Life Cycle, Feeding, and Defecation Patterns of *Triatoma carrioni* (Hemiptera: Reduviidae), Under Laboratory Conditions

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Abstract

Chagas disease is caused by *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). It is transmitted to humans primarily through contaminated feces of blood-sucking vectors of the subfamily Triatominae, known in Ecuador as ‘chinchorros’. Some Triatominae species can adapt to domiciliary and peridomestic environments where *T. cruzi* can be transmitted to humans. *Triatoma carrioni* (Larrousse 1926) colonizes domestic and peridomestic habitats up to 2,242 m above sea level (masl) in southern Ecuador (Loja Province) and northern Peru. This study describes the life cycle, feeding, and defecation patterns of *T. carrioni* under controlled laboratory conditions using mice as hosts. Specimens were collected in Loja Province, Ecuador, and maintained in the laboratory. The life cycle was approximately 385.7 ± 110.6 d. There was a high mortality rate, 40.9% for first instars and 38.9% for fifth instars (NV). Feeding and defecation patterns for each life stage were examined by recording: insertion time of the proboscis into the host, total feeding time, time to first defecation, and weight of the bloodmeal. Total feeding time varied between 20.6 ± 11.4 min for first instars (NI) and 48.9 ± 19.0 min for adult females. The time to first defecation was variable but ranged from 9.8 ± 10.6 min for NI to 39.4 ± 24.7 min for NV during feeding. This suggests that *T. carrioni* has an annual life cycle and is a potential vector of *T. cruzi* in Loja Province. Improved knowledge of populations of *T. carrioni* in domestic and peridomestic environments of Ecuador can have a significant impact on the prevention and control of Chagas disease.

Key words: Chagas disease, Triatominae, Ecuador, *Triatoma carrioni*, life cycle

Currently, in South America, there are 6–7 million people with Chagas disease, and in Ecuador, there is a national seroprevalence of *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) parasite infection of 1.38%, corresponding to 165–170,000 seropositive patients in the country (Aguilar et al. 2001, Dumonteil et al. 2016). In Latin America, there are >152 species of Triatominae (Hemiptera: Reduviidae) (Mendonça et al. 2016, Souza et al. 2016). In Ecuador there are 16 species of Triatominae, at least 13 of them are considered to have medical importance (Abad-Franch et al. 2001, Grijalva and Villacís 2009). These species are distributed in 18 of the 24 provinces of Ecuador. The chain of potential vectors provide a bridge between sylvatic reservoirs of *T. cruzi* and human hosts (Pinto et al. 2006, Coura 2013). Understanding transmission of *T. cruzi* and designing vector control programs must take into account multiple potential vectors.

The Triatominae have been restricted to wild environments where they feed on small mammals, such as opossums, red-tailed squirrels, rats, bats, and other mammals (Tanowitz et al. 1992, Pinto et al. 2006). However, as humans settled in these remote areas, these insects have colonized domestic and peridomestic environments, associated with chicken nests, guinea pigs, and dogs (Azoh 2014, Grijalva et al. 2015, Urioste-Stone et al. 2015).

Several factors must be considered in evaluating a potential vector for the transmission of *T. cruzi*. 1) The ability of the potential vector to colonize rustic houses in rural areas, places where the vector is in close proximity to human hosts. 2) The capacity to establish a population in domestic or peridomestic habitats that is large enough to efficiently vector the trypanosome (Rodríguez et al. 2008, Mosquera et al. 2016). 3) The ability of the potential vector to acquire *T. cruzi* and the infection rate in the triatomine population.

4) The rate of defecation during and after feeding, as defecation on the host during or shortly after feeding facilitates transmission of trypomastigotes (Luitgards-Moura et al. 2005, Cantillo-Barraza et al. 2015).

There is limited knowledge regarding the life cycle, feeding, and defecation patterns of Triatominae in Ecuador, especially for the species found exclusively in Ecuador. The life cycles of *Rhodnius ecuadoriensis* (Lent and León 1958) (Hemiptera: Reduviidae) (Villacís et al. 2008) and *Panstrongylus chinai* (Del Ponte 1929) (Mosquera et al. 2016) have been described. However, no studies concerning the life cycle and vector potential of any species in the genus *Triatoma* have been carried out in Ecuador.

Triatoma carrioni (Larrousse 1926) is endemic to Ecuador and is adapted for coexistence in domestic (human dwellings) and peridomestic habitats. It is distributed in the southern region of the country, in subtropical areas of Loja Province (Abad-Franch et al. 2001; Grijalva et al. 2005, 2015) and northwestern Peru in Piura, near the border with Ecuador (Calderón 1996, Abad-Franch et al. 2001). This species occupies a wide range of arid to humid ecological zones, between 800 and 2,242 meters above sea level (masl) (Grijalva et al. 2015).

In Ecuador, *T. carrioni* has been found inside human dwellings, mainly in bedrooms. In peridomestic environments, this species can be found in chicken coops, guinea pig pens, dog houses and piles of scrap wood, bricks, and firewood (Grijalva et al. 2015). Despite significant search efforts, our group had not found this species in sylvatic habitats; however, Abad-Franch et al. (2001) reported an adult captured by light trapping and a nymph in an epiphytic bromeliad in the canopy of primary cloud forest.

Triatoma carrioni has been found with a *T. cruzi* infection rate of 8.3% in peridomestic habitats (Grijalva et al. 2015). However, the infection rate in dwellings, domestic habitats, was only 4.5%. This level of infection indicates that this species along with other species, *R. ecuadoriensis* (29.7%) (Villacís et al. 2008, Grijalva et al. 2015) and *P. chinai* (13.6%) (Grijalva et al. 2015, Mosquera et al. 2016) should be considered as efficient vectors in the southern valleys of Ecuador. These three species represent a serious health hazard for human populations in this area. The seroprevalence for Chagas disease in this area has been reported to be 3.6% (Black et al. 2009). The main strategy for controlling Chagas disease has been based on prevention and control of vector transmission. Accordingly, understanding the biology and behavior of the local triatomine species is fundamental. This study describes the life cycle, feeding, and defecation patterns of *T. carrioni* under laboratory conditions to enhance our understanding of the vector potential of this species. This information will be important in the design of monitoring and entomological control strategies in Ecuador and the north of Peru.

Materials and Methods

Origin of the Laboratory Colony

Over all, 141 individuals of *T. carrioni* were collected in 2006 in Jacapo, a rural community (4.3166°S, 79.6266°W; 1,509–2,200 m a.s.l.) in Calvas county, Loja Province, Ecuador (Fig. 1). This region has an average annual rainfall of 400 mm, in two rainy seasons, February to May and October to November. There are also two dry seasons, June to September, and December to January (Grijalva et al. 2005).

Fig. 1. Location of the Jacapo community in Calvas County, Loja Province, Ecuador, where the specimens of *T. carrioni* were collected to start the laboratory colony.

Triatomines were collected in domestic and peridomestic habitats as previously described by Grijalva et al. (2005), under collection permit No. 016-07 IC-FAU-DNBAPVS/MA from the Ministerio de Ambiente. Triatomines were sorted by species, sex, and nymphal stage before being transported to the insectary at the Center for Research on Health in Latin America (CISEAL) at the Pontifical Catholic University of Ecuador in Quito. The colony of *T. carrioni* was formed in 2006. We estimated that for this study, the colony was in the ninth generation. For both procedures (life cycle and feeding and defecation patterns), the colony of *T. carrioni* were maintained under controlled conditions of temperature ($24 \pm 6^\circ\text{C}$), humidity ($70 \pm 10\%$), and photoperiod (12 [L:D] h) (Villacís et al. 2008), simulating the environmental conditions in Calvas county.

Life Cycle and Mortality Rate

For the life cycle study, 44 first instar nymphs (NI) were placed in individual plastic bottles (120 ml) with fan-folded Whatman filter paper (10×10 cm) to facilitate movement and absorption of excess humidity. A dead adult insect was added to each container, as a source of endosymbionts for gut colonization. These microorganisms could also be acquired from the egg surface by coprophagy. Maintaining an intestinal symbiont population is crucial for the insect development (Beard et al. 2001, Eichler and Schaub 2002, Oscherov et al. 2004, Huerta-Núñez et al. 2006, Villacís et al. 2008, Mosquera et al. 2016, Azambuja et al. 2017). If insects in the first nymphal stage died, these were replaced to have sufficient numbers for statistical analyzes. During the first nymphal stage of the triatominae, a bloodmeal from female laboratory mice (Swiss) (*Mus musculus*) immobilized in a small plastic mesh (15×15 cm) was offered daily. Each mouse was placed inside a flask, with the edges covered by double-sided tape to prevent the escape of the triatomines. We removed each individual from the culture bottle and placed it on the mouse. Each triatomine took the bloodmeal from one mouse. All the procedures using mice were approved by the protocol 15-H-034 approved by Ohio University Institutional Animal Care and Use Committee (IACUC). Once a NI had fed successfully, a mouse was offered weekly for subsequent blood meals. The nymphal stage was verified daily. The presence of exuviae was used to determine molting dates of each individual. Mortality rate was calculated for each nymphal stage (Villacís et al. 2008, Mosquera et al. 2016).

The adults that completed their life cycle (10 females and 12 males) were paired for mating. The eggs produced by the females were checked daily to estimate the egg development time and egg mortality rate. The adults were maintained under the same controlled conditions (temperature, relative humidity, photoperiod, and bloodmeal source and frequency) as described above.

Feeding and Defecation Pattern

The quantity of ingested blood plays an important role in molting and development of these insects. To determine feeding patterns, we identified multiple events as described by Villacís et al. (2008) and Mosquera et al. (2016). Individual nymphs were weighed before and after each bloodmeal using an analytical balance (Mettler Toledo, AB54-S, Greifensee, Switzerland, with a precision of 0.1 mg). 1) Prefeeding time was defined as the time from introduction of the host to insertion of the proboscis into the mouse skin (from contact with the host to insertion of the mouthparts into the host skin). (ii) Feeding time was defined as the time from insertion of the proboscis to spontaneous detachment from the host (from feeding initiation to withdrawal of mouthparts from the host). (iii) Weight increment was quantified as the difference between the prefeeding and postfeeding

weights. The amount of blood consumed was expressed as milligrams of blood obtained in each feeding.

Time to first defecation and frequency of defecation were evaluated to determine potential vector effectiveness of *T. carrioni*. We recorded the time until the first defecation during feeding and the percentage of insects defecating during feeding (Martínez-Ibarra et al. 2003, Villacís et al. 2008, Reisenman et al. 2011, Mosquera et al. 2016).

Unlike the protocols followed by Villacís et al. (2008) and Mosquera et al. (2016), in which the bloodmeal was offered for a period of 15 min, in this study this prefeeding period was increased to 20 min. This modification was based on the observation that *T. carrioni* required more time to search for the blood vessel in the skin of the mouse with the proboscis.

Statistical Analysis

The data were entered into an Excel database and analyzed using the SPSS (Statistical Package for Social Sciences) for Windows, v. 20.0 (SPSS). The descriptive analysis of life cycle data included the calculation of means and their corresponding standard deviations. Confidence intervals (95%) were calculated for feeding and defecation pattern variables. Mortality rate was calculated by dividing the number of dead insects by the number of initial insects for each stage. A Kaplan–Meier survival analysis was carried out to estimate the median survival time of nymphal stages. The statistical significance of differences in the survival curves was evaluated by the log-rank test.

To determine differences for the prefeeding, feeding, first defecation, and weight increment between the nymphal stages, we conducted a parametric test (one-way analysis of variance [ANOVA]) and for differences between females and males, we used Student's *t*-test. For differences in the percentage of insects defecating during feeding, we conducted a Fisher's exact test.

Results

Life Cycle and Mortality Rate

The study began with 44 nymphs (NI) of *T. carrioni* and finished with 22 adults, although 40 additional insects were added to the sample group after the NI–NII transition. This species was univoltine and completed its development from egg to adult in 385.7 ± 110.6 d (Fig. 2, Table 1). The time between nymphal molts increased as the insects progressed through life stages. First instars (NI) needed 1 mo (27.4 ± 16.6 d), whereas fourth instars (NIV) needed >4 mo (124.9 ± 48.5 d) to complete their development (Table 1).

During the nymphal stages, the highest mortality was observed in the NI–NII (40.9%) and NV–adult (38.9%) stages (Table 1). The cause of death in the 10 insects (55.6%) of the 18 dead insects during the NI stage could be the difficulty in inserting the proboscis into the mouse skin and beginning feeding. A Kaplan–Meier survival analysis (Fig. 3) showed that the median survival times in NI–NII (97 d; interquartile range: 40 to >106 d) and NII–NIII (98 d; interquartile range: 61 to >123 d) were lower than in NIV–NV and adults (log-rank test, $P < 0.001$).

There was a dramatic variation in development time for insects in each stage (Table 1). Some NI insects completed the transition to NII in as little as 5 d, whereas the maximum was 106 d. This pattern, the range of days (minimum and maximum) for development, was repeated in some of the instars.

Egg Development and Fecundity

We monitored the development of 68 eggs from the 10 females that finished their development. The mean time for egg development to

Fig. 2. Developmental stages of *T. carrioni*. The stages illustrated are from left to right, eggs (i.e., pink eggs color indicative the likelihood of hatching), first instar (NI), second instar (NII), third instar (NIII), fourth instar (NIV), fifth instar (NV), and adult female and adult male. The time to complete the life cycle from egg to adult was 385.7 ± 110.6 d under laboratory conditions.

Table 1. Mortality and duration of life cycle of *T. carrioni* from Loja, Ecuador, reared under laboratory conditions

Stage ^a	Started the stage ^b	Finished the stage ^c	Mortality % (No. of dead insects) ^d CI 95% ^f	Development time (d)		
				Min	Max	Mean $\pm$ SD ^e CI 95% ^f
NI–NII	44	26	40.9 (18) ¹ 26.3–56.7	5	106	27.4 $\pm$ 16.6 ¹ 22.8–32.0
NII–NIII	66	48	27.3 (18) ² 17.0–39.6	14	123	51.8 $\pm$ 28.0 ² 43.6–59.9
NIII–NIV	48	41	14.6 (7) ³ 6.1–27.8	17	105	58.9 $\pm$ 24.8 ² 51.2–66.6
NIV–NV	41	36	12.2 (5) ³ 4.1–26.2	40	269	124.9 $\pm$ 48.5 ³ 104.3–138.9
NV–Adult	36	22	38.9 (14) ¹ 24.7–57.9	17	244	145.2 $\pm$ 63.2 ³ 117.2–173.2
Total		22		167	561	385.7 $\pm$ 110.6

Temperature ($24 \pm 6^\circ\text{C}$), relative humidity ($70 \pm 10\%$), and a photoperiod of 12 (L:D) h.

^aStage: First instar nymph I – NI, second instar nymph – NII, Third instar nymph – NIII, Fourth instar nymph – NIV, Fifth instar nymph – NV.

^bN = number of individuals that began the nymphal stage. Because many NI died, 40 additional second instars were added to have sufficient numbers for statistical analyzes.

^cN = number of individuals that finished the nymphal stage.

^dPercentage of mortality: the number of individuals that survived to the subsequent stage divided by initial number of individuals that entered that stage multiplied by 100. Percentage followed by the same number (1, 2, and 3) are not statistically different (pairwise comparisons using Fisher's exact test, $P > 0.05$).

^eMean and SD of development time for each nymphal stage. Means followed by the same number (1, 2, and 3) are not statistically different (Tukey's HSD test, $P > 0.05$).

^fLower–upper 95% CI

the emergence of the NI was 29.0 ± 4.9 d. The mortality of this group of eggs was 20.6% (14 of the 68 eggs). Females produced a minimum of one and a maximum of four eggs per day.

Feeding and Defecation Pattern

Prefeeding times ranged from 8.3 ± 4.4 min for NI insects to 11.5 ± 5.7 min for NIV insects. The differences between prefeeding times for the nymphal stages were not statistically significant (ANOVA, $P = 0.06$; Table 2). Feeding time increased by 77.2% as insects progressed from NI to NV. The feeding time for adults was three times greater than for NI (Table 2). The difference in the feeding time between nymphal stages was statistically significant (ANOVA, $P < 0.001$). In females, the feeding time (48.9 ± 19.0 min; 95% CI 44.3–53.5) was not statistically different from that of males (48.6 ± 23.4 min; 95% CI 44.8–52.4).

The time to first defecation increased progressively from first instars to male adults but was highly variable. This measure dropped slightly in female adults. The mean time to first defecation during the

bloodmeal in first instars was 9.8 ± 10.6 min. It steadily increased as the insects became larger to a maximum of 39.4 ± 24.7 min for NV nymphs (ANOVA, $P < 0.001$). The difference in time to first defecation between females (31.6 ± 31.9 min, 95% CI 25.3–37.9) and males (68.3 ± 7.0 ; 95% CI 67.2–69.4) was statistically significant (Student's *t*-test, $P = 0.04$; Table 2). The percentage of insects that defecated during feeding was variable (Table 2). A lower percentage of the second instars (NII) defecated during feeding (16.9%, 95% CI 7.8–83.1). The percentage of fourth instars (NIV) defecating during feeding was the highest of all the life stages (61%, 95% CI 39.1–63.2; Table 2; Fisher exact test, $P < 0.001$). About half of the adults defecated during feeding, females – 50.0% and males – 41.7%, there was no significant difference between defecation rates for males and females (Fisher's exact test, $P = 0.696$; Table 2).

The amount of blood taken in a single bloodmeal increased with nymphal stage. The differences in the amount ingested at each stage were statistically significant (ANOVA, $P < 0.001$). First instars (NI) ingested a mean of only 3.9 ± 3.3 mg (95% CI 1.9–3.9; Table 2).

Fig. 3. Kaplan–Meier survival curves according to nymphal stages with mortality as failure criteria.

Table 2. Feeding and defecation patterns of laboratory-reared *T. carrioni*, from Loja-Ecuador and fed on *M. Musculus*

Stage ^a	N ^b	Prefeeding ^c	Feeding ^d	First defecation ^e	% Defecation during feeding ^f	Blood meal (mg) ^g	Weight increment ^h
Time (min)							
		Mean ± SD ⁱ	Mean ± SD ⁱ	Mean ± SD ⁱ	%	Mean ± SD ⁱ	Mean ± SD ⁱ
		CI 95% ^j	CI 95% ^j	CI 95% ^j	CI 95% ^j	CI 95% ^j	CI 95% ^j
NI	44	8.3 ± 4.4	20.6 ± 11.4 ¹	9.8 ± 10.6 ¹	36.3	3.9 ± 3.3 ¹	3.0 ± 1.9
		6.9–9.5	17.2–23.9	6.7–12.9	34.0–63.7	1.9–3.9	2.3–3.7
NII	66	9.3 ± 6.3	27.4 ± 13.6 ^{1,2}	16.2 ± 10.7 ^{1,2}	16.9	12.7 ± 12.6 ^{1,2}	3.4 ± 1.7
		7.8–9.5	24.1–31.4	13.6–19.3	7.8–83.1	9.7–16.5	2.9–3.8
NIII	48	10.9 ± 5.8	31.0 ± 19.8 ^{2,3}	29.4 ± 18.1 ^{2,3}	28.1	25.4 ± 15.6 ²	4.2 ± 2.4
		8.5–13.5	25.4–36.8	24.24–34.5	15.4–71.9	20.9–26.6	3.5–4.9
NIV	41	11.5 ± 5.7	34.5 ± 11.3 ^{2,3}	36.1 ± 19.6 ³	60.9	63.4 ± 43.64 ³	3.9 ± 1.6
		10.4–12.6	31.0–37.9	30.1–42.1	39.1–63.2	58.8–67.9	3.4–4.3
NV	36	8.6 ± 4.9	36.5 ± 17.0 ³	39.4 ± 24.7 ³	29.7	119.2 ± 63.0 ⁴	3.5 ± 1.6
		8.4–11.5	30.9–41.9	31.0–47.8	14.9–70.3	86.9–128.9	2.3–4.0
Female	10	11.4 ± 6.4	48.9 ± 19.03	31.6 ± 31.9	50.0	1564.9 ± 508.1	3.3 ± 0.9
		10.1–12.7	44.3–53.5	25.3–37.9	19.0–50.8	1465.3–1664.5	2.7–4.0
Male	12	9.3 ± 5.6	48.6 ± 23.4	68.3 ± 7.0	41.7	1814.9 ± 428.9	3.4 ± 0.9
		8.4–10.2	44.8–52.4	67.2–69.4	13.8–59.1	1744.8–1884.9	2.8–3.9

Temperature (24 ± 6°C), relative humidity (70 ± 10%), and a photoperiod of 12 (L:D) h. 1, 2, 3, and 4 are used to identify the homogeneous subsets of means that are not different from each other (Tuckey HSD test)

^aStage: First instar nymph – NI, second instar nymph – NII, third instar nymph – NIII, fourth instar nymph – NIV, fifth instar nymph – NV.

^bN = number of individuals that began the nymphal stage.

^cPrefeeding = time taken to insert the proboscis into the mouse skin, initiation of feeding.

^dFeeding = time to complete feeding, from insertion of the proboscis until it was removed.

^eFirst defecation = time from the initiation of feeding to first defecation.

^f% Defecation during feeding = percentage of insects that defecated during feeding. There was no significant difference in defecation rates for adult males and females (Fisher's exact test, $P = 0.696$).

^gBlood meal (mg) = weight of ingested blood of *Mus musculus* calculated as post feeding weight minus prefeeding weight.

^hPostfeeding weight divided by prefeeding weight, Weight increments for each transition were not significantly different (ANOVA, $P = 0.091$).

ⁱMeans followed by the same number (1, 2, 3, and 4) are not significantly different (Tukey's HSD test, $P > 0.05$). Adult females and males were compared separately from the immatures and were not significantly different ($P > 0.05$, Student's t -test).

^jLower–upper 95% CI.

In all life stages, the weight gain after taking a bloodmeal ranged between 3 and 4.2 times the initial weight of the insect before feeding (Table 2) and no significant differences between stages were found (ANOVA, $P = 0.091$; Table 2). The mean amount of blood ingested by adults was 1701.2 ± 472.5 mg (95% CI 1659.1–1743.2) with no significant difference between females and males (Student's t -test, $P = 0.210$; Table 2).

Discussion

Triatoma carrioni is a potential vector of the Chagas disease in the province of Loja, Ecuador. The main factor in potential vector capacity of triatomines is the defecation pattern leading to *T. cruzi* transmission in the feces (Chagas 1909). In all nymphal stages and adults, defecation occurred during or right after blood feeding. This pattern would place infected feces in direct contact with the potential host and facilitate infection through mucosal membranes or the bite wound.

In Ecuador, three epidemiological important genera have been reported, *Rhodnius*, *Panstrongylus*, and *Triatoma*, in relation to the transmission of Chagas disease (Grijalva et al. 2015). However, only a few studies of the life cycles of these insects have been carried out. Villacís et al. (2008) demonstrated that *R. ecuadoriensis* is bivoltine as the development time was 181.3 ± 6.4 d. *Panstrongylus chinai* completed its development in 371 ± 22.3 d, indicating that it is an annual species (Mosquera et al. 2016). *Triatoma carrioni* also appears to be univoltine from the data reported in this study.

The development time, from egg to adult was 385.7 ± 110.6 d (~13 mo) under controlled conditions, which are more stable than conditions the insect would encounter in the field. Development time may vary under natural conditions (Martínez-Ibarra et al. 2008). Depending on the species and feeding frequency, the development from egg to adult lasts from 3 to 10 mo (Patterson et al. 2009) and adults can live from 6 mo to 2 yr (Beard 2005). Insects of the genus *Triatoma* generally spend between 6 and 15 mo to develop from egg to adult under controlled conditions (Canale et al. 1999). However, development time for *T. sherlocki* (Hemiptera: Reduviidae) in Brazil was much greater (621 d from eggs to adult) than observed in this study (Lima-Neiva et al. 2017). We attempted to simulate laboratory conditions similar to those encountered by the insect in its natural habitat. The observed and natural life cycles may vary due to factors such as host bloodmeal source and insect metabolism (Zeledón et al. 2010).

In comparison with other species of the same genus, the life cycle of *T. carrioni* is longer than *Triatoma dimidiata* (Latreille 1811) (Hemiptera: Reduviidae) from Colombia, which completes its development in 269 d (9 mo; Reyes and Angulo 2009). In the *T. dimidiata* study, the bloodmeal and feeding procedure was different. Chicken blood was used, and blood meals were offered fortnightly. *Triatoma ryckmani* Zeledón and Ponce, 1972 (Hemiptera: Reduviidae), endemic to Guatemala, is also an annual species which completes its development in 392.5 d (Zeledón et al. 2010), as in our study, insects of this species were fed with rodents.

Eggs required about a month (29.0 ± 4.9 d) for hatching. The time for development from one instar to the next steadily increased through the life cycle. The same trend has been observed in *Triatoma lenti* (Sherlock and Serafim 1967) (Hemiptera: Reduviidae), *T. dimidiata*, *Panstrongylus geniculatus* (Latreille 1811), *P. chinai*, and *T. ryckmani* (Canale et al. 1999, Zeledón et al. 2010, Mosquera et al. 2016). In contrast, the results observed in *T. sherlocki*, the times for instar to instar transitions were 28–47.5 d, and the transition from the NV stage to the adult required a median time of 377 d (Lima-Neiva et al. 2017).

The first nymphal stage in *T. carrioni* needed a mean of 27.4 ± 16.6 d to complete development. This nymphal stage had the highest mortality rate, 40.9%. This can be explained by the difficulty in finding the host blood vessel by NI and the weakness of the proboscis (Mosquera et al. 2016). The NI seems to be frail (Durán et al. 2014). This observation raises questions about hosts used for feeding by NI and mortality rate for young insects under natural conditions. As *T. carrioni* is associated with chickens, the NI may preferentially feed on nestling chickens with thin skin. High mortality in immature *T. carrioni* might be offset by high fecundity in females. However, in our laboratory study, female fecundity was not notably high. High mortality in NI was also observed in *Triatoma pallidipennis* (Stal 1872) (Hemiptera: Reduviidae), *P. geniculatus*, *Rhodnius neglectus* Lent, 1954, and *Rhodnius robustus* Larrousse, 1927 in the laboratory (Galíndez-Girón et al. 1998, Barreto-Santana et al. 2011). Mice were provided for blood meals in the laboratory in this study; however, *T. carrioni* was found in the field mostly associated with chicken nests in the peridomicile and in the bedroom within the domicile (Grijalva et al. 2015). Guarneri et al. (2000) investigated the effect of pigeon and mouse blood on nymphal mortality and life cycle of three *Triatoma* species in Brazil. In *T. braziliensis*, nymphal mortality was significantly higher in the group that fed on pigeons and the life cycle was significantly longer than the group that fed on mice. It may be that *T. carrioni* is adapted to feed on nonmouse hosts in the field and that laboratory mice are a nonoptimal host especially for early instars.

Development of the third nymphal stage (NIII) was completed in a mean of 58.9 ± 24.8 d with mortality of 14.6%. The decrease in the mortality rate could be explained in part by a more effective penetration of the proboscis into the host blood vessel after gaining experience during the previous nymphal stages (Mosquera et al. 2016). The fourth nymphal (NIV) stage required more time for development, 124.9 ± 48.5 d but had a lower mortality rate, 12.2%. However, the NV required the longest time to develop with an average of 145.2 ± 63.2 d. Mortality in this stage increased to 38.9%. In other species, *T. dimidiata*, *Triatoma rubida* (Uhler 1894), and *Triatoma recurva* (Stal 1868) (Hemiptera: Reduviidae), the later nymphal stages needed up to twice as long to develop in comparison to the NI (Reyes and Angulo 2009). The difficulty in obtaining a complete bloodmeal in many species of triatomines might explain the extended development time. Many species can fast for extended time periods between encountering hosts (Zeledón 1983). In the laboratory, resistance to starvation in *T. sherlocki* increased with successive instars, 57.3 d for NI to 156.5 d for NV (Lima-Neiva et al. 2017). In this study of *T. carrioni*, immatures could fast up to 2 mo between blood meals. As the neurosecretory and hormonal systems related to molting respond to the trigger signal of the bloodmeal, fasting will significantly delay the development cycle (Zeledón et al. 1977, Friend and Smith 1985).

It is important to consider some limitations in this study. The number of NI used to start the life cycle study was small and we needed to add additional insects to the study to maintain sufficient sample size. The colony was relatively small and this could have resulted in some degree of inbreeding. This factor should be considered in comparison of domestic and peridomestic populations with laboratory populations (Canale et al. 1999). It can be difficult to compare several species even within the same genus, because of different methodologies and the food sources used in each laboratory. In other species of *Triatoma*, an important parameter for successful development in the laboratory is temperature and humidity (Guarneri et al. 2000, Emmanuelle-Manchado et al. 2002, Damborsky et al. 2005, Martínez-Ibarra et al. 2008). Early in the establishment of the *T. carrioni* colony, variation in temperature and humidity may have resulted in some mortality, especially in NV. The

difficulty in feeding and high mortality in the NI may result from mice being a nonoptimal food source, as in the field, *T. carrioni* was found in peridomestic and domestic habitats in association with chickens and guinea pigs (Grijalva et al. 2015).

Feeding and Defecation Patterns

In other *Triatoma* species, the volume of the bloodmeal was directly related with development rate through the nymphal stages (Carcavallo et al. 1999, Guarneri et al. 2000, Nattero et al. 2013). During the early instars, the insects need less blood, this is due to their smaller size and a lower capacity for blood meals at each feeding. For each instar, the bloodmeal increased the relative weight of the insect three to four times. Similar results were observed for other species, i.e., NV in *P. chinai* (480.3 ± 190.6 mg; Mosquera et al. 2016); *R. ecuadoriensis* (120.6 ± 30.3 mg; Villacís et al. 2008), and *T. rubida* ($5.4\text{--}391.1$ mg; Reisenman et al. 2011) ingested >100 mg of blood to move to the adult stage. But in adults of these three species, the weight of blood meals decreased; this did not happen in *T. carrioni*. *Triatoma carrioni* females and males ingested >1,000 mg of blood to increase their weight by 3.3 and 3.4 times, respectively. The adults took in 10–20 times as much blood as the NV.

In NI, the proboscis was inserted into the host mouse after 8.3 ± 4.4 min. These nymphs fed for a mean of 20.6 ± 11.4 min. First instar (NI) *P. chinai* required just 2.3 min to be able to successfully begin feeding (Mosquera et al. 2016).

In the last nymphal stage, the amount of blood ingested was higher than in earlier instars (119.2 ± 63.0 mg) and the time to introduce the proboscis in the host blood vessel was 8.6 ± 4.9 min. Similar results for annual species such as *P. chinai* were observed with a mean bloodmeal of 480.3 ± 190.6 mg and the introduction of the proboscis after 7.0 ± 3.2 min. However, *R. ecuadoriensis* took a relatively large bloodmeal (120.6 ± 30.3 mg) in the last nymphal stage. This species is significantly smaller than *T. carrioni*, female *T. carrioni* – 1564.9 vs. ~600 mg for *R. ecuadoriensis* females (Villacís et al. 2008).

For vectorial efficiency, it is essential to consider the time it takes to introduce the proboscis to find the blood vessel of the host and the time to first defecation. The less time it takes for a complete bloodmeal and the faster it defecates, the greater the effectiveness as a vector. Another parameter to be considered for vectorial efficiency is the infection rate by *T. cruzi*. In previous studies, *T. carrioni* had lower infection prevalence (4.5%) in domestic habitats than *P. chinai* (13.6%) and *R. ecuadoriensis* (29.7%) (Grijalva et al. 2015). The infection prevalence in *T. carrioni* in peridomestic habitats, primarily chicken coops, was 8.3%. To understand the dynamic of parasite *T. cruzi* circulation, a study of blood sources is being carried out.

Triatoma carrioni should be categorized as a secondary vector of Chagas disease. First, compared with other potential vectors, the infection rate with *T. cruzi* is relatively low. Second, *T. carrioni* apparently prefers peridomestic habitats as opposed to infesting houses directly. This species typically has not been found in association with a primary vector, *R. ecuadoriensis*. If *R. ecuadoriensis* was found in a habitat, *T. carrioni* was not present (Grijalva et al. 2015). Abad-Franch et al. (2001) reported a sylvatic adult collected by light trap and a nymph in epiphytic bromeliad. Despite significant collecting efforts in this area (Loja Province), we have not found *T. carrioni* in any sylvatic habitat. *Triatoma carrioni* is part of the chain of *T. cruzi* transmission from sylvatic to peridomestic to domestic habitats that result in significant human infections. For these reasons, *T. carrioni* should be monitored continuously with *P. chinai* and *R. ecuadoriensis* in the Andean valleys of Loja (Ecuador) and some areas of Peru to understand the transmission dynamics of this disease.

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