Reproductive parameters of *Rhipicephalus sanguineus* sensu lato under controlled conditions

Jorge Ernesto Eliseo Céspedes-Rosas* | Álvaro Enrique de Jesus Peniche-Cardeña* | José Alfredo Villagómez-Cortés* | Francisco Tobías Barradas-Piña* | David Itzcoatl Martínez-Herrera* | Héctor Vivanco-Cid*

*Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Veracruz, Mexico.
*Instituto Tecnológico Superior de Jesús Carranza, Mexico.
*Instituto de Investigaciones Médico Biológicas, Universidad Veracruzana, Veracruz, Mexico.

**Abstract** *Rhipicephalus sanguineus* is an ectoparasite of importance for both animal and public health. Purpose: The objective of this study was to establish a colony of *R. sanguineus* under laboratory conditions in order to characterize its biological cycle and its reproductive parameters while parasitizing the alternative host most frequently used as experimental model. Methods: Adult ticks were collected in veterinary clinics in the Veracruz-Boca del Rio metropolitan area between November 2020 and November 2021 and a rabbit model was used as host under controlled conditions (28°C ± 2°C and 80% ± 10% relative humidity). Results: The biological cycle had a mean duration of 72.5 days (range 43 to 94 days). The larval feeding period ranged from 2 to 8 days. The larva-nymph molting period was between 3 and 5 days. Nymphal feeding time was 4 to 8 days. The molt from nymph to adult took 8 to 22 days. The adult feeding phase required 6 to 9 days with a mean of 6.9 days. The oviposition period needed from 3 to 19 days. The mean egg mass weight was 50.7 mg. The mean percentage of oviposited weight was 58.5%. The mean of number of eggs obtained per tick was 901.2. An average of 693.0 viable larvae per tick was obtained. The hatching rate of the egg mass was estimated at 72.1%. Conclusion: It is concluded that, under this laboratory conditions, *R. sanguineus* reproductive parameters for developing its biological cycle in a rabbit model can favor research under these controlled conditions.

**Keywords**: *R. sanguineus*, hatching rate, feeding chamber, life cycle

1. Introduction

Ticks are currently one of the most widely distributed ectoparasites in urban and rural settings (Parola & Raoult, 2001). In this sense, *Rhipicephalus sanguineus* (R. sanguineus) is a tick with three hosts and its medical importance is centered on its epidemiological role as a vector of infectious diseases in animals and humans (Otoo et al., 2001; Rodriguez-Vivas et al., 2019).

The study of the reproductive parameters as well as the diverse times required to complete *R. sanguineus* biological cycle under laboratory conditions has been described by some authors. Srivastava & Varma, (1964) characterized two biological cycles in the laboratory for *R. sanguineus* fed on rabbits: one with a duration between 86 and 123 days at a temperature of 25°C, and another with a range of 65 to 90 days at a temperature of 29°C, showing the importance of temperature in the speed of reproduction and maturation of the different tick stages.

In Indonesia, Hadi & Adventini (2015) report that the mean weight of females collected directly from dogs was 68.3 mg; the mean egg production by female was 805.4 mg, the mean weight of the egg mass was 32.68 mg and the viability was 53.9% (at a temperature between 27 and 29°C with a humidity of 80 to 90%). The average pre-oviposition period took 4.9 days while the oviposition period needed 14.3 days; the pre-hatching period took 6.9 days and the egg incubation period required 21.2 days.

In turn, Jacobs et al. (2004) report a pre-oviposition period between 4 and 21 days, a 68.3% of oviposited weight with an average weight of 17.4 eggs/mg. The incubation period ranged from 19 to 72 days, and the molting period from larva to...
nymph took from 9.5 to 36.5 days, while the adult nymph molting period required from 15 to 44 days, and the feeding time for adult females was 7 days. The entire time needed for R. sanguineus to complete its life cycle ranged from 99 to 236 days. In Brazil, Guidotti et al. (2013) estimated R. sanguineus reproductive parameters at a temperature of 27°C ± 1°C with a humidity over 70%; under these conditions, they report that the mean weight of the teleogines collected in dogs was 170 mg requiring an average of 4 days for their pre-oviposition period. The mean egg mass weighted 100 mg, and the hatching period mean was 31 days; the weight of the dead teleogines was 30 mg.

The sex ratio is variable and rarely considered in studies oriented to the biology of R. sanguineus (Dantas-Torres & Otranto, 2010); however, its importance lies in males’ ability to cover longer life cycles than those of females. This situation also allows them to mate with females of different generations, which can influence the preservation of male genes resistant to ixodicides. In this sense, a minor proportion of females could influence a smaller population of ticks in the environment. However, and due to the sanitary importance of this species, these reproductive parameters are unknown to Mexican ticks. Therefore, the objective of this study was to obtain data on the life cycle of R. sanguineus under controlled conditions while parasitizing rabbits. This is due to the popularity of rabbits as an experimental model for studying hemoparasites under controlled conditions and the lack of information on certain life cycle parameters of R. sanguineus parasitizing this host.

2. Materials and Methods

2.1. Location and accommodation of animals

The study was carried out in the Campo Experimental La Posta, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located at kilometer 22.5 of the Veracruz-Córdoba highway in the municipality of Medellín, Veracruz, Mexico. The rabbits were housed in a building with concrete walls and ceiling with dimensions of 10 m long, 5 m wide and 3.5 m high. For individual housing, galvanized steel cages of 90 cm long, 60 cm wide and 38 cm high were used in order to provide an individual area of 0.30 m² in accordance with the provisions of NOM-062.-ZOO-1999 (Sagarpa, 2018). The cages were placed at a height of one meter to allow the fall of excreta and urine and facilitate their collection, cleaning and disposal.

2.2. Management and feeding of experimental animals

In this study, rabbits were selected as experimental hosts to reproduce the life cycle of R. sanguineus. This choice was made because rabbits are the most frequently used experimental model for studying the interaction between ticks and their hosts.

Six adult New Zealand rabbits weighing approximately 2.5 kg ± 0.5 kg were used. The animals underwent weekly clinical check-ups starting the week before the first infestation; the foregoing, with the purpose of monitoring and maintaining optimal health status. During the study, the animals were housed in a temporary animal facility under the conditions described above.

They were fed daily and individually with 100 g of a commercial concentrate which was supplied manually in metal feeders; similarly, hay was provided ad libitum. All food residues were removed at the beginning of the daily review.

The water was provided on demand through metal nipple drinkers, connected to a 200 L container. In order to prevent gastrointestinal infectious disorders, sodium hypochlorite was added to the drinking water at a rate of 2 ml per 20 L of water, as recommended by the World Health Organization (WHO, 2013).

2.3. Tick feeding

To provide ticks with blood, a controlled feeding chamber was used to fix it to the rabbit’s body (Barradas-Piña et al., 2017). A small area on the back was shaved and it was adhered to the feeding chamber with the help of a self-adhesive elastic gauze “Hypafix” of the Leukoplast brand and adhesive cloth. Both the elastic gauzes as well as the feeding chambers were removed from the animals during tick molting periods to clean and re-shave the area for the next feeding period. In compliance with NOM-033-ZOO-1995 (Sagarpa, 2015). Once the investigation was concluded, the animals were sacrificed by intracardiac injection with sodium pentobarbital (150 mg per kg/weight) after intramuscularly sedation with ketamine (30 mg per kg/weight) (Carpenter 2018). The disposition of the corpses was carried out by means of deep burial.

2.4. Experimental colony of R. sanguineus

For the establishment of the colony, it was decided to adapt a combination of the methodologies proposed by Morales-Soto (1995) and by Barradas-Piña et al. (2017) (Figure 1).

2.5. Tick collection

Engorged females with a length equal or higher than 8 mm were collected from naturally infected domestic dogs with no history of tick treatment. The collection area was in the Veracruz-Boca del Río conurbation, Veracruz, Mexico. The ticks were transferred into glass jars with perforated lids to the Animal Health Laboratory of the Campo Experimental La Posta, of INIFAP
where they were disinfected by immersion for 10 seconds in a peroxide of hydrogen solution at a concentration of 80 parts per million, to prevent the growth of entomopathogenic microorganisms that could affect ticks. Subsequently, the taxonomic identification was carried out by means of the dichotomous keys proposed by Nava et al. (2019) using the direct observation method with the help of a stereoscopic microscope. Specimens of species other than *R. sanguineus* were killed by freezing and discarded. Selected ticks were weighed on an analytical balance and individually housed in sterile cotton-stoppered Eppendorf tubes at constant temperature and humidity of 28°C ± 1°C and 80-90%, respectively, inside a culture oven until their oviposition. The humidity percentage inside the culture oven was maintained by placing water bowls inside it.

![Figure 1 Methodology for establishing the artificial colony of *R. sanguineus*.](image-url)

2.6. Monitoring

To determine the parameters of adult ticks, daily and individually, the ticks were checked to identify the time elapsed between the start of oviposition and its duration. The time elapsed between the day of collection and the initial day of laying eggs was considered as the pre-oviposition period. The start of oviposition was determined as the day on which the first egg from each tick was observed, and the end date was defined as the date on which the tick did not lay eggs for more than one day.

To prevent contamination of the egg mass, the dead tick was separated and its weight and that of its respective egg mass was determined with the help of an analytical balance of the “Denver instrument” brand. To achieve hatching, the eggs were incubated at 28°C ± 2°C with 80-90% humidity and monitored once a day with the help of a stereoscopic microscope to determine the start and end days of this phase (Morales-Soto, 1995). Day one of the period was considered the date on which at least one live larva was observed and the last, when only empty eggs were observed. The larvae resulting from this process were kept for an additional 14 days in the culture oven to allow them to mature prior to infestation in rabbits. For each female, the hatching rate (ER) of her egg mass was estimated; for it, the number of larvae obtained from it plus the number of unhatched eggs was considered as a sum (Dantas-Torres et al., 2018). The hatching rate was calculated with the formula proposed by Rodriguez-Pacheco et al. (2017):

$$\text{TE} = \frac{L}{H} \times 100.$$  

Where:

- **TE** = Hatching rate of the egg mass
- **L** = Number of hatched larvae
- **H** = Number of incubated eggs

To determine the female/male ratio among the experimental ticks, a group of 251 non-fed adult specimens was separated, killed by freezing to facilitate handling, and sex was determined by direct observation based on the presence of scutum. Males have a complete dorsal scutum and females an incomplete dorsal scutum.
2.7. Artificial infestation

As already mentioned, controlled feeding chambers of 60 mm diameter and 40 mm height were used to carry out artificial infestations in rabbits, following the methodology proposed by Barradas-Piña et al. (2017) (Figure 2). The cameras were monitored once a day to remove fed ticks, to record the time spent doing so, and also to remove dead ticks.

Between 500 and 800 14-day-old larvae were used to infest each rabbit. After their feeding period, the larvae were recovered, counted, and separated into batches of 100 individuals. These batches were weighed before being placed into a culture oven set at 28°C ± 2°C. A water bowl was placed in the culture oven to maintain 80-90% humidity to allow them to carry out their molting process to the nymphal stage. These environmental parameters were used at all stages of the life cycle of the ticks. During this period, they were monitored daily to record the time elapsed until the beginning of their ecdysis, which was considered as the day in which at least one live nymph was observed inside the vial and the end, when all had molted. They were kept in culture under controlled conditions (28°C ± 2°C and 80% ± 10%) for 14 days to allow them to mature. Then, 100 nymphs were used to reinfest each rabbit. Once the nymphs fed, they were withdrew from the animals, weighed on an analytical balance, and separated into groups of 50 in Eppendorf-type vials with a cotton plug to continue their culture. Similarly, their molting time to adults was recorded.

In this sense, the first day of nymphs molting was considered when at least one live adult tick was observed in the vial and the last day when all the nymphs had molted to their adult stage. Non-fed adult specimens were morphologically sexed with the help of a stereoscopic microscope; in the case of males, the criterion used for this was the presence of the complete dorsal scutum. Subsequently, they were separated into groups of five males and 20 females to reinfest each rabbit, allowing them to feed and mate. Their feeding time in this phase was noted; once the teleogins were engorged, they were collected, weighed, individually measured and housed in Eppendorf vials. Males were killed by freezing. During this phase, the start and end days of oviposition were quantified for each female. This methodology was adapted from Barradas-Piña et al. (2017) and is presented in Figure 3.

2.8. Statistical analysis

The results were analyzed with descriptive statistics using the Minitab 17 ® program to obtain the means of the feeding periods duration for the different stages of the biological cycle, as well as the females’ reproductive parameters. To verify if there exists any correlation between the variables, the Pearson correlation test was used with a 95% confidence level using the Minitab 17 ® program.
3. Results and discussion

In total, 103 engorged adult *R. sanguineus* females were collected, of which one died without ovipositing and 102 completed this stage of their life cycle. The total time that its biological cycle lasted under laboratory conditions was 72.5 ± 23 days.

3.1. Teleogins weight

The weight of the females ranged between 30 and 150 mg with a mean of 86.9 ± 25.9 mg (Table 1), which is higher than that reported by Hadi & Adventini (2015), who obtained 68.3 mg as a mean under laboratory conditions; in comparison, Bechara et al. (1995) also obtained an adult weight of 26 ± 17 mg in guinea pigs. Bechara et al. (1995) also calculate the mean for teleogin weight in dogs, foxes and hamsters was it was 126 ± 27, 128±25 and 71 ± 28 respectibly. Furthermore, the weight range found is wider than that mentioned by Morales-Soto (1995), who describes it as between 100 and 132 mg. Levin et al. (2012) calculate a higer average of teleogin weight for ticks feeded on rabbits of 290 ± 5 mg. In this sense, a lower weight was observed in relation to the average of 170 mg reported by Guidotti et al. (2013) both in free-living ticks and in naturally parasitized domestic dogs. Likewise, the weight is lower than the results published by Dantas et al. (2018), who notified a mean of 170.9 mg for ticks grown in the laboratory and 160.8 mg for free-living ticks. Labruna et al., (2017) calculate a mean teleogines weight of 146 mg under summer conditions and 268 mg in winter conditions.

Table 1 Weights average of *Rhipicephalus sanguineus* ingurgitated teleogines, nymphs and larvae artificially fed in rabbits under laboratory conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Max-min*</th>
<th>Mean</th>
<th>SD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teleogin weight (mg)</td>
<td>102</td>
<td>30-150</td>
<td>86.9</td>
<td>25.9</td>
</tr>
<tr>
<td>Teleogin length (mm)</td>
<td>102</td>
<td>8-12</td>
<td>8.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Larv weight (mg)</td>
<td>135</td>
<td>1-86</td>
<td>29.89</td>
<td>18.85</td>
</tr>
<tr>
<td>Nymph weight (mg)</td>
<td>56</td>
<td>3-285</td>
<td>102.83</td>
<td>83.7</td>
</tr>
</tbody>
</table>

* Max-min: range between which all observations are found.
** SD: Standard deviation.

The variations observed between the results obtained in this study and those reported by other authors may be due to the conditions in which the ticks developed. In the present study, they were always kept at 28°C ± 1°C with humidity between 80 and 90%, while Dantas et al. (2018) used 24°C and 70% humidity and Guidotti et al. (2013) cultivated them at 25°C with 70% of humidity. Labruna et al., (2017) use a combinations of different temperature gradients to simulate the annual variation of temperature between 10 °C and 29°C with 80% humidity. Another factor that could influence is the species of host used as a feeding model; in this sense, Dantas et al. (2018) Morales-Soto (1995) Bechara et al. (1995) and Labruna et al., (2017) used rabbits while in the other studies ticks fed on domestic dogs; however, Morales-Soto (1995) and Bechara et al. (1995) mentions that there are no significant differences in the reproductive parameters of *R. sanguineus* depending on the host they are parasitizing. Another possible explanation for the decrease in teleogines weight regarding other studies findings is the time required for females repletion. On this topic, Dantas et al. (2018) report feeding periods between 13.4 and 20.4 days in adults, while the range observed in this study was 6 to 9 days. Longer feeding periods, catalyzed by less favorable environmental variables, may influence the teleogines to reach a greater weight at the end of their repletion.
3.2. Larvae weight

The mean weight of the larval phase was 29.89 ± 18.86 mg (Table 1), ranging between 1 and 86 mg. Szabo et al. (2005) reported a larval weight of 0.47 ± 0.04 mg for Argentinian ticks and 0.29 ± 0.03 mg for Brazilian ticks both fed on rabbits. The results of the present study are similar to the weights of Brazilian ticks but lower than those of Argentinian ticks.

The difference in the parameters calculated in the present study and the results obtained by Szabo et al. (2005) can be explained by the phylogenetic variation of Argentinian ticks in comparison with Brazilian ticks, which is similar to the present study, where the ticks come from Mexico. It is consistent with Sanchez et al. (2016), who report statistical differences in the weight of different \textit{R. sanguineus} phylogenetic populations from different geographical locations.

3.3. Nymphs weight

The nymphs’ weight was 102.83 ± 83.7 mg (Table 1) the range of this was 3-285 mg. For this parameter, Szabo et al. (2005) calculate 5.84 ± 0.95 mg to Argentinian ticks and for Brazilian tick it was 4.08 ± 0.43 mg. The results obtained by Szabo et al. (2006) was different to the present study results, might this be attributable to the different geographical origins of the ticks utilised in both studies.

3.4. Egg mass weight

This parameter ranged from 9 to 100 mg, with a mean of 50.7 ± 19.5 mg (Table 2). Bechara et al. (1995) conducted a study on ticks fed on various hosts and found a mean egg mass weight of 76 ± 27 mg with dogs, 81 ± 30 mg with foxes, 6.6 ± 8 mg with guinea pigs, and 37 ± 21 mg with hamsters. Levin et al. (2012) reported a mean of 190 ± 5 mg in a study with canine hosts. Guidotti et al. (2013) determined a mean egg mass weight of 100 mg for both free-living ticks and those obtained from naturally parasitized domestic dogs, at a temperature between 26 and 28°C with 70% humidity. Similarly, Hadi & Adventini (2015) obtained an average of 32.6 mg for the weight of the egg mass for ticks retrieved from parasitized dogs under laboratory conditions (25 to 27 °C with humidity of 80-90%). Dantas et al. (2018) obtained a weight of 95 mg ± 54.3 mg for this parameter under laboratory conditions (26°C ± 1°C, 70% ± 10% humidity), while in the wild, the mean of this parameter was 49.3 mg ± 27.9.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Max-min*</th>
<th>Mean</th>
<th>SD**</th>
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</thead>
<tbody>
<tr>
<td>Egg mass weight (mg)</td>
<td>102</td>
<td>9-100</td>
<td>50.7</td>
<td>19.5</td>
</tr>
<tr>
<td>Oviposited weight percentage (%)</td>
<td>102</td>
<td>11.3-91.0</td>
<td>58.5</td>
<td>16.2</td>
</tr>
<tr>
<td>Number of eggs (no)</td>
<td>52</td>
<td>0-1753</td>
<td>901.2</td>
<td>400.9</td>
</tr>
<tr>
<td>Larvae per tick (no)</td>
<td>52</td>
<td>0-1753</td>
<td>693.0</td>
<td>449.3</td>
</tr>
<tr>
<td>Hatching rate (%)</td>
<td>52</td>
<td>0-100</td>
<td>72.1</td>
<td>35.8</td>
</tr>
</tbody>
</table>

* Max-min: range between which all observations are found.
** SD: Standard deviation.

In this regard, the results proposed by Bechara et al. (1995) for ticks fed on dogs and foxes are consistent with the findings of the present study. Similarly, for free-living ticks, Dantas et al. (2018) reported a comparable result for the average weight of the egg mass (49.3 mg), which is higher than the 32.6 mg reported by Hadi & Adventini (2015). This similarity may be attributed to similarities in the methodologies used during the growth and feeding of ticks in the laboratory. The disparity found with the results of Guidotti et al. (2013) can be attributed to the collection of teleogines under natural conditions rather than using laboratory models, as in the present investigation. Additionally, the difference with Levin et al. (2012) could be explained by the use of dogs as hosts.

3.5. Oviposited weight percentage

This indicator ranged from 11.3% to 91.0%, with a mean of 58.5% ± 16.2% (Table 2). In this regard, the obtained result is similar to the 55.7% reported by Dantas et al. (2018) for ticks maintained under laboratory conditions at 26°C ± 1°C and 70% humidity. For free-living ticks, these same authors obtained an average of 38.9% for this parameter. In a similar study, Levin et al. (2012) reported a comparable result, with 65.4 ± 9.3% for ticks fed on dogs under similar temperature and humidity parameters. Guidotti et al. (2013) mentioned in their study an average of 61% for ticks in the wild, which aligns with the findings of the present work. The consistency of this reproductive parameter across the studies suggests its relative stability in both natural and laboratory conditions.

3.6. Number of eggs per tick

Between 0 and 1753 eggs per tick were estimated with a mean of 901.2 ± 400.9 (95% CI: 772.4 - 1019.4) (Table 2). Rodriguez-Vivas et al. (2023) report a range between 1000 and 4000 eggs per tick under free-living conditions. Dantas et al.
(2018) obtained a mean of 468.6 ± 222.1 eggs under laboratory conditions and 281.7 ± 221.4 for free-living ticks. Guidotti et al. (2013) determined an approximate of 2300 eggs per tick; however, this number was not a direct count but rather an estimate from the relationship between the weight of the egg mass and the number of eggs/mg. Hadi & Adventini (2015) report an average of 805.4 eggs per tick with a range between 135-1707, in ticks parasitizing dogs under laboratory conditions. Szabo et al. (2005) obtained a mean of egg production of 3822 ± 782.9 per tick for ticks fed on rabbits under controlled conditions. The difference with the results of Dantas et al. (2005), which may be due to the more controlled laboratory conditions used in both investigations to maintain ticks during their oviposition period. Some differences were noted with the results of Dantas et al. (2018), which could be related to the environmental variables used in their laboratory study and, on the other hand, to the conditions under which the free-living teleogines were found, resulting in a much lower mean for this parameter. As for the differences identified with Guidotti et al. (2013), they can be related to the methodology used for obtaining this data through a calculation based on the weight of the egg mass, while in the present investigation, it was determined by direct counting.

3.7. Hatching rate

The hatching rate ranged from 0.0% to 100.0% with a mean of 72.1% ± 35.8% (Table 2). In this regard, Bechara et al. (1995) obtained a mean of 92.2% ± 13.5% for ticks fed on dogs, 83.7% ± 31.2% on foxes, 73.2% ± 31.8% on guinea pigs, and 91.4% ± 8.3% on hamsters. Dantas et al. (2018) reported under laboratory conditions a hatching rate of 84.6% ± 15.1% with a range of 57.8%-99.3%, while for free-living species, it was 20.1% ± 14.9% with a range of 0.3%-40.7%. Guidotti et al. (2013) mentioned a hatching rate of 94% in free-living ticks in the environment and 95% in ticks parasitizing dogs. Hadi & Adventini (2015) reported a hatching rate of 53.9% for teleogines under laboratory conditions. In a study published by Szabo et al. (2005), the mean hatching rate was 95.94% ± 5.15% for ticks fed on dogs, and 0.06% ± 0.04% for ticks fed on rabbits. Labruna et al. (2017) found that the hatching rate was 68% under warm conditions, but under cold conditions, it was 80%. The differences between the results obtained in this study and those of Dantas et al. (2018), Guidotti et al. (2013), Szabo et al. (2005), and Labruna et al. (2017) could be due to the environmental variables used in each study.

3.8. Pre-parasitic period

The pre-oviposition period ranged from 1 to 33 days with a mean of 6.6 ± 7.3 (Table 3). In a study with four different hosts, Bechara et al. (1995) obtained a mean of 4.2 ± 1.4 days for ticks on dogs, 4.3 ± 2.1 days on foxes, 6.8 ± 2 days on guinea pigs, and 3.3 ± 1.4 days on hamsters. Levin et al. (2012) obtained a mean of 7.42 ± 1.99 days for ticks fed on rabbits. Hadi & Adventini (2015) reported in their study a mean of 4.6 days. Guidotti et al. (2013) estimated a mean of 4.1 days for ticks fed on dogs and 3.9 days for ticks in free life. Dantas et al. (2010) estimated the pre-oviposition period to be between 1 and 4 days with a mean of 2.5 days for ticks in laboratory conditions, and between 2 and 12 days with a mean of 4.7 for free-living ticks. Similarities were observed between the results obtained in this work and those of these authors. This can demonstrate that this parameter is not affected by environmental conditions and different hosts.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>SD**</th>
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<td>Pre-oviposition (days)</td>
<td>102</td>
<td>1-33</td>
<td>6.6</td>
<td>7.3</td>
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<tr>
<td>Oviposition (days)</td>
<td>102</td>
<td>3-19</td>
<td>10.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Incubation (days)</td>
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<td>11-28</td>
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<td>3.2</td>
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<td>Time to hatching (days)</td>
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<td>4.4</td>
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<tr>
<td>Larval feeding (days)</td>
<td>135</td>
<td>2-8</td>
<td>3.6</td>
<td>1.0</td>
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<td>Molt larva-nymph (days)</td>
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<td>1-15</td>
<td>9.6</td>
<td>3.8</td>
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<td>Nymph feeding (days)</td>
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<td>3-8</td>
<td>5.1</td>
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<tr>
<td>Molt nymph-adult (days)</td>
<td>56</td>
<td>8-22</td>
<td>14.1</td>
<td>4.1</td>
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<tr>
<td>Adult feeding (days)</td>
<td>127</td>
<td>6-9</td>
<td>6.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Max-min: range between which all observations are found.
** SD: Standard deviation.

The oviposition period lasted between 3 and 19 days with a mean of 10.4 ± 3.3 (Table 3). Hadi & Adventini (2015) observed a range from 9 to 19 days with a mean of 14.3 days. Dantas et al. (2018) for ticks in the laboratory, found an average of 11.3 days, while for free-living ticks it was 8.4 days. The results obtained in this study were similar to those reported by Dantas et al. (2018), but they were well below what was published by Hadi & Adventini (2015). For that reason, it could be assumed that this parameter is not very susceptible to environmental factors such as humidity and temperature, which are the most common variables considered in comparative studies.
The incubation period lasted between 11 and 28 days with a mean of 18.8 ± 3.2 (Table 3). Dantas et al. (2018) calculates a mean of 23.4 days for ticks under controlled conditions and a mean of 29.6 days for free-living ticks. Bechara et al. (1995) obtain a means of 22.9 ± 3 whit a dog host, 24 ± 2.2 using foxes like hosts, 21 ± 3.6 for guinea pigs, and 24. ± 1.7 using similar temperature parameters to the present study. Szabo et al (2005) using similar temperature and humidity parameters than this study obtain a mean of 17.22 ± 0.60 days using dogs like hosts and 29.44 ± 5.7 on rabbits. Hadi & Adventini (2015) refer to it with an average of 21 days in ticks kept in controlled conditions. The differences in this parameter with the referenced studies could be an indication that the incubation period of R. sanguineus is highly influenced by environmental temperature and not so much by relative humidity.

3.9. Feeding and molting period

The mean time for larval feeding was 3.6 ±1.0 days. The molting period from the first larva to nymphal stage lasted 9.6 ± 3.8 days (Table 3). Dantas et al. (2018) reports an average of 2 days of feeding and 10 days for molting in larvae under laboratory conditions and 3 days of feeding and 10 days for molting in free-living larvae. Quiroz (2009) mentions that the larval feeding period of R. sanguineus in free life goes from 3 to 7 days with a molting time between 6 and 23 days, a situation that agrees with the results obtained in the present study. These parameters ranges are shown in Table 3. Likewise, the results coincide with those described by Morales-Soto (1995), who determined this period ranged between 2 to 6 days; nevertheless, this author finds a molting time of 8 to 9 days, which is greater than the range of 3-5 days observed in this study.

On the other hand, the feeding of the nymphs took from 3 to 8 days with a mean of 5.1 ± 1.2 days, while the period of molting to the adult stage ranged between 8 and 22 days with a mean of 14.1 ± 4.1 days. These times coincide with the results presented by Morales-Soto (1995), who observed a feeding period of 3 to 6 days and a molting period of 16 days. The adult feeding phase in our study had a mean duration of 6.9 ± 0.7 days while Dantas et al. (2018) determined means of 20.4 and 13.4 days for ticks under controlled and free-living conditions, respectively, while the molting period to the adult stage ranged between 15 and 22 days with a mean of 17.6 days.

In general, when comparing the results obtained in this study with those published by other authors, it can be deduced that humidity levels above 80% are crucial for the development of R. sanguineus under laboratory conditions. Contrary to this, it was also observed that temperature, although it is an important variable, does not have a significant impact on the reproductive parameters of this species. Similarly, it was shown that rabbits are a good experimental model for keeping this tick under controlled conditions, both for its practicality and for allowing the biological cycle of this species to be maintained in a similar way to that developed in dogs, which are its usual hosts.

3.10. Male/Female ratio

The male/female ratio (Table 4) was 52.98 % (95% CI = 46.61-59.29) for male ticks (m) and 47.02% (95% CI = 40.70-53.38) for female ticks (f). This is consistent with the results of Levin et al. (2012), that calculate m=55.5% and f=44.5 while Brophy et al. (2022) calculate m=55.8 and f=44.2.

Table 4 Male/Female ratio of adult *Rhipicephalus sanguineus* under controlled conditions, fed on a rabbit host.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Ratio (%)</th>
<th>CI95%* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>133</td>
<td>52.98</td>
<td>46.61-59.29</td>
</tr>
<tr>
<td>Female</td>
<td>118</td>
<td>47.02</td>
<td>40.70-53.38</td>
</tr>
</tbody>
</table>

*CI* = confidence interval.

3.11. Correlation between parameters

Positive correlations were identified between the weight of the egg mass and the weight of the teleogines, as well as between the weight of the egg mass and the percentage of oviposited weight (Table 5). This is consistent with Giannelli et al. (2012), who state that the weight of the *R. sanguineus* egg masses and the number of eggs are influenced by the weight of the engorged teleogines prior to oviposition in *R. sanguineus* ticks.

Table 5 Correlation coefficients between parameters of adult *R. sanguineus* teleogines.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Teleogin weight</th>
<th>Oviposited weight percentage</th>
<th>Egg mass weight</th>
<th>Number of eggs</th>
<th>Larvae per tick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviposited weight percentage</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg mass weight</td>
<td>0.875*</td>
<td>0.590*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of eggs</td>
<td>0.360*</td>
<td>0.176</td>
<td>0.367*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae per tick</td>
<td>0.309*</td>
<td>0.211</td>
<td>0.341*</td>
<td>0.792*</td>
<td></td>
</tr>
<tr>
<td>Hatching rate</td>
<td>0.171</td>
<td>0.054</td>
<td>0.147</td>
<td>0.307*</td>
<td>0.739*</td>
</tr>
</tbody>
</table>

* *p* value ≤ 0.05
4. Conclusions

The reproductive parameters of *R. sanguineus* under laboratory conditions using a rabbit model for the development of its biological cycle are like those published by other authors who used other models. The use of feeding chambers to establish tick colonies under laboratory conditions was a very useful tool to accuracy study of the life cycle and some interactions between parasites and its hosts, including incidental hosts that can be rarely seen associated to free living parasites. The rabbits like alternative host to *R. sanguineus* under laboratory conditions were a suitable replacement for the natural host of these ticks. High temperature and humidity gradients are determinant so *R. sanguineus* can complete its life cycle in shorter periods, nevertheless this can cause decreased reproductive parameters on *R. sanguineus*; more studies with different humidity and temperature gradients are necessary to confirm this. The difference between reproductive parameters in this study and similar studies may be caused by the difference in temperature and humidity gradients as well as the strain of the ticks analyzed in the different studies. It is necessary to investigate if exists a correlation between the tick strain and its susceptibility to environmental parameters.

Ethical considerations

To conduct this study, the authors obtained permission 02/23 from the Bioethics Committee of the Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, which confirmed that both the maintenance and the activities carried out on the animals complied with the requirements of the law in México in order to the NOM-033-ZOO-1995, section 6.1.b. (Sagarpa, 2015).

Conflict of Interest

The authors have no financial or non-financial interests to disclose.

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