Rev Soc Bras Med Trop 50(1):121-125, January-February, 2017
doi: 10.1590/0037-8682-0250-2016

Short Communication

The development of *Panstrongylus herreri* under fluctuating environmental conditions

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Abstract

Introduction: *Panstrongylus herreri* is a main Chagas disease vector, and its success as a vector stems from its ability to establish domiciliated colonies; we aimed to explore its biology and reproduction. **Methods:** The average amount of blood ingested and the time from the beginning of a blood meal to the production of feces were recorded. **Results:** Females exhibited a higher blood ingestion rate than males, but similar defecation times and frequencies were observed. **Conclusions:** Despite the detected decrease in oviposition rates, *P. herreri*'s potential as a Chagas disease vector in environments other than the Amazon forest cannot be discounted.

Keywords: *Panstrongylus herreri*. Biology. Life cycle.

Chagas disease, which is caused by the protozoan *Trypanosoma cruzi*, affects millions of people around the world. The disease can be transmitted by different routes, but the route involving insect vectors known as triatomines represents the primary means of infection in endemic countries[1]. Generally, the insect defecates after it bites a vertebrate host and ingests blood, and the host can subsequently become infected when parasites in the feces enter its body through disrupted mucous membranes or through the skin[2].

The Triatominae subfamily is comprised of approximately 140 species, and 109 of these species, which belong to the *Rhodnius*, *Triatoma*, and *Panstrongylus* genera, are major members of this group[3]. These species tend to live in either sylvatic or urban areas, but evolutionary pressures might allow some species to migrate from sylvatic areas and to adapt to domestic environmental conditions. Furthermore, migration from one ecosystem to another might be related to the introduction of different *T. cruzi* strains to urban areas[4]. *Panstrongylus herreri,* also described as *P. lignarius*[5], is primarily found in the Amazonian forests of Peru, Ecuador, and Brazil, and it is one of the main vectors of Chagas disease in Peru[6]. However, this triatomine has the ability to successfully establish domiciliated colonies[7], thus demonstrating its great potential as a Chagas disease vector and reinforcing the need to study its biology and reproductive aspects under varied environmental conditions.

Since one of the main characteristics of triatomines is their ability to adapt to and develop in distinct geographical areas under either sylvatic or domestic conditions, a better understanding of their biology, behavior, and ability to acclimatize to different environmental conditions may reveal important strategies that can be used to control both the vector and the spread of disease.

To this end, we conducted experiments to assess biology, reproduction, and feeding behavior; to study the impact of fluctuating environmental and geographical conditions on the development of *P. herreri*, 80 nymphs were monitored from egg eclosion to the adult stage. Insects used in this study were kindly donated by São Paulo State University, Araraquara, São Paulo, Brazil; they were raised and maintained in small plastic flasks (6.0cm height × 7.0cm diameter) at the insectary of the Federal University of Triângulo Mineiro [*Universidade Federal do Triângulo Mineiro* (UFTM)] until the 4th nymphal stage was reached. Older nymphs and adults were maintained in intermediate-sized flasks (7.5cm height × 11.0cm diameter). All triatomines were provided with a natural light cycle, fluctuating environmental temperatures (max: 31.03°C ± 0.81°C; min: 18.87°C ± 1.387°C), and humidity conditions (56.85% ± 25.66%) in Uberaba, Minas Gerais, Brazil (19° 44′ 52″ S, 47° 55′ 55″ W; 823m above sea level). The experiments were

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Received 15 June 2016
Accepted 18 October 2016

conducted from September 2013 to March 2014, and specimens were fed weekly on immobilized and anesthetized Swiss mice. The mice were obtained from the animal facility at the Parasitology Division of the UFTM in Uberaba, Minas Gerais, Brazil, and were anesthetized using sodium thiopental (40mg/kg via intraperitoneal injection).

After oviposition, eggs were collected and monitored until eclosion. Eighty nymphs were then divided into colonies, and the time required to reach the adult phase was recorded. The 1st nymphal stage insects were fed 10 days after eclosion or molting (2nd and 4th nymphal stages), and were then monitored until the next molt. Insects at the 5th nymphal stage were fed twice (10 and 20 days after molting). After these feedings, only nymphs that were able to develop into adults were considered viable, and nymphs at the 5th stage were considered viable after the second feeding.

Various aspects associated with the feeding behavior of the insects were examined, including the average feeding duration, the amount of blood ingested, the elapsed time between a blood meal and excretion, and the frequency of feces per meal. Twenty adult insects (10 males and 10 females) that were fasted for 28 days were randomly selected from pre-existing colonies. The rate of blood ingestion was calculated for each individual by dividing the amount of blood ingested by the feeding duration. The triatomines were individually housed in plastic flasks, and were weighed before and after each blood meal using an analytic scale (UniBloc Shimadzu AUY220).

To evaluate the effect of fasting on egg production, oviposition was supervised during specific fasting times. Therefore, eight adult couples were randomly chosen, isolated, and fed only once, and oviposition was subsequently monitored daily over a 21-day period. Eggs were collected on days 7, 14, and 21 after oviposition, and were kept in different containers for analysis.

The normal distribution and homogeneous variance of each variable were tested, and the size of the groups warranted the use of a non-parametric Mann-Whitney test to compare the time of feeding, amount of blood ingested, and the blood ingestion rate. Moreover, Fisher's exact test was used to address the differences between the percentage of males and females that produced feces. Since only eight couples were analyzed, a non-parametric Friedman test was used to compare oviposition between weeks. The results were expressed as mean ± standard error of the mean, and differences were considered statistically significant when $p < 0.05$ (5%). All analyses were performed using GraphPad Prism 5.0 software (San Diego, CA).

The mean time for eclosion was 19.09 ± 0.66 days (**Table 1**). The results indicated that the developmental time from one stage to the next (specifically the five stages between the 1st nymphal stage and the adult stage) was slightly longer than that observed at the previous developmental stage (18.61 ± 0.91, 21.38 ± 0.80, 22.74 ± 0.82, 24.82 ± 0.90, and 34.91 ± 1.86 days), respectively (**Table 1**). Hence, *P. herreri* seems to be able to acclimatize and reach sexual maturity under environmental conditions that differ from their normal conditions.

Feeding behavior was assessed because *P. herreri* individuals were able to reach the adult stage under local environmental conditions, and because blood meals are key to understanding disease transmission and triatomine survival. The results indicated that females required more time than males to complete blood meals, ingested larger amounts of blood, and had higher blood ingestion rates (**Table 2**). Furthermore, 80% of the adult males defecated within 19.27 ± 6.03 minutes after starting a blood meal at a rate of 0.9 ± 0.56 feces/meal (**Table 2**). In contrast, females excreted within 18.81 ± 7.41 minutes at a rate of 1.0 ± 0.00 feces/meal (**Table 2**).

TABLE 1

Time required to complete different developmental stages.

Developmental stage	Median ± SD (days)
Eggs–N1 (n = 80)	19.09 ± 0.66
N1–N2 (n = 70)	18.61 ± 0.91
N2–N3 (n = 45)	21.38 ± 0.80
N3–N4 (n = 38)	22.74 ± 0.82
N4–N5 (n = 28)	24.82 ± 0.90
N5–adult (n = 11)	34.91 ± 1.86
Total	141.29 ± 5.81

SD: standard deviation; **n:** number of individuals at each developmental stage.

We then investigated the ability of *P. herreri* to lay eggs (**Table 3**). Our results indicated that 123, 83, and 40 eggs were laid during the first, second, and third weeks, respectively (**Figure 1A**), and the oviposition rate was 15.37 ± 11.96, 10.37 ± 4.59, and 5.00 ± 4.92 for the first, second, and third weeks, respectively (**Figure 1B**). Furthermore, daily oviposition was also verified, and the rates were 2.19 ± 1.70, 1.48 ± 0.65, and 0.71 ± 0.70 for the first, second, and third weeks, respectively (**Figure 1C**). Despite the fact that no statistical differences were identified, the >60% reduction in oviposition indicated a dramatic decrease between the 1st and 3rd weeks of the study.

In the present study, *P. herreri* was able to reach sexual maturity and feed, and individuals only required a slight increase in developmental time from one stage to the next under varying conditions. However, the ability of *P. herreri* to lay eggs was reduced during the fasting period, which suggests that the reproductive aspects of this triatomine species are highly sensitive to food shortages.

Data concerning the life cycle of *P. herreri* are scarce in the literature, but our results were similar to those observed in a previous study that used comparable temperature conditions[8]. Silva and Silva[8] demonstrated the impact of two distinct temperatures on the development of several triatomine species, including *P. herreri*, and they concluded that triatomines exposed to temperatures as high as 30°C exhibited a shortened life cycle compared to those exposed to a lower temperature (25°C). These results are meaningful given that other important species for vectorial transmission in Brazil such as *T. brasiliensis, T. infestans,* and *T. pseudomaculata* have life cycles of 119.7, 161.9, and 232.6 days, respectively[9]. Furthermore, *T. sordida*, which is considered the most important species for vectorial

TABLE 2

Feeding behavior, time post-meal, and frequency of defecation.

	Time of feeding (min)	Blood ingestion (mg)	Blood ingestion rate (mg/min)	Time of defecation (min)	Insects producing feces (%)	Frequency of defecation (%)
P. herreri (M)	18.73 ± 7.77*	97 ± 38.10*	5.67 ± 2.47*	19.27 ± 6.03	80.0	0.90 ± 0.56
P. herreri (F)	30.06 ± 10.34*	258.7 ± 76.22*	9.23 ± 3.10*	18.81 ± 7.41	100.0	1.0 ± 0.00

P.: *Panstrongylus;* **M:** adult males; **F:** adult females. Number of individuals/sex = 10. A non-parametric Mann-Whitney test was used to compare the time of feeding, blood ingestion, and the blood ingestion rate. Fisher's exact test was used to address the differences between the percentage of males and females that produced feces. Data are depicted as mean ± standard deviation, and an asterisk (*) indicates a significant result ($p < 0.05$).

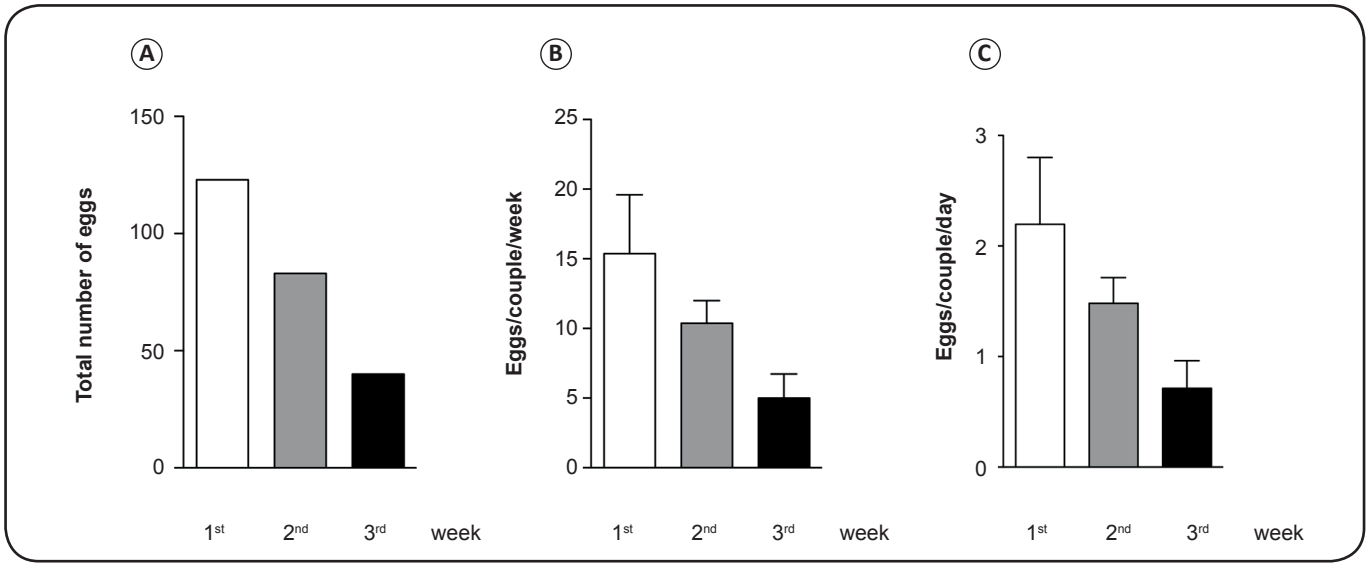

FIGURE 1. **Oviposition of *Panstrongylus herreri* during the fasting period**. After a blood meal, eight couples were separated into individual plastic flasks for three weeks as previously described. (A). Total number of eggs. (B). Eggs per couple/week. (C). Average eggs per couple per day. A Friedman test was used to compare the differences in oviposition between the weeks. The results were expressed as mean ± standard error of the mean.

transmission in the Uberaba region, had a life cycle of 183 ± 36 days under conditions similar to those used in our study[10]. Therefore, the fact that *P. herreri* exhibited an intermediate life cycle reinforces its potential as a vector in areas that are distant from its regular ecological niche.

Of the factors that dictate the ability of triatomines to adapt to different environments and to transmit disease, their feeding behavior is perhaps one of the most important. The results demonstrated that females not only required more time to complete blood meals, but they also ingested higher amounts of blood than their male counterparts. Although no differences were detected between the sexes regarding the post-meal time of defecation, the proportion of females producing feces tended to be greater than males (not significantly different), thus suggesting that females might have a greater capacity to transmit disease than males. In this context, because both blood meals and defecation are essential to the transmission of Chagas disease, triatomines with longer feeding times (such as those of the females observed in our study) are of great epidemiological importance[11]. However, the importance of triatomines with shorter feeding times cannot be disregarded[12].

Taken together, the results presented here indicated that *P. herreri* is able to develop and reach sexual maturity in distant geographical areas. Food availability had a large influence on the rate of oviposition, which suggests that food shortages may have a great impact on the reproductive biology of the species. Furthermore, because females ingested greater amounts of blood than males, it is reasonable to assume that they are potentially stronger vectors. Lastly, because this triatomine species was able to reach sexual maturity under fluctuating environmental conditions similar to those of distant geographical areas, the role of this insect as a Chagas disease vector in environments other than the Amazon forest cannot be overlooked.

TABLE 3

Weekly Oviposition of *Panstrongylus herreri* during the fasting period.

1st week				
Couple (number)	**Number of eggs**	**Standard deviation**	**Daily oviposition**	**Standard deviation**
1	6	11.96348611	0.857142857	1.709069445
2	7		1.000000000	
3	9		1.285714286	
4	5		0.714285714	
5	36		5.142857143	
6	18		2.571428571	
7	11		1.571428571	
8	31		4.428571429	
Total	123		2.196428571	
2nd week				
Couple (number)	**Number of eggs**	**Standard deviation**	**Daily oviposition**	**Standard deviation**
1	17	4.596194078	2.428571429	0.656599154
2	11		1.571428571	
3	10		1.428571429	
4	9		1.285714286	
5	10		1.428571429	
6	11		1.571428571	
7	14		2.000000000	
8	1		0.142857143	
Total	83		1.482142857	
3rd week				
Couple (number)	**Number of eggs**	**Standard deviation**	**Daily oviposition**	**Standard deviation**
1	11	4.928053803	1.571428571	0.704007686
2	2		0.285714286	
3	6		0.857142857	
4	6		0.857142857	
5	2		0.285714286	
6	0		0	
7	0		0	
8	13		1.857142857	
Total	40		0.714285714	

Ethical aspects

All procedures were scrutinized and approved by the Institutional Animal Care and Use Committee of the Federal University of Triângulo Mineiro (Brazil) under protocol 307.

Conflict of interest

The authors declare that there is no conflict of interest.

Financial support

This work was supported by the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), and the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

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