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Technical note

# Comparison of two devices for *in vivo* feeding of the *Rhipicephalus sanguineus* tick under controlled conditions in an experimental host

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**Abstract**:

*Rhipicephalus sanguineus* is a tick considered a vector of diseases for both animals and humans. Its life cycle is complex to study under laboratory conditions due to its feeding and reproductive habits, which limits *in vitro* research. This study aimed to compare two different models of *in vivo* feeders for ticks by using the biological cycle of *R. sanguineus* fed on rabbits as an evaluation method. Two artisanal feeding chambers measuring 60 x 40 mm and 25 x 40 mm were designed and placed on rabbits. The results revealed that the 60 x 40 mm chamber provided better reproductive parameters and a higher recovery rate for both nymphs (82.3 %) and adults (26 %), compared to the 25 x 40 mm chamber (34.7 % for nymphs and 10 % for adults) ($P \leq 0.05$). The biological cycle had a similar length in both chambers (74.4 days for the large chamber and 74.1 d for the small one), but there were differences ($P \leq 0.05$) in the weight of larvae, nymphs, adult females, and the length of the larval feeding period.

**Keywords:** Ticks, Feeding chambers, Reproductive parameters, *In vivo* infestation, Biological cycle.

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Research into ticks' biology, pathology, and control methods requires simulating the natural scenarios in which these ectoparasites develop under controlled conditions[1,2]. The complexity of *in vitro* feeding methods in terms of their implementation makes them unfeasible[3]. *In vivo* artificial feeding allows for more accurate observations by mimicking a controlled natural infestation[2,3].

The use of feeding chambers on hosts is an *in vivo* method[4] that consists of placing devices on animals that are infested in a controlled manner; thus, the escape of ticks is prevented, and the collection of specimens, once they finish their feeding process, is facilitated[5].

*Rhipicephalus sanguineus* (*R. sanguineus*) is a hard tick of the Ixodidae family with a cosmopolitan distribution that parasitizes domestic dogs, although it can be found in other species[6]. It is of great relevance both for veterinary medicine and for public health due to its role as a vector for transmitting infectious diseases to animals and humans[7,8]. Each stage of its life cycle (larva, nymph, adult) requires a different host to feed. Its copulation occurs only on the host in turn during the feeding process of adults[9]; this complicates the maintenance of its colonies in the laboratory for research purposes[9].

Therefore, the present study aims to compare two artisanal models of *in vivo* feeding chambers, using the biological parameters and the length of the biological cycle of *R. sanguineus* as an evaluation method.

The present work was conducted in a provisional vivarium located in the La Posta Experimental Field, INIFAP, km 22.5 Veracruz-Córdoba federal highway, Paso del Toro, Municipality of Medellín, Veracruz, Mexico.

A total of 82 adult female ticks were collected in three veterinary clinics located in the Veracruz-Boca del Río metropolitan area, Veracruz, Mexico, by manual collection from naturally parasitized dogs.

The taxonomic key proposed by Nava *et al*[10] was used to identify the ticks as *R. sanguineus*. Ticks were housed at 27 °C in a culture oven until the eggs were obtained[11]; they were incubated for 20 d at 80 % moisture to obtain larvae.

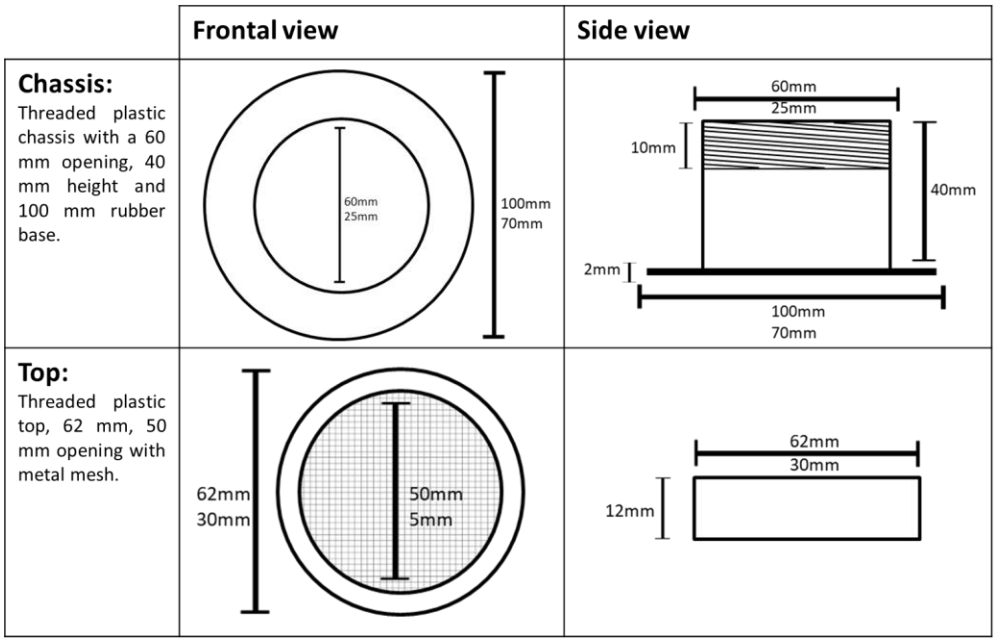
Two groups of three adult New Zealand rabbits (*Oryctolagus cuniculus*) were used without distinction of sex. The 25 x 40 mm chamber model was placed in group one, and the 60 x 40 mm chamber was placed in group two. A brush was used to infest them with larvae, and the larvae were allowed to feed until they detached themselves from the host. They were collected again and stored in Eppendorf® tubes with cotton stoppers until they changed to their nymphal stage. The process described was repeated with the nymphs until adult ticks were obtained for three consecutive generations, as proposed by Morales[11]. The experimental animals were subjected to clinical examinations before each infestation period to verify that their health status was suitable for experimentation.

The six rabbits used in this study were individually housed in galvanized steel cages 90 cm long, 60 cm wide, and 38 cm high in accordance with the provisions of NOM-062-ZOO-1999[12]. The daily feed consisted of 100 g of commercial feed, pangola grass hay on demand, and water on demand. Clinical examinations were carried out before each infestation period to guarantee the health status of the animals. To carry out the study, permission with number 01/21 was obtained from the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the University of Veracruz to verify that all procedures were in accordance with the provisions of NOM-062-ZOO-1999[12].

Two models of feeding chambers were made: one of 60 x 40 mm (large) and the other of 25 x 40 mm (small), according to what was proposed by Thomas[13] and Mateos-Hernández *et al*[14].

60 x 40 mm feeding chamber**.** They were made from Edigar® 120 ml plastic cups, drilling a hole of 5 cm in diameter in the lid to add a metal mesh of 120 threads per inch. The bottom of the vessels was removed to allow direct access to the rabbits' skin. A rubber base was added to facilitate the attachment of the chamber to the animals' skin (Figure 1).

**Figure 1:** Diagram of 60 x 40 mm and 25 x 40 mm infestation chambers




25 x 40 mm feeding chamber**.** Fifty-milliliter polypropylene test tubes were modified by cutting them at 40 mm from the mouth of the tube; a rubber base was added for easy attachment. A 5 mm diameter hole was drilled in the lid, adding a wire mesh of 120 threads per inch (Figure 1).

For chambers placement, an area of 30 x 30 cm on the back of the animals was shaved with an electric razor. The contour of the chambers was marked with a marker to adjust and place a Hypafix® adhesive dressing (Leukoplast® laboratory). A contact adhesive was used on both the base of the chambers and the Hypafix®, allowing it to dry for 5 min, then, the chamber was placed, and finally, micropore tape was placed around it to prevent rabbits from biting the base of the chambers. A large feeding chamber (60 x 40 mm) was placed on each animal; for the small ones (25 x 40 mm), two were placed on each animal.

In each of the instars, the ticks were placed inside the feeding chamber using a brush. The rabbit was infested with between 500 and 800 14-d-old larvae in both infestation chamber models for the larval stage. As for ticks in the nymph stage, the host was infested with 200 individuals 14 d post-molt for the 60 x 40 mm chambers and 100 individuals for the 25 x 40 mm chambers. In the case of adult ticks, the rabbit was infested with 100 specimens of 14 d post-molt maturation for the large chambers and 50 specimens for the small chambers.

The recovered ticks were organized in batches within Eppendorf tubes: larvae 100 individuals per batch, nymphs 50 individuals per batch, and adults one individual per batch.

They were then housed in a culture oven at 28 ± 1 °C and moisture of 80 ± 10 % until their molting process was completed.

The parameters measured were: live weight per batch of larvae and nymphs, individual live weight of adults, egg mass weight, percentage of oviposited weight, weight loss rate, number of eggs, larvae per tick, hatching rate, preoviposition, oviposition, incubation, larval feeding period, larval-nymph molt, nymph feeding period, nymph-adult molt, adult feeding period, cycle length, nymph recovery rate, adult recovery rate.

The parameters and length of the biological cycle with the two types of feeding chambers were compared with the Student's t-test using the Minitab® 17 program, considering comparisons with a *P*-value ≤0.05 as significant. Recovery rates were compared using the Mann-Whitney test with the Minitab® 17 program.

The weight of the larvae in the small chamber was 17.44 ± 10.30 mg, whereas in the large chamber, it was 23.74 ± 6.83 mg (*P*=0.00) (Table 1). The difference may be due to competition for the feeding area. A smaller feeding area can cause each larva to gain access to a smaller amount of blood, limiting its weight. Szabo *et al*[15] reported a larval weight of 0.47 ± 0.04 mg for ticks fed on rabbits. The difference in the results with these authors[15] is due to a methodological difference since they weighed the larvae individually, whereas in the present study, the weight was obtained in batches.

**Table 1:** Comparative analysis of biological parameters obtained in the feeding chamber

| **Parameter** | **n** | **25 x 40 mm (small)** | **60 x 40 mm (large)** |
|---|---|---|---|
| Live weight larvae, mg | 93 | 17.44 [a] | 23.74 [b] |
| Nymph live weight, mg | 37 | 47.80 [a] | 117.40 [b] |
| Adult live weight, mg | 31 | 113.40 [a] | 131.81[b] |
| Egg mass weight, mg | 31 | 95.60 [a] | 91.58 [a] |
| Percentage of oviposited weight | 31 | 80.90 [a] | 69.75 [a] |
| Weight loss rate, % | 31 | 37.23 [a] | 30.65 [a] |
| Number of eggs, n | 234 | 1,923.20 [a] | 1,783.54 [a] |
| Larvae/tick, n | 234 | 1,646.60 [a] | 1,421.38 [a] |
| Hatching rate, % | 234 | 81.52 [a] | 78.49 [a] |

[ab] Different letters indicate significant differences (*P*≤0.05).

The live weight of the nymphs in the small chamber was 47.80 ± 51.80 mg, and in the large chamber, it was 117.40 ± 88.90 mg (*P*=0.00) (Table 1); this difference is attributed to the competition of the nymphs for feeding sites, the irrigation of the area may have been insufficient for the nymphs to reach a higher weight. Szabo *et al*[15] calculated a weight of 5.84 ± 0.95 mg for ticks of the Argentine strain and 4.08 ± 0.43 mg for ticks of the Brazilian strain. The difference with the results of these authors is due to the methodology used between the studies, as mentioned.

The live weight of adult ticks was 113.40 ± 34.40 mg for the small chamber and 131.81 ± 35.13 mg for the large one (*P*=0.00) (Table 1). This difference is attributed to increased tick competition for the feeding area, as each female would have access to less blood flow. This could be counterproductive to reproduce ticks under laboratory conditions using the small chamber model since the weight of adult females is related to their ovipositing abilities and fertility. Under laboratory conditions, Hadi and Adventini[16] observed a slightly lower result of 68.4 mg; in contrast, Kamani[17] obtained an average weight of 184.1 ± 154 mg. The difference in parameters could be because Kamani used domestic dogs as hosts, which could favor a higher weight at the end of the feeding period.

The small chamber obtained a mean of 95.60 ± 48.00 mg for the weight of the egg mass and the large chamber had 91.58 ± 36.92 mg (Table 1) (*P*=0.83). Guidotti *et al*[18] obtained an egg mass weight of 100 mg, whereas another study[17] obtained a weight of 95.8 ± 89.8 mg and reported 73.7 ± 57 mg in natural infestation. The similarity suggests that this parameter is not related to the host species of *R. sanguineus* and is stable in relation to the feeding method.

The percentage of oviposited weight in the small and large chambers was 80.90 ± 25.90 % and 69.75 ± 26.04 %, respectively (Table 1) (*P*=0.38). A similar study reports 49.9 ± 13.7 % in the laboratory and 39.7 ± 12.7 % in free-living conditions[17]. When comparing the results of both studies, it is concluded that this parameter does not present significant alterations depending on the type of diet used.

The weight loss rate was 37.23 ± 16.76 % in the small chamber and 30.65 ± 10.25 % in the large chamber (Table 1) (*P*=0.24). It can be assumed that there is no relationship between the rate of weight loss and the feeding methods analyzed. Kamani[17] obtained superior results, 75.1 ± 8.1 % in the laboratory and 62.1 ± 19.8 % in free-living conditions.

Averages of 1,923.20 ± 1,079.58 eggs and 1,782 ± 696.97 eggs were obtained for the small and large chambers, respectively (Table 1) (*P*=0.70); this suggests that this indicator is stable with respect to the type of feeding chamber. Under controlled conditions, 1,687 ± 1,274 eggs were reported, while in free-living conditions, 2,179 ± 1,952[17].

For larvae born per tick, results of 1,646.60 ± 1,145.93 were recorded for the small chamber and 1,421.38 ± 664.61 for the large one (Table 1) ($P$=0.54). Bechara *et al*[19] determined that, in ticks fed on guinea pigs, each tick produced an average of 586 ± 491 larvae; since in this study the methodology was similar to that of the present study, it can be assume that the variation in the results is the product of the difference in host used.

The small feeding chamber obtained 81.52 ± 18.19 % hatching rate, whereas the large feeding chamber had 78.49 ± 15.85 % ($P$=0.70) (Table 1). A similar result was reported by Hanan *et al*[20], who mentioned 94.4 ± 7.3 %. Bechara *et al*[19] report similar results for ticks fed on dogs (90.2 ± 13.5 %), foxes (83.7 ± 31.2 %), hamsters (81.1 ± 23.7 %), and guinea pigs (92.3 ± 12.5 %) by using feeding chambers similar to the 25 x 40 mm model.

A length of 3.80 ± 0.83 d for preoviposition period was observed in the small chamber and 4.00 ± 1.26 d in the large one (Table 2); these differences were not significant ($P$=0.73) and were similar to other studies where lengths of 4 ± 0.81 d[20] and 4.9 d[16] were observed; this suggests that this parameter is stable and not influenced by the type of diet.

**Table 2:** Parameters of the biological cycle of *R. sanguineus* obtained in feeding chambers

| Parameter | n | 25 x 40 mm (small) | 60 x 40 mm (large) |
|---|---|---|---|
| Preoviposition | 31 | 3.80 [a] | 4.00 [a] |
| Oviposition | 31 | 3.20 [a] | 4.73 [a] |
| Incubation | 31 | 27.60 [a] | 27.42 [a] |
| Larval feeding period | 93 | 5.50 [a] | 4.57 [b] |
| Larva-nymph molt | 93 | 4.85 [a] | 5.00 [a] |
| Nymph feeding period | 37 | 6.16 [a] | 5.89 [a] |
| Nymph-adult molt | 37 | 15.55 [a] | 15.36 [a] |
| Adult feeding period | 31 | 7.40 [a] | 8.03 [a] |
| Cycle length | | 74.83 | 75.30 |

[ab] Different literals indicate significant difference ($P$≤0.05).

The oviposition period in the small chamber, it was 3.20 ± 0.44 d and in the large chamber, 4.73 ± 2.27 d (Table 2). The difference was not statistically significant ($P$=0.14), suggesting that the type of feeding chamber does not influence the tick's free-living phases. Results of 7.5 ± 1.72 d[20] and 14.3 d[16] have been obtained.

The length of incubation period was 27.60 ± 2.51 d for the small chamber and 27.42 ± 1.72 d for the large chamber (*P*=0.84) (Table 2) (*P*>0.05) Under laboratory conditions[16], they obtained 6.9 d, whereas, in free-living conditions, they mention that the incubation of the eggs varies from 6 to 23 d[21].

The larval feeding period was 5.50 ± 1.56 d for the small chamber and 4.57 ± 1.08 d for the large one (*P*=0.00) (Table 2). This difference could be associated with the feeding area, as it was larger in the 60 x 40 mm chamber, the larvae were subjected to less competition for an ideal site to anchor, which made it easier for them to carry out this part of their biological cycle in less time. Hanan *et al*[20], in a study using *in vivo* feeding, obtained very similar results, with a mean of 4.5 ± 1.16 d. This suggests that this period is shortened in the absence of hair and the sebum layer.

The larva to nymph molting period lasted 4.85 ± 0.70 d for the small chamber and 5.00 ± 0.72 d for the large one (Table 2); this difference was not significant (*P*=0.47) because this is a free-living stage where the feeding chambers do not have a considerable influence. Under laboratory conditions[20], a period of 11.6 ± 1.41 d is reported. These differences indicate that this phase of the tick cycle can be shortened under laboratory conditions.

The feeding period of nymphs was 6.16 ± 1.09 d in the small chamber and 5.89 ± 1.10 d in the large one (Table 2) (*P*=0.45). Although intraspecific competition for feeding sites within small chambers should be greater, the results suggest that, with the number of nymphs used for infestation, it is not possible to see significant alterations in this parameter. Under laboratory conditions, Hanan *et al*[20] obtained results of 5.5 ± 1.1 d, very similar to those of the present study.

The molting period in nymphs had a mean of 15.55 ± 0.92 d and 15.36 ± 0.83 d in the small and large feeding chamber, respectively (Table 2). This parameter is also a free-living phase, so as mentioned, it is unlikely that the type of chamber can influence it, which explains why the difference obtained between the two is not significant (*P*=0.52). Under laboratory conditions[20], they obtained an average of 12.5 ± 2.0 d in this stage, while in free-living conditions[22], they reported between 9 and 47 d. The similarity with Hannan *et al*[20] results suggests that this parameter is not affected by host change for ticks.

The adult feeding period in the small chamber, this time was 7.40 ± 0.89 d and in the large chamber, it was 8.03 ± 0.77 d (Table 2) (*P*=0.17), which could be explained by the low density of ticks used at this stage for infestations. Under laboratory conditions[20], they obtained 9.0 ± 1.1 d. In free-living conditions, adult females have a feeding period ranging from 5 to 21 d[22].

The total length of the biological cycle was 74.1 ± 8.3 d for the small chamber and 74.4 ± 8.2 d for the large one (Table 2), results similar to those published of 41 to 73 d for ticks kept under controlled conditions[20]. In free-living conditions, Louly[23] reported between 63 and 91 d. The similarity of the results obtained in this work with the data published by other authors suggests that the *in vivo* artificial feeding model allows *R. sanguineus* to complete its biological cycle in an amount of time similar to that it would take in free-living conditions.
Throughout the study, it was observed that 40 % of the small feeding chambers intended for nymphs and 50 % of those intended for adults were damaged by rabbits. This incident did not occur with any of the large feeding chambers. This situation leads to reflect that the experimental animals showed a certain ease, or preference for trying to remove or get rid of the small feeding chambers, affecting their integrity and functionality.

For nymphs, recovery rates of 34.73 ± 29.25 % were obtained for the small chamber, whereas in the large chamber, the recovery rate was 82.30 ± 60.71 % ($P$=0.13). Despite this, the findings suggest that higher recovery rates can be achieved with large feeding chambers than with small ones.

For adults, a recovery rate of 10.04 ± 4.24 % was obtained in the small chambers, while in the large chambers, it was 26.72 ± 5.66 % ($P$=0.14). These results, although not statistically significant, suggest that large feeding chambers under laboratory conditions facilitate obtaining higher recovery rates.

In conclusion, both feeding chambers are functional for the maintenance of *R. sanguineus* colonies; however, the use of the large chamber model (60 x 40 mm) allowed the ticks to maintain biological parameters consistent with those reported in the literature for *R. sanguineus* under free-living conditions. While both feeding chamber models were functional, the large one (60 x 40 mm) did not suffer any damage caused by rabbits compared to the small chambers (25 x 40 mm), where they damaged some of them, which resulted in compromising their functionality and could be considered as an advantage of large chambers.

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