Chagas disease parasite induces behavioural changes in the kissing bug *Mepraia spinolai*

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Abstract

Parasites have been shown to manipulate the feeding behaviour of their invertebrate vectors, which results in an increased probability of transmission to definitive hosts. Most evidence for this hypothesis comes from protozoan species with salivary transmission but evidence for stercorarian parasite transmission is lacking. We present experimental evidence that infection of the kissing bug *Mepraia spinolai* (Hemiptera; Reduviidae) with the protozoan *Trypanosoma cruzi* reduced the time to detect potential hosts in comparison to control insects. Infected bugs bit about twice more often than uninfected nymphs and defecated 8 min after the last blood meal whereas uninfected bugs needed 11 min. The behaviour of male and female nymphs did not differ significantly. Since all of these traits relate to parasite transmission, we suggest that parasite-mediated changes in the foraging and defecation behaviour of *M. spinolai* may promote the spread of *T. cruzi* toward definitive hosts.

Keywords: Triatomine; Reduviidae; Hemiptera; Insect vector; Protozoa; *Trypanosoma cruzi*

1. Introduction

The way parasites induce host changes that increase the likelihood of parasite transmission has long attracted the attention of parasitologists and behavioural ecologists (Poulin, 1998; Moore, 2002). Many studies have described that parasites can modify a wide range of physiological, behavioural, and morphological host traits (e.g., Holmes and Bethel, 1972; Moore and Gotelli, 1990; Hechtel et al., 1993; Mouritsen and Jensen, 1994; Ballabeni, 1995; Moore, 1995; Vance, 1996; Poulin and Thomas, 1999). However, whether host alterations represent simple pathological side-effects of parasite infection, consequences of parasite manipulation to increase its own transmission to another host, or host adaptations to minimize the costs of being parasitized is still a source of controversy in the literature (e.g., Poulin, 2000; Wilson, 2000).

Changes in host behaviour or appearance often increase intermediate host susceptibility to predation by definitive hosts (see review in Moore, 2002), or increase parasite inoculation by modifying the feeding behaviour of insect vectors (Hurd, 1990). Transmission by inoculation is represented by haematophagous insects, which often exhibit altered feeding behaviour and increased blood intake when parasitized (Molyneux and Jeffries, 1986; Moore, 1993; Hurd, 2003). In haematophagous vectors, the foraging process is central to parasite transmission, and reported host modifications include the promotion of salivary pathology (Schaub, 1992), an increase

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in the probing behaviour (Killick-Kendrick et al., 1977; Añez and East, 1984; Beach et al., 1985; Wekesa et al., 1992; Garcia et al., 1994), and a decrease in blood vessel location ability (Moore, 2002).

In protozoan parasite–vector associations, such as those of trypanosomes and insects, two mechanisms have been suggested to explain the increase in the number of attacks of infected bloodsucking insects upon hosts. First, trypanosomatids and insect vectors compete for metabolites in the ingested blood, and resource depletion would lead to new feeding attempts by the insect (Schaub, 1992; Kollien and Schaub, 2000). Second, trypanosomes would interfere in the ingestion process of the insect, causing digestive tract disturbances, especially in the foregut and the anterior midgut, leading to new feeding attempts (Schaub, 1992; Borges et al., 2006). Experimental evidence for trypanosome-induced change in triatomine feeding behaviour is scarce and mostly limited to salivary-transmitted parasites (see review in Schaub, 1992). Whether stercorarian parasites induce changes in vector feeding behaviour has been rarely investigated (but see D'Alessandro and Mandel, 1969).

In this study, we focus on an insect-trypanosomatid system, the reduviid vector *Mepraia spinolai* and the protozoan parasite *Trypanosoma cruzi*, an endemic association from the arid zones of Chile (Lent et al., 1994). *Mepraia spinolai* is a strictly haematophagous and diurnal species. It distributes between 18 and 34°S, and its main habitat includes stay grounds, bird nests, rock crevices, and caves, although it has been found in rustic and abandoned houses (Lent and Wygodzinsky, 1979; Schofield et al., 1982; Canals et al., 1997). This species requires the blood of vertebrates to complete its life cycle, which includes an egg, five nymphal stages, and the adult (Sagua et al., 2000; Canals et al., 2001). In many triatomine species one full engorgement is sufficient for moulting from one stage to the next (Kollien and Schaub, 2000).

The protozoan parasite *T. cruzi* is the causative agent of Chagas disease, a serious human parasitic disease in America (Panzera et al., 2004). This heteroxenous trypanosomatid possesses a life cycle that involves several morphologically different stages that can be found in insect vectors and mammalian hosts (Kollien and Schaub, 2000). The trypanosomatid multiplies and differentiates in the digestive tract of the insect vector. The infection of the definitive vertebrate host occurs by contamination of mucous membranes with insect faeces, which contains the infectious metacyclic trypomastigote stage of the flagellate (Kollien and Schaub, 1997).

Here, we evaluate the effect of *T. cruzi* on the foraging and defecation behaviour of its insect vector *M. spinolai*. More specifically, we assess the following questions: (1) does the parasite modify the time needed by the vector to detect and bite a potential mammal host? (2) Does the parasite modify the biting rate or the time for fecal drop deposition of its vector? (3) Are male and female vectors evenly affected by the infection? If behavioural alterations are detected, a more general question can be addressed: (4) would behavioural modifications suggest an increase in the probability of parasite transmission?

2. Materials and methods

2.1. Control and infected nymphs

Mepraia spinolai eggs were obtained from laboratory matings between adults collected at Las Chinchillas National Reserve (31°30′S, 71°06′W, IV Region, Chile). Eggs were daily isolated from the mating jars, cleaned and placed in sterile plastic containers. Once the first-instar nymphs emerged, a random assignment to infected (fed on *T. cruzi*-infected hosts) or control (fed on uninfected hosts) was performed. Each nymph was individually housed in a 3.2 cm × 3.6 cm clear plastic compartment of an 18-compartment box (11.4 cm × 20.5 cm). Olfactive and visual stimuli among insects were allowed by setting small holes on the walls of compartments. Each compartment was provided with sandy bottom and a folded piece of paper as refuge. All insects were reared under optimal growing conditions in a climatic chamber at 26 °C, 65–70% relative humidity, and 14-h light:10-h dark regime (Ehrenfeld et al., 1998).

Nymphs were infected at the first feed, 3–4 weeks after eclosion, using *T. cruzi*-infected laboratory mice (C_3H mouse strain, Central Breeding Ground, Faculty of Medicine, University of Chile, Santiago, Chile). The *T. cruzi* strain used was isolated in May 2002 from *M. spinolai* collected at the field (Las Chinchillas National Reserve, Chile). Trypanosomes in faeces and urine of field-captured insects were used to infect mice by intraperitoneal inoculation. The *T. cruzi* strain was maintained by cyclical transmission across mouse generations (10^4 blood trypomastigotes, Wallace et al., 2001). Only infected mice with increasing parasitaemia were used for feeding purposes. Control nymphs were fed 3–4 weeks after eclosion on uninfected laboratory mice. After each moult, infected and control nymphs were starved for 3 weeks and then fed on infected and uninfected mice, respectively, until the emergence of fifth-instar nymphs. This feeding procedure ensured that trypanosomes reached the stage of being infective to mammal hosts before behavioural experiments were carried out. Additionally, to ensure that infected nymphs were truly

infected, the presence of *T. cruzi* in fecal samples of fifth stage nymphs was verified by light microscopy (NIKON Diaphot-FXA). All kissing bugs fed on infected mice ($N = 101$) showed evidence of *T. cruzi* in their faeces.

Because insect behaviour may be affected by the gender of the insect, once fifth-instar nymphs reached maturity, sex was determined according to diagnostic characters (Lent and Wygodzinsky, 1979). Infected group included 60 female and 41 male nymphs, and control group included 86 female and 69 male nymphs.

2.2. Behavioural experiments

Experiments were carried out 7 weeks after insects moulted to the fifth stage. Infected and control insects were starved since their last bloodmeal as fourth stage. Experiments of 101 infected and 155 uninfected nymphs were performed in an experimental chamber provided with a video camera recorder (SONY DCR-TRV27). The chamber was artificially lighted, and temperature and relative humidity kept constant at 26 °C and 70%, respectively. At the beginning of the experiment, the nymph was kept under an upside down black tube placed on one randomly assigned corner of a square glass container (20.7 cm × 20.7 cm). After a 5-min habituation, insects were released and allowed to feed on an uninfected anaesthetized laboratory mouse placed at the center of the container. Recording started with insect locomotion and ended with insect defecation. In order to avoid any host effect on insect behaviour (e.g., odour stimuli), only 2-month old male mice (ca. 30 g) from the same genetic line were used for experimental purposes. Mice were used as host only once. The glass container was washed with distilled water and alcohol 70% between experiments. Nymph weight was recorded at the beginning and end of the feeding experiment to ensure engorgement.

We quantified the following behavioural variables: (i) host detection (i.e., time elapsed between insect locomotion and antenna orientation towards the host), (ii) first bite (i.e., time elapsed between host detection and first bite attempt), (iii) biting rate, calculated as the number of bites divided by the feeding time (i.e., time elapsed between the first bite and the end of blood ingestion), and (iv) time of defecation (i.e., time elapsed between the end of blood ingestion and fecal drop deposition). The end of blood ingestion was considered as the moment of cessation of biting attempts after engorgement. Experiments were analysed by an independent observer unaware of the infection status and sex of the insect tested.

2.3. Statistical analyses

All the variables measured were analysed in a two-way MANCOVA, with status of infection and host sex as single factors and starvation as covariate (Sokal and Rohlf, 1995). To examine the separate effects on each dependent variable, two-way ANCOVAs were performed with status and sex as single factors and starvation as covariate. Starvation was computed as the number of days elapsed between the last blood ingestion, i.e., engorgement as fourth stage nymph, and the blood ingestion during the feeding experiment as fifth stage nymph. All response variables were checked for homogeneity of variance by using the F_{max} test. To obtain normality, variables were log or square root-transformed (Sokal and Rohlf, 1995).

3. Results

Results from a two-way MANCOVA on measured variables showed significant differences between infected and uninfected insects (Wilks' lambda = 0.91, $P < 0.001$). However, no significant effect was detected between male and female nymphs (Wilks' lambda = 0.98, $P = 0.263$). Separate data analysis revealed that infected insects detected and orientated to a potential host almost twice faster than those uninfected (mean time of detection ± S.E. (s): infected nymphs = 20.92 ± 6.03, $N = 100$; uninfected nymphs = 40.04 ± 9.48, $N = 155$), but sex had no significant effect on this variable (ANCOVA: status, $F_{1,234} = 7.35$, $P = 0.007$; sex, $F_{1,234} = 0.92$, $P = 0.339$; status × sex, $F_{1,234} = 1.01$, $P = 0.316$). The infection status and sex did not affect the time required for the first bite (mean time for first bite ± S.E. (min): infected: male nymphs = 3.57 ± 0.65, female nymphs = 3.17 ± 0.42; uninfected: male nymphs = 5.36 ± 0.57, female nymphs = 3.52 ± 0.41; ANCOVA: status, $F_{1,234} = 2.38$, $P = 0.125$; sex, $F_{1,234} = 1.38$, $P = 0.241$; status × sex, $F_{1,234} = 1.812$, $P = 0.180$). The biting rate was 45% higher in infected insects as compared to control individuals (mean biting rate ± S.E. (bites/min): infected nymphs = 0.27 ± 0.02, $N = 100$; uninfected nymphs = 0.15 ± 0.01, $N = 155$), but no sex effect was detected (ANCOVA: status, $F_{1,234} = 11.53$, $P < 0.001$; sex, $F_{1,234} = 1.36$, $P = 0.244$; status × sex, $F_{1,234} = 0.10$, $P = 0.756$). Finally, the infected insects dejected 3 min faster on the average than uninfected individuals (mean time of defecation ± S.E. (min): infected nymphs = 8.06 ± 0.79, $N = 100$; uninfected nymphs = 10.82 ± 1.37, $N = 154$), but no sex effect was detected (ANCOVA: status, $F_{1,227} = 5.71$, $P = 0.018$;

sex, $F_{1,227} = 1.61$, $P = 0.205$; status × sex, $F_{1,227} = 0.05$, $P = 0.821$).

4. Discussion

Previous studies have suggested that competition between parasites and vectors may result in hungry insect vectors more alert to potential host stimuli (Schaub, 1992; Kollien and Schaub, 2000). In this study, we report experimental evidence of parasite-induced alterations in host location ability in a trypanosome–triatomine system.

Similar to other parasite–vector systems (see review in Moore, 2002), *T. cruzi*-infected insects had an increased biting rate as compared to control insects. For example, in *Leishmania–sandfly* associations, a system with salivary parasite transmission, blocked foreguts have been reported. The pharynx of sandflies can be blocked for its entire length with a plug of parasites implying that only small quantities of blood can be uptake (Killick-Kendrick et al., 1977; Schaub, 1992). Another feeding alteration produced by a salivary-transmitted parasite was reported by Jenni et al. (1980), where *Trypanosoma congolense*-infected tsetse flies seemed to be more voracious and probed significantly more times than uninfected flies (but see Moloo, 1983). For kissing bugs, it has been shown that the probing behaviour of the triatomines *Rhodnius robustus* and *R. prolixus* was increased by infection with the salivary-transmitted *Trypanosoma rangeli* (Añez and East, 1984; Garcia et al., 1994). *Rhodnius prolixus* naturally infected with *T. rangeli* and/or *T. cruzi* fed less frequently than uninfected insects (D'Alessandro and Mandel, 1969). In our system, because *T. cruzi* transmission occurs through defecation, no physiological blockage or salivary gland pathology can be invoked to explain this altered behaviour. Once inside the triatomine vector, *T. cruzi* colonizes the posterior midgut and rectum of the insect (Kollien and Schaub, 2000), which makes unlikely that a migration of parasites toward the foregut and anterior midgut occurs at the time of bloodfeeding. Therefore, this altered behaviour can be a mere consequence of a higher level of starvation of infected individuals due to trace nutrient depletion, which translates into infected insects eager to bite. Impaired feeding may have several consequences for haematophagous insect fitness. For example, infected insects may be malnourished, produce smaller egg clutches, and incur more risk from defensive hosts as they attempt to feed (Moore, 2002).

The time elapsed between the end of blood ingestion and fecal drop deposition was affected by infection status. Notoriously, as it would be predicted by the manipulative parasite hypothesis (see review in Poulin, 1998), in this study we found that infected insects defecated significantly faster than uninfected controls. When insects defecate, infective trypanosomes are released and parasite transmission can occur. Apparently, *T. cruzi* decreases the time between blood ingestion and defecation in *M. spinolai*. Fresh bloodmeals favour a *T. cruzi* detach from the vector's rectum (Schaub and Lösch, 1988). If the reduction in defecation time and the increase in the biting rate are considered altogether, the chance of parasite transmission may be enhanced substantially because more bites imply more potential wounds for parasite contamination from fecal drops shortly deposited. This scenario is especially plausible if the insect remains on the host or at least nearby after engorgement to allow more chance of contact between the host skin and fecal drops deposition.

In summary, we have presented experimental evidence of parasite-induced changes in the foraging and defecation pattern of *M. spinolai*. It is possible that most of these behavioural alterations are mere side-effects of the infection as a consequence of a resource curtailment experienced by the insect during its ontogeny, rather than parasite adaptations to increase transmission. However, irrespective of the ultimate cause, the parasite-induced behavioural modifications reported here might have profound epidemiological consequences for Chagas disease transmission.

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