Life Cycle, Feeding, and Defecation Patterns of *Panstrongylus chinai* (Hemiptera: Reduviidae: Triatominae) Under Laboratory Conditions

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Abstract

Chagas disease is caused by the protozoan *Trypanosoma cruzi*. *Panstrongylus chinai* (Del Ponte) is highly domiciliated in the Peruvian and Ecuadorian Andes and has been found naturally infected with *T. cruzi*. The objective of this study was to describe the life cycle, feeding, and defecation patterns of *P. chinai* in the Loja province within southern Ecuador. To characterize its life cycle, a cohort of 70 individuals was followed from egg to adult. At each stage of development, prefeeding time, feeding time, weight of ingested meal, proportional weight increase, and the time to the first defecation were recorded. *Panstrongylus chinai* completed its development in 371.4 ± 22.3 d, (95% CI 355.4–387.4), which means that it is likely a univoltine species. Prefeeding time, feeding time, and weight of ingested meal increased as individuals developed through nymphal stages. Moreover, time to first defecation was shortest in the early nymphal stages, suggesting higher vector potential in the early developmental stages. Data obtained in this study represent an important advance in our knowledge of the biology of *P. chinai*, which should be considered as a secondary Chagas disease vector species in the Andean valleys of Loja (Ecuador) and in the north of Peru, and included in entomological surveillance programs.

Key words: Chagas disease, Triatominae, Ecuador

Chagas disease, also known as American Trypanosomiasis, is a parasitic disease caused by the protozoan *Trypanosoma cruzi* (Coura 2013), which is mainly transmitted through infected feces of insects belonging to the family Reduviidae, subfamily Triatominae (Coura 2007). Chagas disease is present in 18 countries in the Americas, and its vectorial transmission occurs from the southern United States to southern Argentina (Moncayo and Silveira 2009). The disease is an important public health problem in Latin America, including Ecuador. There are 16 species of triatomines reported in Ecuador, seven of which are considered epidemiologically important (Abad-Franch et al. 2001, Villacís et al. 2010). Limited knowledge exists regarding the biology and vector capacity of most of these species.

Most triatomines are obligate blood feeders in all stages and therefore susceptible to infection by *T. cruzi* through their life cycle, which highlights the importance of studying their biology as well as their feeding and defecation patterns. In particular, the interval between the start of feeding and defecation of a triatomine species is a key factor in determining its *T. cruzi* vector competence (Zeledón et al. 1977). *Panstrongylus* species were previously limited to wild

habitats, although there are increasing reports of different species inhabiting synanthropic environments (intradomicile and peridomicile), thereby increasing their epidemiological importance (Santos et al. 2003, Villacís et al. 2015).

Panstrongylus chinai (Del Ponte) is distributed in Ecuador, Peru, and Venezuela (Patterson et al. 2009). In Peru, this species is reported to be the primary household vector in the Department of Piura (Barrett 1991). In Ecuador, it has been reported to occupy peridomestic environments, including chicken nests and guinea pig pens, as well as inside human dwellings, particularly in bedrooms in the southern provinces of Loja and El Oro in altitudes ranging from 175 to 2,003 m above sea level (masl; Abad-Franch et al. 2001, Grijalva et al. 2005, 2015). Despite considerable efforts, there are no reports of sylvatic populations of *P. chinai* in Ecuador. However, in the case of Venezuela, it was reported only from one sylvatic locality in Mérida State as *Panstrongylus turpiali* Valderrama, Lizano, Cabello Valera (Patterson et al. 2009).

In the absence of vaccines and effective treatments, the main strategy for controlling Chagas disease is based on vector transmission prevention. This can be accomplished mainly by controlling

synanthropic triatomine populations using properly designed campaigns grounded in a solid understanding of the biology and behavior of local triatomine species (Villacís et al. 2008, Suarez-Davalos et al. 2010). This study describes the life cycle, feeding, and defecation patterns of laboratory-reared *P. chinai*, with the aim to orient vector management interventions in the Andean region of Ecuador and Peru.

Materials and Methods

Source of Triatomines

Individuals reared for this study were collected in rural communities of Loja province in southern Ecuador, at altitudes ranging from 991 to 1,937 masl. This range encompasses inter-Andean temperate valleys, with an average rainfall of 400 mm/yr and two rainy seasons and two dry seasons (Villacís et al. 2010). Triatomines were collected in domestic and peridomestic habitats as previously described by Grijalva et al. (2005), under collection permit number 016-07 IC-FAU-DNBAPVS/MA. Triatomines were transported to the insectary at the Center for Infectious and Chronic Disease Research at Pontifical Catholic University of Ecuador, where they were maintained under controlled conditions of $24 \pm 6^\circ\text{C}$, $70 \pm 10\%$ relative humidity, and a photoperiod of 12:12 (L:D) h.

Life Cycle

A cohort was initiated with 73 eggs, which were checked daily to record hatching dates. When nymphs I (NI) emerged (70 individuals), they were placed in plastic vials fitted with fan-folded Whatman filter paper to facilitate movement of individuals and absorption of excess humidity, and a dead adult was added as an endosymbiont source for gut colonization. The vials were checked daily, and the condition of triatomines was recorded, including if they were alive or dead, and the sex of each individual as they reached adulthood. Bloodmeals were offered daily, using immobilized mice (*Mus musculus*), until the first meal, thereafter, bloodmeals were offered weekly. The presence of exuviae was used to determine molting dates of each individual and mortality rate calculated for each stage (Villacís et al. 2008). This work was conducted using protocol 15-H-034 approved by Ohio University Institutional Animal Care and Use Committee.

Feeding and Defecation Patterns

To determine feeding patterns, we identified multiple events as previously described for *Triatoma rubrovaria* (Blanchard) in Almeida et al. (2003), *Meccus picturatus* Usinger in Martínez-Ibarra et al. (2003), and *Rhodnius ecuadoriensis* (Lent & Leon) in Villacís et al.

(2008). Prefeeding time was defined as the time taken by the individual from introduction to the host to insertion of the proboscis into the mouse skin. Feeding time was defined as the time taken by an individual from proboscis insertion to spontaneous detachment from the host. The time to first defecation was recorded for each individual as the time that had elapsed from the initiation of feeding up to 15 min after the completion of the bloodmeal (Martínez-Ibarra et al. 2003, Villacís et al. 2008, Reisenman et al. 2011). The percentage of those insects that successfully fed and defecated during the feeding period or up to 15 min after feeding were calculated. To determine the amount of blood ingested at each feeding, the triatomines were weighed (mg) individually using an analytical balance (Mettler Toledo AB54-S, Switzerland, with a repeatability and readability of 0.1 mg) before and after each meal (Villacís et al. 2008). Because only 11 individuals (nine males and two females) from the life cycle cohort reached adulthood, feeding and defecation patterns were analyzed in an additional group of 29 adults (11 males and 18 females from the same colony) that were added, which were kept under the same conditions described during the development of the life cycle (temperature, relative humidity, photoperiod, and blood-meal source and frequency).

Statistical Analyses

Descriptive statistics for all study variables were calculated using SPSS (Statistical Package for Social Sciences) for Windows, version 19.0 (SPSS Inc., Chicago, IL). Means, their corresponding standard deviation, and 95% CI: limits were obtained. Percent mortality was calculated based on the number of dead insects divided by number of initial insects per stage. Finally, to determine differences in the time to first defecation between females and males, a *t*-test was conducted using STATA version 11 (StataCorp LP, College Station, TX).

Results

Seventy, first-instar nymphs (NI) were obtained from the initial cohort of 73 eggs. The average egg hatching time was 25.6 ± 1.5 d (95% CI 23.7–27.5). The time between nymphal molts increased as individuals progressed through life stages (Table 1). *Panstrongylus chinai* required an average of 371.4 ± 22.3 d (95% CI 355.4–387.4) to complete its life cycle from egg to adult (339 ± 8 and 379 ± 15 d for females and males, respectively) with a total mortality of 84.9% (Fig. 1); the stages with the highest mortality were NI (50%) and fifth-instar nymph (NV; 52.2%; Table 1). Of the 35 NI that died, only eight NI (22.9%) had blood fed.

Fig. 1. Developmental stages of *P. chinai*. Days to complete its life cycle from egg to adult (371.40 ± 22.34). (Online figure in color.)

Table 1. Duration of life cycle (d) and percent mortality of *P. chinai* from Loja, Ecuador, reared under laboratory conditions

Stage ^a	N ^b	Mortality (%) ^c	Developmental days ^d				
			Min.	Max.	Median	Avg. $\pm$ SD	Lower–upper 95% CI ^e
Egg–NI	70	4.1	24	28	25	25.6 $\pm$ 1.5	23.7–27.5
NI–NII	35	50	20	58	37	38.6 $\pm$ 9.0	35.5–41.7
NII–NIII	30	14.3	29	67	42	42.9 $\pm$ 8.7	39.6–46.2
NIII–NIV	29	3.3	26	76	48	51.5 $\pm$ 17.9	44.7–58.3
NIV–NV	23	20.7	43	97	73	73.6 $\pm$ 13.5	67.8–79.5
NV–Adult	11	52.2	107	219	166	168.9 $\pm$ 41.6	139.1–198.7
Total	11	84.9	330	400	371	371.4 $\pm$ 22.3	355.4–387.4

^a Stage: Nymph I (NI), Nymph II (NII), Nymph III (NIII), Nymph IV (NIV), and Nymph V (NV).

^b N=number of individuals ending a stage transition alive. Cohort began with 73 eggs.

^c Percent mortality—the number of individuals that survived to the subsequent stage divided by initial number of individuals that entered that stage multiplied by 100.

^d Temperature (24 $\pm$ 6°C), relative humidity (70 $\pm$ 10%), and photoperiod (12:12 [L:D]) h.

^e Lower and upper 95% CI: limits for a confidence level of 95%.

Table 2. Feeding and defecation patterns of *P. chinai* reared under laboratory conditions collected as eggs from Loja, Ecuador, and fed upon *M. musculus*

Stage ^a	N ^b	Time (mean decimal min $\pm$ SD) ^c			Defecation event (%) ^d 95% CI	Bloodmeal (mg) ^e 95% CI	Proportional increase ^f
		Prefeeding 95% CI	Feeding 95% CI	First defecation 95% CI			
NI	70	2.4 $\pm$ 3.0 1.5–3.44	15.9 $\pm$ 7.8 13.3–18.5	20.3 $\pm$ 9.3 15.3–25.3	62.5 35.9–89.1	8.4 $\pm$ 2.6 7.4–9.4	14.9
NII	35	4.0 $\pm$ 3.4 2.8–5.2	25.7 $\pm$ 12.7 21.2–30.3	23.0 $\pm$ 10.2 18.6–27.4	78.3 60.0–96.5	24.4 $\pm$ 9.5 20.9–27.9	4.7
NIII	30	6.7 $\pm$ 4.0 5.2–8.2	35.7 $\pm$ 16.9 29.4–42.0	27.5 $\pm$ 9.8 24.0–31.0	81.3 67.0–95.6	127.4 $\pm$ 91.0 91.4–163.4	6.8
NIV	29	6.2 $\pm$ 4.0 4.7–7.8	33.7 $\pm$ 16.4 27.2–40.2	33.9 $\pm$ 19.7 24.7–43.1	70 48.0–92.0	215.3 $\pm$ 128.8 151.3–279.3	2.9
NV	23	6.9 $\pm$ 3.2 5.4–8.4	45.4 $\pm$ 23.1 34.6–56.2	56.9 $\pm$ 11.3 47.5–66.4	62.5 19.2–105.8	480.3 $\pm$ 190.6 344.0–616.6	1.5
Adult ♀	20	2.6 $\pm$ 1.8 1.7–3.5	47.2 $\pm$ 27.1 33.8–60.7	31.7 $\pm$ 14.6 24.2–39.2	88.2 71.2–105.3	187.4 $\pm$ 0.04 165.3–209.5	1.9
Adult ♂	20	2.9 $\pm$ 3.5 0.7–5.2	26.8 $\pm$ 17.5 15.7–37.9	32.3 $\pm$ 5.8 25.1–39.4	40 0.0–108.0	105.5 $\pm$ 0.07 63.2–147.8	2.2

Temperature (24 $\pm$ 6°C), relative humidity (70 $\pm$ 10%), and a photoperiod of 12:12 (L:D) h. 95% CI = Lower and upper 95% confidence interval.

^a Stage: Nymph I (NI), Nymph II (NII), Nymph III (NIII), Nymph IV (NIV), and Nymph V (NV).

^b N=number of individuals ending a stage transition alive. Cohort began with 73 eggs.

^c Prefeeding: the time taken by the individual from introduction to the host to insertion of the proboscis into the mouse skin; Feeding: the time taken by an individual from proboscis insertion to spontaneous detachment from the host; First defecation: the time that had elapsed from the initiation of feeding up to 15 min after the completion of the bloodmeal.

^d Defecation event = percent of individuals that defecated during or within 15 min after completion of feeding.

^e Bloodmeal: blood of immobilized mice *M. musculus*.

^f Proportional increase = weight of individual postfeeding divided by the weight of the individual before blood feeding.

The prefeeding time increased with nymphal stage progression, but decreased by 56% in adult males and 64% in adult females as compared with NV (Table 2). Feeding time varied with stage, being highest in NV (45.4 $\pm$ 23.1 min) and adult females (47.2 $\pm$ 27.1 min). Interestingly, while the amount of bloodmeal ingested increased with advancing nymphal stage, blood ingestion decreased markedly in adult males and females with respect to NV (Table 2). The proportion of blood to body weight generally decreased as the nymphal stage advanced.

The time to first defecation increased as individuals progressed from NI to NV and then decreased in adults. Most defecation events occurred while individuals were feeding (Table 2). Additionally, we found that a higher percentage of females (88%) defecated during

feeding and a higher percentage of males defecated soon thereafter (60%; data not shown). The difference in the time of the first defecation during feeding between females (31.7 $\pm$ 14.6, 95% CI 24.2–39.2) and males (32.3 $\pm$ 5.8, 95% CI 25.1–39.4) was not statistically significant.

Discussion

The results of this study suggest that *P. chinai* is a competent *T. cruzi* vector, for in most of the developmental stages, defecation occurs during or right after feeding. Taking into account that vector transmission is the primary mode of acquiring *T. cruzi* (Cook and Zumla 2009), and that in endemic areas >80% of new infections are

associated with this type of transmission (Coura 2013), an appropriate triatomine control campaign depends on understanding their biology and behavior (Miles et al. 2003).

Our results indicate that *P. chinai* requires ~12 mo to complete the development, from egg to adult. This period is within the parameters described for triatomines by Canale et al. 1999 (6–15 mo for multiple species), and with what has been reported specifically for *Panstrongylus tupynambai* Lent [455 d (Salvatella 1986) in Patterson et al. (2009)], but is longer than previous reports on the life cycle of other *Panstrongylus* species, such as *Panstrongylus megistus* (Burmeister) [260–324 d (Neiva 1910) and 100–159 (Perlowagora-Szumlewicz 1976) in Patterson et al. 2009], *Panstrongylus rufotuberculatus* (Champion) [223 and 180 d in female and males, respectively (Wolff et al. 2004)], and *Panstrongylus geniculatus* (Latreille) [275 d (Cabello and Galíndez 1998)]. It is noteworthy that although the laboratory rearing conditions in this study were chosen to mimic natural environmental conditions, natural variations in the field certainly influence the metabolism of the insect, their feeding needs, and consequently their life cycle (Canale et al. 1999, Zeledón et al. 2010). Nevertheless, knowledge about the development period and mortality rate is particularly important when dealing with anthropophylic species with a tendency for domiciliation (Canale et al. 1999), as is the case of *P. chinai* (Grijalva et al. 2005).

The life stages that had the highest mortality were NI (50%) and NV (52.2%). This could be explained by the difficulty of NI to successfully feed as their mouthparts are delicate and that 77% of the dead NI individuals had not blood fed. For NV, mortality was probably due to challenges during the final molt, which has been observed in other species (Villacís et al. 2008, Barreto-Santana et al. 2011, Martínez-Ibarra et al. 2012, Durán et al. 2014).

The prefeeding time increased as individuals progressed through their nymphal stages. According to Zeledón et al. (1977), the time that triatomines are in contact with their host before they bite is between 5–10 min. The shortest prefeeding time for *P. chinai* was found in NI and the longest in NV, with a decline in the adult stage. Nevertheless, *P. chinai* were aggressive toward the evaluated host with a relatively rapid onset of feeding (average 4.55 min) compared with other triatomine species such as the adults of *Triatoma protracta* (Uhler) (12.45 min) and *Triatoma rubida* (Uhler) (9.45 min; Martínez-Ibarra et al. 2012).

With regard to feeding time, prolonged contact with the food source (host) creates a greater chance to defecate on the host (Rabinovich et al. 1979). Species that tend to feed for longer than 10 min are considered potential vectors of *T. cruzi* (Zeledón et al. 1977). In the present study, it was evident that the feeding time increased as individuals developed, recording the longest feeding time in NV (final nymphal instar; 45.4 ± 23.1 min). In addition, feeding time is an epidemiologically relevant variable when the host is infected with *T. cruzi*, and the longer time that the triatomine needs to feed, the greater opportunity for ingestion of the parasite (Oliveira et al. 2009). Prolonged contact between the vector and the host also increases the likelihood of abrupt interruptions, due to host shaking, and therefore increases the risk of exposure to fecal matter (Zeledón et al. 1977, Zárate et al. 1983).

The long feeding times could be explained because sometimes the restrained mouse moved, forcing the insect to reinitiate the feeding process. Species like *Triatoma brasiliensis* Neiva and *Triatoma pseudomaculata* Correa & Espínola have been shown to feed for long periods if undisturbed, with some bugs feeding for >2 h (Soares et al. 2000). On the other hand, it is important to mention that the

time until repletion is affected by the host species and the size of the species, among other variables (Arévalo et al. 2007).

The amount of blood ingested at each feeding depends on the species of triatomine, the nymphal stage, and environmental conditions in which triatomines develop (Carcavallo et al. 1999). Similar to feeding time, the amount of blood ingested by individuals increased as nymphal development progressed and decreased in adulthood. The major requirement of blood for the NV stage is related to the acquisition of new anatomical structures and physiological changes during the molt to adulthood (Arévalo et al. 2007, Barreto-Santana et al. 2011). Additionally, we found that females of *P. chinai* consumed more blood than males. According to Gonçalves et al. (2000), females generally ingest a larger volume of blood to fulfill the energy demands involved in egg development.

Our results regarding weight gained after a bloodmeal are consistent with Schofield (1994), who reported that after a bloodmeal, the nymphs increase eight to nine times their own weight, while adults increase it from two to four times. Schofield argues that this may be related to the fact that adults have completed their biological development and all food ingested would be oriented only to maintenance, survival, and reproductive activities.

According to Zeledón et al. (1977), defecation events occurring during feeding or just after it are important in determining the vector competence of triatomines. Triatomines that defecate during this period, such as *Triatoma infestans* (Klug), *Rhodnius prolixus* Stål and *Triatoma dimidiata* (Latreille), are considered efficient vectors for transmission of *T. cruzi* to humans (Zeledón et al. 1977, Martínez-Ibarra et al. 2001, Arevalo et al. 2007, Rodríguez et al. 2008). Interestingly, in our study, 62–81% of *P. chinai* nymphs defecated during feeding. Previous studies by Noguera-Torres et al. (2000) have described that females defecate sooner than males in seven different species [*R. prolixus*, *T. infestans*, *Triatoma barberi* Usinger, *T. dimidiata*, *Triatoma pallidipennis* (Stål), *Triatoma phyllosoma* (Burmeister), and *Triatoma picturata* Usinger]. However, no statistically significant differences between females over males were found in our study in the time to first defecation during feeding.

Information on the feeding and defecation patterns of little studied triatomine species is relevant to the epidemiology of Chagas disease because it is indicative of the vectorial competence to transmit *T. cruzi*. Despite that *P. chinai* has been cataloged as a candidate vector for the transmission of the disease in the arid areas nearby the Andean western border with Peru (Abad-Franch and Aguilar 2003), no previous studies concerning its biology have been conducted.

Because of the speed in obtaining food, the amount of blood ingested, the rapidity of some stages in producing the first defecation (characteristics of a competent vector), and its high *T. cruzi* natural infection rate (14%; Grijalva et al. 2015), our results indicate that *P. chinai* should be categorized as a potential vector of Chagas disease, and should be monitored continuously in the Andean valleys of Loja (Ecuador) and associated sectors of Peru. Furthermore, this species may move to occupy habitats that species considered as primary vectors could be vacating due to vector control interventions. Currently, additional studies related to morphometric, antennal phenotype, and cytogenetic analyses are being conducted. Altogether, these studies will serve to inform and orient the implementation of control interventions designed to reduce the risk of transmission of Chagas disease in Loja province.

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