Artificial feeding of partially engorged *Amblyomma sculptum* females through capillaries*

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ABSTRACT. Abel I., Cunha N.C., Rangel C.P., Corrêa F.N. & Fonseca A.H. **Artificial feeding of partially engorged *Amblyomma sculptum* females through capillaries**. [Alimentação artificial de fêmeas parcialmente ingurgitadas de *Amblyomma sculptum*, por meio de tubos capilares.] *Revista Brasileira de Medicina Veterinária, 38(supl. 3):113-118, 2016*. Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465, km 7, Campus Seropédica 23897-970, Seropédica, RJ, Brazil. E-mail: isisabel@ufpa.br

This study presents the standardization of an artificial feeding technique using capillaries and discusses its effects on biological parameters of partially engorged *Amblyomma sculptum* females. Partially engorged females were sorted for mean baseline weight (71.94 mg, group I; 167.58 mg, group II). Females were detached from rabbits after 7 days of feeding and then exposed to capillary tubes containing citrated bovine blood for 6, 12, and 24 h. Biological parameters were analyzed for each weight group, after each period. All ticks fed on this system took blood meal. Weights before and after artificial feeding were compared, and significant difference was observed. Mean weight gain for group I females artificially fed for 6, 12, and 24 h was 56.05, 86.75 and 192.89 mg, respectively. Weight gain in group II females fed for 6, 12 and 24 h was 133.73, 182.09 and 368.77 mg. Results indicate that capillary feeding may be used routinely in studies on pathogen transmission by *A. sculptum* females. The ideal initial weight range is discussed in terms of the kind of study design.

KEY WORDS. Ticks, *in vitro* feeding, capillaries, *Amblyomma sculptum*.

RESUMO. Este estudo apresenta a padronização de uma técnica de alimentação artificial de carrapatos, por meio de capilares e discute seus efeitos nos parâmetros biológicos de fêmeas de *Amblyomma sculptum*. As fêmeas parcialmente ingurgitadas foram classificadas para o peso médio inicial (71,94 mg, grupo I e 167,58 mg, grupo II). As fêmeas foram coletadas dos coelhos após 7 dias de alimentação e depois expostas a tubos capilares contendo sangue bovino citratado durante o período de 6, 12 ou 24 h. Os parâmetros biológicos foram analisados para cada grupo de pesos, após cada período. Todos os carrapatos alimentados com este sistema ingeriram sangue. Os pesos antes e após a alimentação artificial foram comparados. O ganho de peso médio para fêmeas do grupo I alimentadas artificialmente por 6, 12 e 24 h foi 56.05, 86.75 e 192.89 mg, respectivamente. O ganho de peso em fêmeas do grupo II

*Received on July 21, 2016.
Accepted for publication on November 17, 2016.

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alimentadas por 6, 12 e 24 horas foi 133,73, 182,09 e 368,77 mg. Os resultados indicam que a alimentação capilar pode ser utilizada rotineiramente em estudos sobre a transmissão de patógenos por *A. sculptum* fêmeas. O intervalo ideal de peso inicial é discutido em termos do tipo de desenho do estudo.

PALAVRAS-CHAVE. Carrapato, alimentação artificial, tubos capilares, *Amblyomma sculptum*.

INTRODUCTION

In vitro feeding of ticks is a valuable tool to investigate feeding habits and to analyze their role in pathogen transmission (Bouwknegt et al. 2010). Artificial feeding methods afford to reduce the number of animal hosts required for research and kept in laboratory. Additionally, artificial feeding systems are useful in efforts towards shedding new light on attachment stimuli, nutritional requirements and host resistance factors, and have been used mainly to investigate pathogen transmission (Howarth & Hokama, 1983, Broadwater et al. 2002, Kocan et al. 2005).

It is known that in their feeding cycle ixodid ticks consume the largest volume of blood meal in the last 24 h, a phenomenon in which mating plays an established role (Sonenshine 1991, Sanches et al. 2012). Therefore, depending on the objective of a given study, artificial techniques to feed partially engorged females may stand as an important investigation tool. In this sense, studies have investigated how to improve ingested blood volumes, with a view to providing a more comprehensive body of evidence of the likely effects thereof on pathogen transmission and vaccine antigen selection (Gonsioroski et al. 2012). Nevertheless, artificial feeding techniques have to be standardized concerning individual requirements by different tick species, with no consequence to their biology and guaranteeing reproducibility and applicability of experimental results. Moreover, carefully finding out the most appropriate moment artificial feeding should be implemented is an essential part of the design such research efforts.

Although artificial feeding techniques using capillaries differ from natural feeding habits and do not enable total engorgement of specimens, they allow reducing the number of variables surrounding the tick feeding cycle. What's more, capillary-based techniques allow a variety of experimental procedures, an advantage over other less flexible related methods (Fingerle et al. 2002). These techniques are also safe, inexpensive, and may be used in the selection of vaccine targets, since polyclonal or monoclonal, isolated or associated antibodies may be offered using one same capillary (Almazán et al. 2005, Gonsioroski et al. 2012). Other benefits include the possibility (i) to adjust inoculum volume as required, (ii) to test molecules that inhibit or promote tick development, (iii) to phenotypically test pathogenic agents not infectious to hosts, and even (iv) to abolish experimental infections of hosts in laboratories (Broadwater et al. 2002, Bouwknegt et al. 2010, Pohl et al. 2011).

Amblyomma sculptum is a three-host tick with low infestation specificity — an important characteristic concerning the transmission of zoonotic pathogens, including *Rickettsia rickettsii.* This tick species may be kept in laboratory, reared on rabbits. However, pathogen transmission studies require susceptible hosts, and the synchronism between parasitemia and the right moment that tick should take the blood meal. In addition, many laboratory animals have to be infected, sometimes with lethal pathogens, in order to assess the transmission by a few ticks (Scoles et al. 2011, Soares et al. 2012). In this sense, artificial feeding may be a new promise in terms of preventing infections in laboratory animals, as long as the technique used preserves tick biology.

In this context, the present study describes the standardization of a capillary-based artificial feeding technique for partially engorged *A. sculptum* females with bovine blood. The biological parameters of females obtained after capillary tube artificial feeding were analyzed to determine the efficiency of the method. The aim was to consider *A. sculptum* as a study model to discover whether the method can be used routinely in studies on pathogen transmission or on vaccine target research with other *Amblyomma* species.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Parasitic Diseases, Department of Epidemiology and Public Health, School of Veterinary Medicine, Federal Rural University of Rio de Janeiro and was approved by the University's ethics committee for research using animals, protocol number 142/2011.

Adult *A. sculptum* ticks were fed on rabbits (*Oryctolagus cuniculus* New Zealand x Californian) for seven days. Then, females were manually detached from hosts, washed in sodium hypochlorite 5% and weighed. Next, integrity of mouthparts of females was inspected. Ticks were sorted into baseline weight groups I (between 44.8 and 108.0 mg; mean 71.94 mg) and II (112.20 and 246.10 mg; mean 167.58 mg). Three experimental groups with 11 ticks were then formed for each weight group, each subjected to a different artificial feeding time (6, 12, and 24 h).

For the artificial feeding process, tick females were fixed on a polystyrene plate, ventral face upwards, and exposed to capillary tubes containing citrated bovine blood. Capillaries were replaced whenever they got empty or serum was the only remaining content, throughout the feeding period. Plates were maintained under controlled conditions (27 ± 1ºC and relative humidity over 80%). After established experimental feeding times had elapsed, ticks were washed, once again weighed and fixed on Petri dishes to analyze oviposition and larval eclosion biological parameters. *A. sculptum* females reared on rabbits and collected after natural detachment, were weighed, fixed on a Petri dish, kept under the same conditions as the experimental groups for analysis of biological parameters, and used as controls.

Final mean weights after *in vitro* feeding of the two groups were compared to baseline values using the Student's t test with 5% significance level. Comparisons across feeding times were carried out using the Tukey test at 5% significance level. The same test was used to compare biological parameters across groups. All statistical calculations were made using the software Graph Pad InstatTM (copyright 1990-1994).

RESULTS AND DISCUSSION

All ticks artificially fed using capillary tubes took blood meal, with significant differences between baseline and weights measured after *in vitro* feeding (Table 1). These results corroborate the successful use of this technique by our research team in feeding other ixodid ticks (Rangel et al. 2008, Cunha et al. 2010, Gonsioroski et al. 2012). In such studies, as a rule ticks start or end their feeding cycle on natural or laboratory hosts, for periods that vary for different tick species and the specific objectives of the study being conducted. This pre-feeding period has been reported to reach 21 days (Bouwknegt et al. 2010, Gonsioroski et al. 2012). In the present study, the pre-feeding period stipulated was seven days after infestation challenge, when *A. sculptum* ticks were about to naturally detach from hosts. The intention was to manually detach females so as to make sure that they were still at the beginning of the fast feeding period. According to Sonenshine, (1991), ticks at this stage of the feeding cycle take a considerable volume of blood meal.

During the artificial feeding process, *A. sculptum* females quickly consumed the whole contents of capillaries (70 µL). In total, 54 mL of citrated blood was used to feed all 66 partially engorged tick females, of both weight groups (I and II). No blood warming was required, as demonstrated in previous studies (Bouwknegt et al. 2010), although all the experiment was conducted under controlled temperature and humidity conditions (27ºC; >80% RH).

Previous studies have aimed to improve the artificial feeding technique in ixodids, demonstrating that fasting *A. sculptum* females gained on average 5.30 mg in weight after daily 6-h exposure periods to capillaries, for 8 days (Abel et al. 2008). It should also be observed that this technique caused weight gains of 146.0 mg, 45.1 mg, and 40.9 mg in partially engorged females of *Dermacentor nitens* (Rangel et al. 2008), *Rhipicephalus sanguineus* (Cunha et al. 2010) and *R. microplus* (Gonsioroski et al. 2012), respectively. Here, mean weight gain in group I females fed for 24 h was 192.89 ± 104.83 mg, while group II females increased weight to 368.77 ± 440.51 mg on average, which means more than 300% of the baseline weight, as demonstrated by Rangel et al. (2008). Additionally, in the present study all mouthparts were inserted in capillaries, differently from studies that adopted the insertion of the hypostome (Broadwater et al. 2002, Bouwknegt et al. 2010). In spite of the long feeding periods, no obstruction of capillary tubes was observed, similarly to what was reported by Cunha et al. (2010). Also, we observed that the blood used was constantly exposed to salivary secretions, which prevented clotting and obstruction of capillaries. This may be due to the fact that mouthparts of species of the genus *Amblyomma* characteristically are rather resistant, and that all mouthparts were inserted in tubes, basically occupying the whole internal diameter. Our results indicate that the limited success in using this technique previously reported elsewhere may

Table 1. Mean weight of *Amblyomma sculptum* females before (baseline) and after artificial feeding by capillaries (weigh group I).

Feeding periods	Mean weight		
	Baseline	After feeding	Weight gain
6 h	$71.95\pm20.21^{A,b}$	$128.00\pm44.25^{C,a}$	56.05 ± 24.86^{C}
12 h	$71.93\pm19.82^{A,b}$	$158.67\pm75.96^{B,a}$	86.75 ± 58.54^{B}
24 h	$71.90\pm19.73^{A,b}$	$264.79\pm119.05^{A,a}$	192.89 ± 104.83^{A}

Means followed by at least one capital letter repeated along lines and one lowercase letter repeated on lines did not differ statistically in the Student's t test and Tukey test, respectively ($p<0.05$).

Table 2. Mean weight of *Amblyomma sculptum* females before (baseline) and after artificial feeding by capillaries (weigh group II).

Feeding periods	Mean weight		
	Baseline	After feeding	Weight gain
6 h	$167.56\pm48.78^{A,b}$	$301.29\pm79.37^{B,a}$	133.73 ± 59.08^{B}
12 h	$168.59\pm48.61^{A,b}$	$350.68\pm51.21^{B,a}$	182.09 ± 68.45^{B}
24 h	$166.58\pm34.02^{A,b}$	$535.35\pm123.92^{A,a}$	368.77 ± 110.51^{A}

Means followed by at least one capital letter repeated along lines and one lowercase letter repeated on lines did not differ statistically in the Student's t test and Tukey test, respectively ($p<0.05$).

be linked to size of mouthparts, not to the insertion of palps in capillaries (Billeter et al. 2012). This obstacle may be overcome by replacing capillaries more often (Cunha et al. 2010).

Compared to control specimens, all weight group II females submitted to artificial feeding for one same given period (6, 12 or 24 h) presented engorging values (%) higher than the weight group I counterparts. Final weight of group II ticks was 98% of the mean weight of control females (Table 1). Oppositely, when mean weights are compared before and after feeding, group I ticks are seen to have gained more percent weight than group II ticks (Table 2). These findings show that ticks of lower baseline weights were able to take more blood meal. However, *A. sculptum* females of higher baseline weights (group II) managed to reach greater mean weights after feeding, comparable to those of control ticks. Similarly, to the present study, partially engorged *D. nitens* females of higher baseline weights were shown to gain more weight with artificial feeding (Rangel et al. 2008). Even with no feeding restrictions in a given environment, virgin ixodid females may reach only 50% of the weight of mated females. It is possible that some of the partially engorged female ticks used here had not mated before they were detached from their hosts. Apart from blood meal, ovary development requires mating, when peptide pheromones are transferred to females as chemical stimuli (Sanches et al. 2012, Sonenshine 1991). It is possible that females of group II had already mated, which explains resultant higher final mean weights.

Based on these findings, it may be said that ideal weight range of partially engorged *A. sculptum* females under an artificial feeding regime depends on what the research effort in question intends to prove. If in a given experimental scenario large volumes of ingested blood meal are required, then the mean baseline weight indicated is between 44.8 and 108.0 mg. It is feasible as a model to study pathogen migration inside the tick or in vaccination trials, even for other *Amblyomma* species, like *A. variegatum* and *A. americanum*. The amount of blood that tick in this study could uptake underlines the fact that this technique can be applied for this purpose since, in this kind of research, the higher the amount taken, the better.

However, if in a particular study on oviposition parameters the objective is to obtain weights comparable to those of naturally engorged females, baseline weight of ticks should lie within 112.0 and 246.10 mg. In agreement with this, results of larvae eclosion from weight group II ticks did not differ statistically from those of control ticks, reared to full engorgement on rabbits. Females within this baseline weight range can be used in studies on the transovarial transmission of different pathogens, like *Rickettsia* spp, *Ehrlichia*, *Theilieria* sp, *Borrelia* and *Borrelia*-like spirochetes, minimizing laboratory animal infection (Ribeiro et al. 2011, Scoles et al. 2011, Soares et al. 2012).

Here, biological parameters of artificially fed ticks were compared to those of tick attached to rabbits. Artificial feeding did not influence pre-oviposition period of ticks in any experimental group. In turn, the oviposition period was shorter for weight group I tick females artificially fed for 6 and for 12 h, while no difference was observed in this parameter for females whose final weight was comparable to that of control females (Table 3).

Except for weight group I females fed for 6 h, all other experimental groups presented nutrient index (NI) similar to the control group. However, although weight group II females fed for 24 h reached 98% of the engorged weight of naturally fed females, they exhibited lower egg mass, little weight loss and consequent low egg production index (EPI). So, the data indicate that artificial feeding does not affect the ability of females to convert

Table 3. Parameters of the non-parasitic stage of *Amblyomma sculptum* females artificially fed using capillary tubes and control group.

Biological parameters	Weight range and artificial feeding periods						
	Weight group I			Weight group II			
	6 h	12 h	24 h	6 h	12 h	24 h	Control
Weight (mg)	128.00±44.25^{c}	158.67±75.96^{c}	262.15±125.15^{c,b}	301.29±79.37^{b}	350.68±51.21^{b}	535.35±123.92^{a}	543.77±138.58^{a}
Pre-oviposition period (days)	6.78±1.56^{c}	8.20±1.32^{b,c}	9.73±2.41^{a,b}	8.73±0.47^{b}	7.18±0.75^{b,c}	7.27±0.90^{b,c}	6.73±0.79^{b,c}
Oviposition period (days)	10.27±5.62^{c}	12.91±7.18^{b,c}	16.91±3.39^{a,b}	18.82±2.96^{a,b}	20.09±3.42^{a}	21.55±5.70^{a}	22.73±3.69^{a}
Total egg weight (mg)	42.44±31.46^{e}	66.59±49.63^{e,d}	109.56±63.95^{e,c}	144.18±46.94^{c,d}	163.46±42.72^{b,c}	231.71±63.05^{b}	332.08±88.31^{a}
Female's weight loss (mg)	65.75±39.80^{d}	92.56±59.37^{d}	150.62±75.77^{c,d}	199.11±55.60^{c}	224.25±28.41^{b,c}	314.77±84.14^{a,b}	402.56±114.58^{a}
EPI (%)	29.11±16.63^{c}	35.97±15.91^{b,c}	40.26±5.89^{b}	47.32±6.69^{a,b}	46.37±9.52^{b}	43.29±6.78^{b,c}	61.24±8.02^{a}
NI (%)	53.23±28.61^{b}	63.23±22.25^{a,b}	70.11±7.48^{a,b}	71.65±7.40 ^{a,b}	72.31±14.18^{a,b}	73.89±7.48^{a}	83.06±8.26^{a}
Eclosion (%)	13.55±30.79^{c}	26.36±33.85^{b,c}	4.55±12.14^{c}	73.18±25.03^{a}	49.55±30.45^{a,b}	62.73±27.24^{a}	68.18±30.27^{a}

EPI=egg production index; NI=nutrient index

Means followed by at least one lowercase letter repeated on lines did not differ statistically in Tukey test, respectively ($p<0.05$).

blood meal into energy, but it has negative effects on conversion in eggs, under the conditions adopted in the present study. In spite of the lower egg mass, the technique did not influence percent larval eclosion values in weight group II ticks, indicating that embryogenesis was likewise unaffected.

It may be hypothesized that results were affected by the fact that feeding started on rabbits and ended with bovine blood. Moreover, De la Vega et al. (2000) tested an artificial feeding regime in *R. microplus* using defibrinated or heparinized blood. The authors observed that heparinized blood negatively affected tick biology. Therefore, considering that no study on this specific aspect has been published in current literature, the negative effect of sodium citrate phosphate dextrose used as anticoagulant cannot be ignored. The differences in feeding regimes, like type of anticoagulant and phagostimulant agents used, have been held to influence feeding, mortality and biological performance of several hematophagous arthropods (Galun 1967, Schwan et al. 1991, Waladde et al. 1993). Further studies should indicate the best anticoagulant agent for use in a capillary-based artificial feeding regime.

Previous studies have clearly demonstrated weight gains in capillary-fed ticks, though weight gain values observed in the present study are higher in comparison to many published values. This may be explained in the light of the fact that *A. sculptum* ticks have low host specificity and therefore are able to feed effectively under a variety of different conditions. Weight gain in partially engorged *A. sculptum* females used in the present study was higher than that previously reported for fasting females of the same tick species (Abel et al. 2008). This may be due to the fact that partially engorged females were in the fast feeding period and that fasting females had not mated before the beginning of the *in vitro* feeding process.

Although it is well known that ticks can be removed from a host and re-attach on another (Scoles et al. 2011), the present study shows that this technique can be used to minimize the use of laboratory animals in pathogen transmission experiments. Indeed, it is less expensive and offers some advantages when compared with artificial infestations in naturally infected animals. Ticks do not take blood meal throughout attachment to the host. They interrupt the feeding process in order to concentrate the blood already taken. On the other hand, some pathogens do not remain in peripheral blood all the time. So it is too difficult to synchronize parasitemia in naturally infected animals with the best period for tick blood meal. In this scenario, capillary feeding can be a useful tool, even in studies designed to quantify the pathogen.

CONCLUSIONS

Artificial feeding may be used as an alternative in studies with *A. sculptum* females initially reared on rabbits. In this sense, *A. sculptum* ticks are able to uptake large amounts of blood artificially. Investigations requiring large volumes of ingested blood should use females weighing between 44.8 and 108.0 mg, while studies about oviposition parameters ought to be carried out using females between 112.20 and 246.10 mg.

Acknowledgements. The authors are grateful to Dr. Rafael de La Vega Ruibal, *in memoriam*, for the substancial support in the conduction of this study, and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Pró-Reitoria de Pesquisa e Pós-Graduação da Universidade Federal do Pará (PROPESP/UFPA) and Fundação de Amparo e Desenvolvimento de Pesquisa (FADESP) for financial support.

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