *T. cruzi* improves the vector competence of *T. infestans* by altering the feeding and excretion/defecation patterns of the insects. The main observational consequences tested were that infected insects begin to defecate earlier, defecate more times, ingest more food and show more feeding attempts than uninfected insects. For this, different variables involved in the processes of feeding and excretion/defecation in *T. infestans* uninfected and infected with *T. cruzi* were determined.

Materials and methods

Insects and parasites

The *Triatoma infestans* strain originated from the village of 25 de Mayo, in the department of Quitilipi (26°52′42″S, 60°13′52″W), province of Chaco, Argentina. Second-generation descendants of field-collected insects were used for experiments. The insects were reared under controlled temperature (26 ± 1°C), relative humidity (50–60% RH), and LD (12:12 h photoperiod) in the Centro de Referencia de Vectores (CeReVe) (Santa María de Punilla, Córdoba, Argentina), and fed every 15 days on hens (*Gallus gallus domesticus*).

Trypanosoma cruzi, Telahuen strain, DTU VI (Zingales *et al.*, 2009) trypanosomes were used to infect insects. The strains were maintained by cyclical passages in adult mice BALB/c.

Infection by Trypanosoma cruzi

Infection by *T. cruzi* was induced in third instar nymphs (15 ± 5 days old) to ensure a chronic infection in the fifth instar nymphs. For infection with trypomastigotes, nymphs were fed *ad libitum* on mice infected with 1×10^6 parasites ml^{-1} of blood. Control group insects were fed on uninfected mice. In order to confirm the blood ingestion, the nymphs of both experimental groups were individually weighed both before and immediately after feeding. Infection status was confirmed for all insects used in the experiments through microscopic examination of a fecal drop 30 days after infection. The same procedure was carried out for the control of uninfected insects. After infection, the insects were maintained under rearing conditions and were fed fortnightly on hens until the molt to the fifth-instar stage. To confirm the occurrence of the infection throughout the experiment, a new parasitological analysis of the insects of the infected group was performed 5 days after the experimental determination of the feeding and defecation patterns. Only insects that were confirmed positive after the experiment were considered for the analysis.

Feeding and excretion/defecation patterns

The experiments were carried out with infected ($n = 31$) and uninfected ($n = 44$) fifth-instar nymphs, 15–20 days old and fasted since the last molt. In order to characterize the feeding and excretion/defecation patterns, each insect was fed *ad libitum* on pigeons and its behavior was observed during and after feeding according to Lobbia *et al.* (2018). Briefly, a pigeon was placed on a device that kept the pigeon 20 cm away from the laboratory table with its ventral side exposed underneath. An insect was placed inside a transparent plastic container of known weight with circular section (2.75 × 3.78 cm) which was open at one end and had a folded cardboard inside that allowed the insect to walk upwards. The open end of the tube was then attached to the ventral side of the pigeon allowing the insect to access the skin of the pigeon and feed *ad libitum*. As it fed, the feeding variables and the number of defecations were recorded. After feeding, the insect was gently placed on a filter paper (9 cm in diameter) where it could walk freely for an hour. A glass ring (9 cm in diameter) on the filter paper prevented the insect from escaping. During that hour, the insect was observed and the number and time of defecations were registered. For all insects, the initial length (L1), the initial weight (W1), the weight after feeding (W2), the weight after the post-feeding hour (W3) were recorded. The initial nutritional status for each insect was calculated as W1/L1 according to Schofield (1980). The length of each insect was measured from the clypeus to the tip of the abdomen using a handheld Vernier caliper. The weight of each insect was determined with an analytical balance (Ohaus PA214). The feeding variables recorded for each insect were: (1) attack

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Table 1. Initial nutritional status and feeding variables for *Triatoma infestans* uninfected and infected with *Trypanosoma cruzi*.

Variable	Uninfected Mean ± SE ($n = 44$)	Infected Mean ± SE ($n = 31$)	Statistical values
Initial nutritional status	5.09 ± 0.11 a	5.37 ± 0.11 a	$F = 3.17$, df = 1.73, $P = 0.0791$
Number of probing attempts	6.07 ± 1.34 a	6.74 ± 1.58 a	$F = 0.52$, df = 1.73, $P = 0.4737$
Attack time (min)	0.40 ± 0.08 a	0.35 ± 0.07 a	$F = 0.24$, df = 1.73, $P = 0.6245$
Feeding time (min)	15.48 ± 0.92 a	15.72 ± 1.36 a	$F = 0.02$, df = 1.73, $P = 0.8826$
Weight gain	2.44 ± 0.10 a	2.53 ± 0.15 a	$F = 0.30$, df = 1.73, $P = 0.5859$
Feeding rate (g/min)	14.37 ± 0.89 a	16.77 ± 1.30 a	$F = 2.48$, df = 1.73, $P = 0.1196$

Same letters within a row indicate non-significant differences between uninfected and infected insects by one-way test ($P > 0.05$ for nutritional status, $P > 0.01$ for feeding variables).

time (min) (i.e. time elapsed since the insect was placed in feeder until the insect placed its proboscis on the pigeon for the first time), (2) total feeding time (min), (3) number of probing attempts, (4) weight gain = (W2−W1)/(W1), 5) feeding rate (g min^{-1}) = (W2−W1)/total feeding time. The defecation variables registered for each insect were: (1) number of defecations during feeding, (2) time to first defecation since the end of feeding (min), (3) weight of defecation during the first post-feeding hour relativized to the blood meal weight = (W3−W2)/(W2−W1), (4) number of defecations in each interval of 10 min during the first post-feeding hour, (5) percentage of insects that defecated during in each interval of 10 min during the first post-feeding hour, and (6) Defecation index (DI) at 10 min = (% insects that defecated at 10 min post-feeding) × (average of defecations per insect at 10 min post-feeding)/100 (Zeledón *et al*., 1977). Each insect was one replicate.

Ethics statement

All experiments using live animal were performed in accordance with resolution 1047/2005 of the National Council of Scientific and Technical Research (CONICET) on the National Reference Ethical Framework for Biomedical Research with Laboratory, Farm, and Nature Collected Animals, and National Law 14346 on Animal Welfare.

Data analysis

One-way tests were used to analyze the initial nutritional status and the following variables grouped by functional meaning: feeding (attack time, total feeding time, number of probing attempts, weight gain, feeding rate) and defecation (time to first defecation, weight of total feces, accumulated number of defecations at 10 and 60 min post-feeding). The continuous variables were evaluated by ANOVA and the assumptions of homogeneity of variances and normality were tested using the Levene's test and the modified Shapiro–Wilks's test, respectively. The number of probing attempts and number of defecations at 10 and 60 min were evaluated by the generalized linear model (GLM) (function glmer() in "lme4" package) by the negative binomial distribution using "log" as link function. The goodness-of-fit of the models was visually inspected using the residual plots and overdispersal was also checked. The percentage of insects that defecated at each 10-min interval was analyzed using the *G*-test of independence with Williams's correction. The number of defecations per insect over each 10-min interval was analyzed by means of a two-way ANOVA with measures repeated over time as a factor followed by the Tukey's test when a posteriori comparisons were required. The Bonferroni correction was applied to adjust the significance level for the one-way tests of each of the variables within each functional grouping (i.e. overall α level for each grouping = 0.05); thus the differences were considered statistically significant if $P < 0.01$ for feeding group and $P < 0.0125$ for defecation group. For the rest of the test, significance was determined at $P < 0.05$. The repeated measures ANOVA was performed with the STATISTICA program (StatSoft, Inc. 2011). The one-way ANOVAs and GLMs were carried out with the statistical program InfoStat, version 2008 (Di Rienzo *et al*., 2008).

Results

Table 1 shows the mean values for the initial nutritional status and all feeding variables for each experimental group. The infected and uninfected fifth-instar nymphs showed the same nutritional status before the experimental feeding. There were no significant differences between the experimental groups in the attack time, the feeding time, the number of probing attempts, the weight gain and the feeding rate.

Fig. 1 shows the time to first defecation of each experimental group. The results showed significant differences between infected and uninfected insects ($F = 9.56$, df = 1.73, $P = 0.0028$). Although both experimental groups emitted the first fecal drop, on average, before 10 min post-feeding, the infected insects began to defecate before half the time required by the uninfected insect.

Fig. 2 shows the numbers of defecations per insect and the percentages of insects that defecated during feeding and during the first hour after feeding for each experimental group. No insects in any experimental groups defecated during feeding, as evidenced by the defecation parameters equal to zero at the beginning of the post-feeding hour (time 0 in fig. 2b, d). Numbers of defecations per insect in each 10-min interval during the post-feeding hour are shown in fig. 2a. No 'Infection' × 'Time' interaction was observed ($F = 1.4076$, df = 5.365, $P = 0.2206$). The infected insects emitted significantly more feces than the uninfected insects during all the time intervals [main effect 'Infection', $F = 48.748$, df = 1.73, $P < 0.0001$ (lower case letters in fig. 2a)]. The defecations per insect ranged from 0.45 to 1.61 in infected insects and 0.27 to 0.95 in uninfected insects. Finally, there were significant differences between the 0–10 min interval and the rest of intervals, between the 10–20/20–30 min intervals and the 50–60 min interval and between the 20–30 min interval and the 30–40 min interval [main effect 'Time', $F = 19.597$, df = 5.365, $P < 0.0001$; Tukey's test $P < 0.05$ (not shown in fig. 2a)]. Accumulated numbers of defecations per insect during the post-feeding hour are shown in fig. 2b. The infected insects progressively accumulated more

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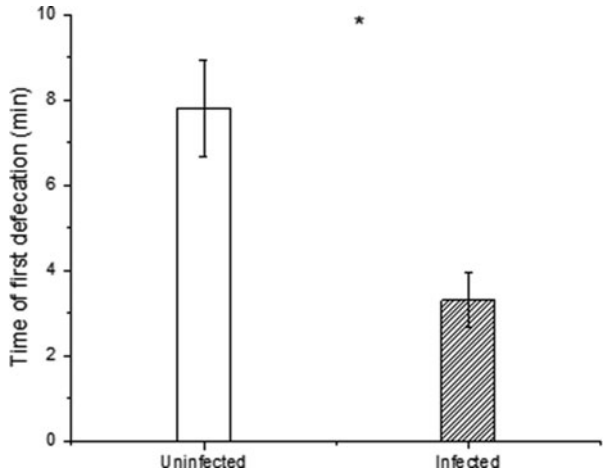

Fig. 1. Time to first defecation after feeding for *Triatoma infestans* uninfected ($n = 44$) and infected ($n = 31$) with *Trypanosoma cruzi*. Assays were conducted on fifth-instar nymphs. Bars = means, vertical lines = standard errors. * indicate significant differences by one-way ANOVA ($P < 0.0125$).

defecations than uninfected insects during post-feeding hour. The accumulated defecations per insect at the end of the post-feeding hour for infected insects (mean ± SE = 5.19 ± 0.24) was significantly higher than for uninfected insects (mean ± SE = 3.16 ± 0.18) [$F = 18.44$, df = 1.73, $P < 0.0001$ (* in fig. 2b)].

The percentages of insects that defecated in each 10-minute interval during the first hour after feeding are shown in fig. 2c. The proportion of infected insects that defecated was higher than the proportion of uninfected insects in all time intervals, with significant differences in the 0–10, 20–30 and 30–40 min intervals [G_{adj} 0–10 = 4.65, df = 1, $P < 0.05$; G_{adj} 10–20 = 0.24, df = 1, $P > 0.60$; G_{adj} 20–30 = 3.93, df = 1, $P < 0.05$; G_{adj} 30–40 = 9.87, df = 1, $P < 0.0025$; G_{adj} 40–50 = 3.62, df = 1, $P > 0.05$; G_{adj} 50–60 = 1.70, df = 1, $P > 0.15$; (* in fig. 2c)]. The percentages ranged from 42 to 94 in the infected group and 25 to 75 in the uninfected group. Accumulated percentages of insects that defecated during the post-feeding hour are shown in fig. 2d. For infected group, more than 90% of the insects defecated in the first 10 min and the 100% of insects defecated at 20 min post-feeding, whereas the 75% of the uninfected insects defecated in the first 10 min and reached 100% at 30 min post-feeding.

Fig. 3 shows the weight of total feces per insect emitted during the first hour after feeding relativized to the blood meal weight for each experimental group. The weight excreted by infected insects was significantly higher than the weight excreted by uninfected insects ($F = 14.56$, df = 1.73, $P = 0.0003$).

Fig. 4 shows the Defecation Index (DI) at 10 min post-feeding for each experimental group. The infected insects showed a DI higher compared to uninfected insects. This result reflects the differences observed in the number of defecations and the proportion of defecating insects in the first 10 min post-feeding (0–10 min interval in fig. 2a, c). In both variables, the infected insects showed higher values than the uninfected insects ($F = 6.28$, df = 1.73, $P = 0.0144$; $G_{adj} = 4.65$, df = 1, $P = 0.0276$).

Discussion

In the present study, the patterns of feeding and defecation/excretion in *T. infestans* infected and uninfected with *T. cruzi* were determined. The study demonstrated that the feeding pattern was not different between the two experimental groups. On the other hand, the infected fifth-instar nymphs began to defecate earlier, defecated in greater quantity and showed a greater proportion of defecating individuals in relation to uninfected nymphs. These differences were observed in times of great epidemiological relevance such as 10 min after feeding. This is the first report of the effect *T. cruzi* on the defecation/excretion pattern in *T. infestans*.

The infected and uninfected *T. infestans* behaved similarly during the feeding process. Modifications in the process and feeding behavior have been descripted for different parasite-vector systems (Koella *et al.*, 1998; Smallegange *et al.*, 2013). Effects of infections with trypanosomatids have been observed in different insect species. *Phlebotomus doboscqi* infected with *Leishmania major* probed at least three times or more and ingested only a little blood, while uninfected phlebotomines probed only once or twice and swelled completely in 10 min (Beach *et al.*, 1985). *Glossina morsitans morsitans* infected with *T. brucei* probed more frequently, fed more voraciously and needed more time for blood ingestion than uninfected flies (Jenni *et al.*, 1980), although these effects could not be observed in tsetse flies by other authors (Makumi & Moloo, 1991; Schaub, 2006). In triatomines, *R. prolixus* and *R. robustus* infected with *T. rangeli* probed more often, fed less frequently and ingested less blood than uninfected insects (Schaub, 2009). Few works studied the feeding process in triatomines infected with *T. cruzi*. In agreement with the present investigation, *T. cruzi* did not affect the feeding time and ingestion attempts in *R. prolixus* (Takano-Lee & Edman, 2002). In *M. spinolai*, the *T. cruzi* did not affect the time until the first feeding attempt but the number of attempts of ingestion was higher in infected individuals than in uninfected individuals (Botto-Mahan *et al.*, 2006). Although, meanwhile the amount of blood ingested in *M. spinolai* was lower in infected insects than in uninfected ones (Botto-Mahan *et al.*, 2008), a later study showed that blood intake was not affected by *T. cruzi* (Botto-Mahan, 2009). The speculation about the possible causes of the differences between the results of each study is complex due to the multiple experimental differences between them. The studies differed in factors as important as the triatomine species, stage in which the insect was infected and in which the effects were observed, duration of infection, strain of *T. cruzi*, and experimental design. Several studies showed that the behavior and development of *T. cruzi* on triatomines, including transmissibility, varies with the strain or genetic variant of the parasite and with the vector species (Lana *et al.*, 1998; Campos *et al.*, 2007; Noireau *et al.*, 2009; Vallejo *et al.*, 2009). In addition, different combinations of a species of vector and a species of parasite from different places can show different effects on the physiology of the vector (Schaub, 2009). On the other hand, like all experimental studies, the conditions in which the infection is carried out and the assays where feeding and defecation are evaluated are determining factors in the results obtained.

The main defecation variables in fifth-instar nymphs of *T. infestans* were affected by the infection with *T. cruzi*. The modification of the defecation/excretion pattern in triatomines has great epidemiological importance due to the central role that the defecation/excretion process has in the natural transmission of *T. cruzi* to the mammalian host. This relevance is enhanced when the factor that modifies such pattern is the parasite since, depending on the type of the alteration, the presence of the parasite inside the insect could promote its

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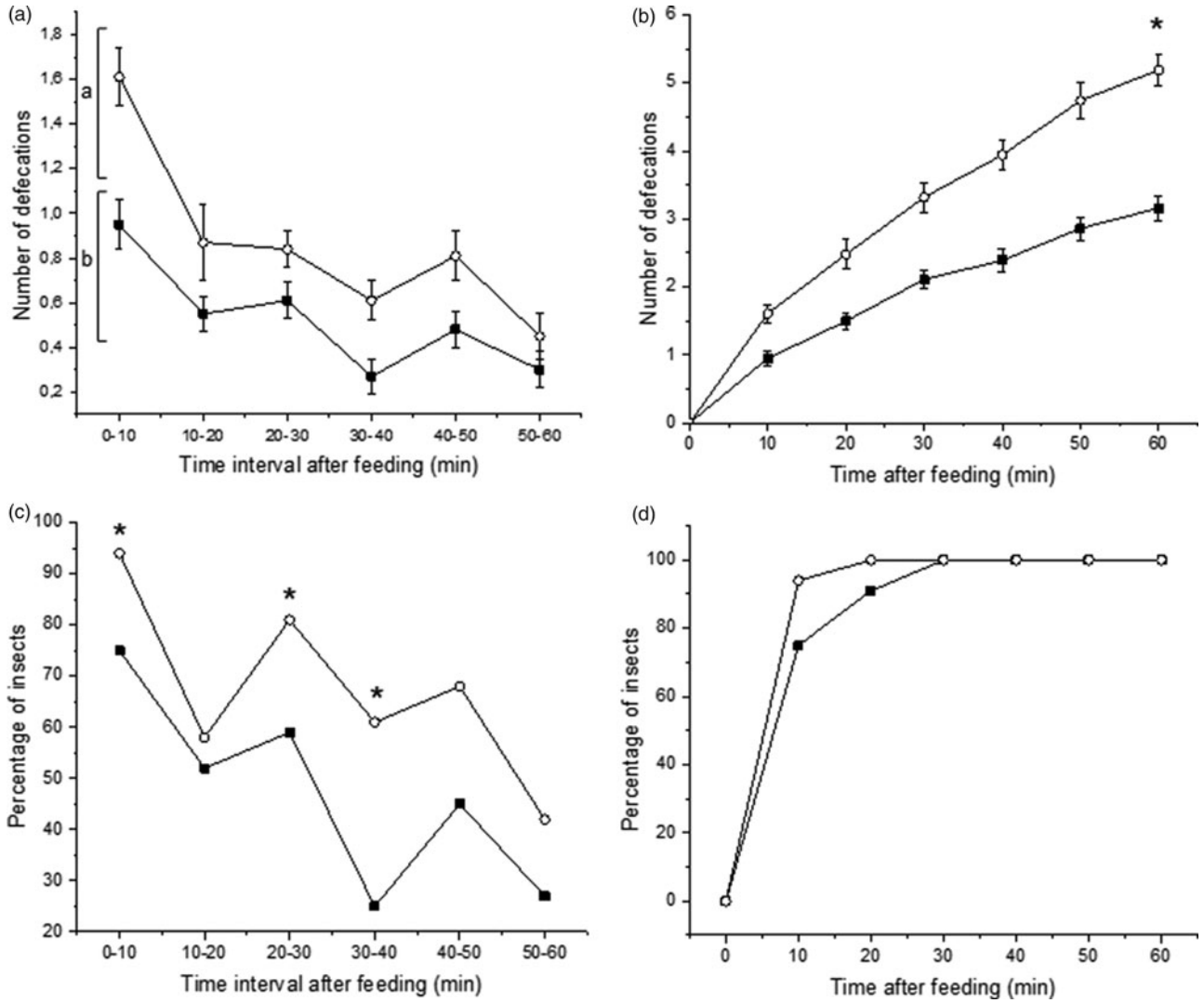

Fig. 2. Number of defecations per insect and percentage of insects that defecated during feeding and during the first post-feeding hour for *Triatoma infestans* uninfected ($n = 44$) and infected ($n = 31$) with *Trypanosoma cruzi*. Assays were conducted on fifth-instar nymphs. Top panels show the number of defecations per insect (mean ± SE) in each 10 min interval (a) and accumulated every 10 min (b). Bottom panels show the percentage of insects that defecated in each 10 min interval (c) and accumulated every 10 min (d). Black square = uninfected insects, white circle = infected insects. Different lowercase letters in panel A indicate significant differences in the main effect 'Infection' of the two-way ANOVA with repeated measures ($P < 0.05$). * in panel b indicates significant differences between uninfected and infected insects at 60 min by one-way GLM with a negative binomial distribution and 'log' as link function ($P < 0.0125$). * in panel c indicate intervals with significant differences between uninfected and infected insects by *G*-test adjusted by Williams's correction ($P < 0.05$).

own transmission. Because vector transmission occurs when the triatomines defecate during feeding or after finishing while they are still on the host, one of the most relevant variables is the time between feeding and the beginning of defecation. According to Zeledón *et al*. (1977), a species that defecates within the first ten minutes of the end of feeding has a high probability of transmitting the parasite to the host. In addition, the amount of feces emitted by each insect and the proportion of insects that defecate are also of great epidemiological relevance since they determine the amount of parasite release events that would occur in a population of insects. The present study showed that both experimental groups started defecating before ten minutes, but the infected insects did so 4.47 min before the uninfected insects. In addition, the infected insects emitted more defecations than the uninfected insects at 10 min post-feeding and during the rest of the time studied. This effect was also evidenced in the weight of the total of the feces emitted during the post-feeding hour, which was greater in the infected insects. Finally, the proportion of infected insects that defecated after feeding was greater than the proportion of uninfected insects. The epidemiological relevance of these differences is evidenced in the defecation index (DI) which is a measure of the number of parasite release events that a population of a triatomine species will produce in a given time. The DI of the infected insects was 50% higher that of the uninfected at 10 min post-feeding. So, the present study shows that

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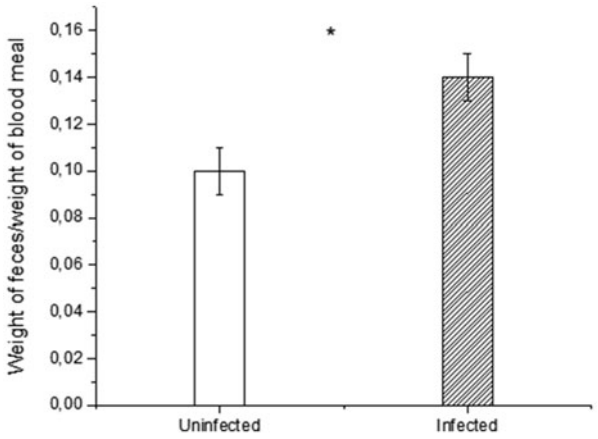

Fig. 3. Weight of total feces emitted for one insect during the first hour after feeding relativized to the blood meal weight for *Triatoma infestans* uninfected ($n = 44$) and infected ($n = 31$) with *Trypanosoma cruzi*. Assays were conducted on fifth-instar nymphs. Bars = means, vertical lines = standard errors. * indicate significant differences by one-way ANOVA ($P < 0.0125$).

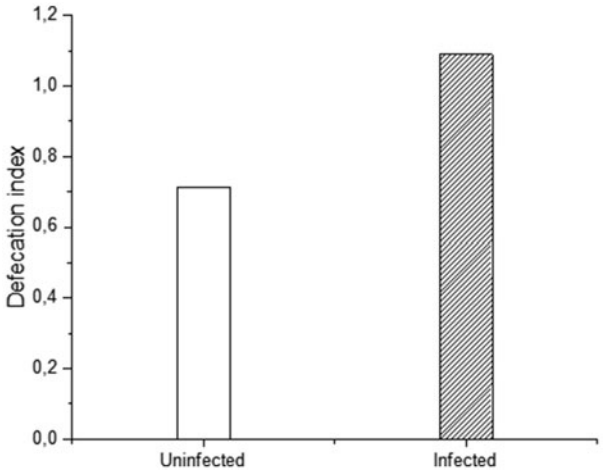

Fig. 4. Defecation Index at 10 min after feeding for *Triatoma infestans* uninfected ($n = 44$) and infected ($n = 31$) with *Trypanosoma cruzi*. Assays were conducted on fifth-instar nymphs. Bars = Defecation index.

T. cruzi affected the excretion/defecation pattern in fifth-instar of *T. infestans* in a way that would increase the probability of contact between infective feces and the mammalian host and, therefore, the probability of parasite transmission. Finally, as there were no effects of the parasite on the feeding pattern, the modification in the excretion/defecation pattern will be a consequence of the effect of *T. cruzi* on the excretion/defecation process itself. Future studies on the possible mechanisms underlying this effect will deepen the knowledge of the interaction *T. cruzi*-*T. infestans*.

Only two previous works studied the effects of infection with trypanosomatids on the excretion/defecation processes in triatomines. Takano-Lee & Edman (2002) did not found differences in the time to the first fecal drop and the number of defecations between *R. prolixus* (fourth and fifth instar nymph and adults) infected and uninfected with *T. cruzi*. In addition, the authors discussed that the defecation index tended to be lower in the infected insects, but this difference was marginal and did not occur in all stages and experimental conditions evaluated. On the other hand, according with the present study Botto-Mahan *et al.* (2006) showed that fifth instar nymphs of *M. spinolai* infected with *T. cruzi* defecated three minutes before than uninfected nymphs, i.e. 25.6% of the average time used by non-infected insects (in the present study, this percentage was 57.5%). Again, the variation in the strain of parasite, triatomine species, and experimental design and contradictory results prevent making general conclusions.

On the other hand, the results of the insects without infection of the present study agree with the results of previous studies that evaluated the feeding and defecation pattern in fifth-instar nymphs of *T. infestans*. The previous studies showed an attack time of 0.5 min (Loza-Murguía & Noireau, 2010), feeding time between 14.19 and 27.45 min (Zeledón *et al.*, 1977; Trumper & Gorla, 1991; Rodriguez *et al.*, 2008; Loza-Murguía & Noireau, 2010), weight gain of 2.67 (Rodriguez *et al.*, 2008), time to first defecation between 3.35 and 8.27 min (Zeledón *et al.*, 1977; Trumper & Gorla, 1991; Rodriguez *et al.*, 2008; Loza-Murguía & Noireau, 2010), percentage of insects that defecated during the first 10 min after feeding between 80 and 90% (Zeledón *et al.*, 1977; Rodriguez *et al.*, 2008) and number of defecations per insect at 10 min after feeding between 0.94 and 1.3 (Zeledón et al, 1977; Rodriguez *et al.*, 2008).

The presence of the parasite inside the vector can modify a wide range of physiological processes of the insect. These modifications may be adaptive consequences of the infection or merely a by-product of infection that by chance and under certain conditions can benefit one of the organisms of the parasite-host system (Poulin, 2010). The first case could be a response of the host that decreases the negative effects of the infection or affects the parasite (i.e. compensatory response by the host) or a modification of some aspect of the host's phenotype which results in an increase in the rate of transmission of the parasite (i.e. adaptive host manipulation by the parasite). It is complex to classify a certain effect of a parasite on a host within these categories. However, the identification of possible benefits that an effect would mean for the parasite or the host would allow proposing the hypothesis by which that effect is effectively an adaptive consequence. Considering the way in which transmission of *T. cruzi* occurs naturally, defecation near feeding clearly would increase the probabilities of transmission of the parasite to the vertebrate host. In turn, the increase in the amount and volume of droppings would also increase the probabilities of transmission of the parasite since more parasites would be released from the insect but, precisely for this same reason, these modifications would also benefit the vector because it would decrease the amount of parasites in its internal environment. To distinguish between these two alternatives, an important criterion is whether the parasite develops the infective stage before the modified event occurs. If this happens, the increased release of parasites will result in an increase in transmission. In triatomines, the defecation occurs when the urine is released and includes the contents that are located in the rectum, the region where the infective stage of *T. cruzi* develops (i.e. metacyclic tripomastigote), which are also released. Then, an increase in the number and/or volume of excreta would release a greater number of infective parasites. In this way, and although a

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by-product of infection is not ruled out, this study proposes the hypothesis that the modifications in the process of defecation/excretion in *T. infestans* by *T. cruzi* are the result of adaptive host manipulation by the parasite. The present research does not allow evaluate this hypothesis and future studies that consider the empirical verification that the observed alteration increases the probability of transmission of the parasite are needed (Poulin, 2010).

In conclusion, this is the first work that describes the effect of *T. cruzi* on the patterns of feeding and defecation/excretion in *T. infestans*. The close link between feeding and defecation/excretion with the transmission of the parasite determines that these processes are relevant components of the vector competence of the triatomines. The present study demonstrated that *T. cruzi* modified the dynamics of the excretion/defecation process in *T. infestans* in a sense that it would increase the probability of contact between feces and the mammal. Thus, considering only the defecation/excretion pattern, *T. cruzi* increased vector competence of *T. infestans* nymphs suggesting that the infection increases the probability of parasite transmission.

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References

Beach, R., Kiilu, G. & Leeuwenburg, J. (1985) Modification of sand by biting behavior by *Leishmania* leads to increased parasite transmission. *The American Journal of Tropical Medicine and Hygiene* **34**, 278–282.

Biral dos Santos, C.B., Leite, G.R., Sessa, P.A. & Falqueto, A. (2006) Dynamics of feeding and defecation in *Triatoma vitticeps* (Stal, 1859) (Hemiptera: Reduviidae, Triatominae) and its potential in the transmission of *Trypanosoma cruzi*. *Memórias do Instituto Oswaldo Cruz* **101**, 543–546.

Botto-Mahan, C. (2009) *Trypanosoma cruzi* induces life-history trait changes in the wild kissing bug *Mepraia spinolai*: implications for parasite transmission. *Vector-Borne and Zoonotic Diseases* **9**, 505–510.

Botto-Mahan, C., Cattan, P.E. & Medel, R. (2006) Chagas disease parasite induces behavioural changes in the kissing bug *Mepraia spinolai*. *Acta Tropica* **98**, 219–223.

Botto-Mahan, C., Ossa, C.G. & Medel, R. (2008) Direct and indirect pathways of fitness-impact in a protozoan-infected kissing bug. *Physiological Entomology* **33**, 25–30.

Campos, R., Acuna-Retamar, M., Botto-Mahan, C., Ortiz, S., Cattan, P.E. & Solari, A. (2007) Susceptibility of *Mepraia spinolai* and *Triatoma infestans* to different *Trypanosoma cruzi* strains from naturally infected rodent hosts. *Acta Tropica* **104**, 25–29.

Crocco, L.B. & Catalá, S.S. (1996) Feeding and defecation patterns in *Triatoma sordida*. *Memórias do Instituto Oswaldo Cruz* **91**, 409–413.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M. & Robledo, C. (2008) W. InfoStat versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available online at http://www.infostat.com.ar.

Eichler, S. & Schaub, G.A. (2002) Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. *Experimental Parasitology* **100**, 17–27.

Elliot, S.L., Rodrigues, J.O., Lorenzo, M.G., Martins-Filho, O.A. & Guarneri, A.A. (2015) *Trypanosoma cruzi*, etiological agent of Chagas disease, is virulent to its triatomine vector *Rhodnius prolixus* in a temperature-dependent manner. *PLoS Neglected Tropical Diseases* **9**, e0003646.

Fellet, M.R., Lorenzo, M.G., Elliot, S.L., Carrasco, D. & Guarneri, A.A. (2014) Effects of infection by *Trypanosoma cruzi* and *Trypanosoma rangeli* on the reproductive performance of the Vector *Rhodnius prolixus*. *PLoS ONE* **9**, e105255.

Heil, M. (2016) Host manipulation by parasites: cases, patterns, and remaining doubts. *Frontiers in Ecology and Evolution* **4**, 80.

Jenni, L., Molyneux, D.H. Livesey, J.L. & Galun, R. (1980) Feeding behavior of tsetse flies infected with salivarian trypanosomes. *Nature* **283**, 383–385.

Koella, J.C., Sørensen, F.L. & Anderson, R.A. (1998) The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proceedings of the Royal Society of London. B* **265**, 763–768.

Lana, M.D., Pinto, A.D.S., Barnabé, C., Quesney, V., Noël, S. & Tibayrenc, M. (1998) *Trypanosoma cruzi*: compared vectorial transmissibility of three major clonal genotypes by *Triatoma infestans*. *Experimental Parasitology* **90**, 20–25.

Lent, H. & Wygodzinsky, P. (1979) Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. *Bulletin of the American Museum of Natural History* **63**, 236–238.

Libersat, F., Delago, A. & Gal, R. (2009) Manipulation of host behavior by parasitic insects and insect parasites. *Annual Review of Entomology* **54**, 189–207.

Lobbia, P., Calcagno, J. & Mougabure-Cueto, G. (2018) Excretion/defecation patterns in *Triatoma infestans* populations that are, respectively, susceptible and resistant to deltamethrin. *Medical and Veterinary Entomology* **32**, 311–322.

Loza-Murguía, M. & Noireau, F. (2010) Vectorial capacity of *Triatoma guasayana* (Wygodzinsky & Abalos) (Hemiptera: Reduviidae) compared with two other species of epidemic importance. *Neotropical Entomology* **39**, 799–809.

Makumi, J.N. & Moloo, S.K. (1991) Trypanosoma vivax in *Glossina palpalis gambiensis* do not appear to affect feeding behaviour, longevity or reproductive performance of the vector. *Medical and Veterinary Entomology* **5**, 35–42.

Marliére, N.P., Latorre-Estivalis, J.M., Lorenzo, M.G., Carrasco, D., Alves-Silva, J., Rodrigues, J.O., de Lima Ferreira, L., de Melo Lara, L., Lowenberger, C. & Guarneri, A.A. (2015) Trypanosomes modify the behavior of their insect hosts: effects on locomotion and on the expression of a related gene. *PLoS Neglected Tropical Diseases* **9**, e0003973.

Nattero, J., Crocco, L. & Rodríguez, C. (2002) Feeding and defaecation behaviour of *Triatoma patagonica* (Del Ponte, 1929) (Hemiptera: Reduviidae). *Memórias do Instituto Oswaldo Cruz* **97**, 1063–1065.

Noireau, F., Diosque, P. & Jansen, A.M. (2009) *Trypanosoma cruzi*: adaptation to its vectors and its hosts. *Veterinary Research* **40**, 1–23.

Oliveira, T., Carvalho-Costa, F., Gomes, T., Sarquis, O., Sposina, R. & Lima, R. (2010) Developmental and reproductive patterns of *Triatoma brasiliensis* infected with *Trypanosoma cruzi* under laboratory conditions. *Memórias do Instituto Oswaldo Cruz* **105**, 1057–1060.

Poulin, R. (2010) Parasite manipulation of host behavior: an update and frequently asked questions. *Advances in the Study of Behavior* **41**, 151–186.

Reisenman, C., Gregory, T., Guerenstein, P. & Hildebrand, J. (2011) Feeding and defecation behavior of *Triatoma rubida* (Hemiptera: Reduviidae) under laboratory conditions and its potential role as vector of Chagas disease in Arizona, USA. *The American Journal of Tropical Medicine and Hygiene* **85**, 648–656.

Rodríguez, C., Carrizo, S. & Crocco, L.B. (2008) Comparison of feeding and defecation patterns between fifth instar nymphs of *Triatoma patagonica* (Del Ponte, 1929) and *Triatoma infestans* (Klug, 1934) under laboratory conditions. *Revista da Sociedade Brasileira de Medicina Tropical* **41**, 330–333.

Schaub, G.A. (2006) Parasitogenic alterations of vector behaviour. *International Journal of Medical Microbiology* **296**, 37–40.

Schaub, G.A. (2009) Interactions of trypanosomatids and triatomines. pp. 177–242 *in* Simpson, S.J. & Casas, J. (*Eds*) *Advances in Insect Physiology*, vol. **37**. Burlington, Academic Press.

Schofield, C.J. (1980) Nutritional status of domestic populations of *Triatoma infestans*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 770–778.

Schofield, C.J., Jannin, J. & Salvatella, R. (2006) The future of Chagas disease control. *Trends in Parasitology* **21**, 583–588.

Smallegange, R.C., van Gemert, G.J., van De Vegte-Bolmer, M., Gezan, S., Takken, W., Sauerwein, R.W. & Logan, J.G. (2013) Malaria infected mosquitoes express enhanced attraction to human odor. *PLoS ONE* **8**, e63602.

StatSoft. Inc. (2011) STATISTICA (data analysis software system). Version 10. Available online at http://www.statsoft.com.

Takano-Lee, M. & Edman, J.D. (2002) Lack of manipulation of *Rhodnius prolixus* (Hemiptera: Reduviidae) vector competence by *Trypanosoma cruzi*. *Journal of Medical Entomology* **39**, 44–51.

Trumper, E.V. & Gorla, D. (1991) Density-dependent timing of defecation by *Triatoma infestans*. *Transactions of the Royal Society Tropical Medicine and Hygiene* **85**, 800–802.

Vallejo, G., Guhl, F. & Schaub, G. (2009) Triatominae-*Trypanosoma cruzi/t. Rangeli*: vector–parasite interactions. *Acta Tropica* **110**, 137–147.

Wood, S. (1951) Importance of feeding and defecation times of insect vectors in transmission of Chagas' disease. *Journal of Medical Entomology* **44**, 52–54.

Zeledón, R., Alvarado, R. & Jirón, L. (1977) Observations on the feeding and defecation patterns of three triatomine species (Hemiptera: Reduviidae). *Acta Tropica* **34**, 65–77.

Zingales, B., Andrade, S.G., Briones, M.R.D.S., Campbell, D.A., Chiari, E., Fernandes, O., Guhl, F., Lages-Silva, E., Macedo, A.M., Machado, C.R., Miles, M.A., Romanha, A.J., Sturm, N.R., Tibayrenc, M. & Schijman, A.G. (2009) A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. *Memorias do Instituto Oswaldo Cruz* **104**, 1051–1054.