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ABEL, ISIS; CORRÊA, FABÍOLA N.; CASTRO, ABISAIR A.; CUNHA, NATHALIE C.; MADUREIRA, RENATA C.; FONSECA, ADIVALDO H.

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ARTIFICIAL FEEDING OF *Amblyomma cajennense* (ACARI: IXODIDAE) FASTING FEMALES THROUGH CAPILLARY TUBE TECHNIQUE*

ISIS ABEL[1]; FABÍOLA N. CORRÊA[2]; ABISAIR A. CASTRO[2]; NATHALIE C. CUNHA[2]; RENATA C. MADUREIRA[2]; ADIVALDO H. FONSECA[3]

ABSTRACT:- ABEL, I.; CORRÊA, F.N.; CASTRO, A.A.; CUNHA, N.C.; MADUREIRA, R.C.; FONSECA, A.H. **Artificial feeding of *Amblyomma cajennense* (Acari: Ixodidae) fasting females through capillary tube technique.** [Alimentação artificial de fêmeas de *Amblyomma cajennense* (Acari: Ixodidae) em jejum por meio da técnica de tubos capilares.] *Revista Brasileira de Parasitologia Veterinária*, v. 17, n. 3, p.128-132, 2008. Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Cx. Postal 74548, Km 7 da BR 465, Seropédica, RJ 23890-970, Brasil. E-mail: adivaldo@ufrrj.br

The present study aimed to adjust the artificial feeding technique through capillaries and to verify its influence over the biology of *Amblyomma cajennense* females. Five groups of 20 female ticks were formed. Females were starved for 45 days and then fed with citrated bovine blood using capillary tubes in different periods of time. Females were divided in five experimental groups with 20 individuals each and fed as follows: groups uninterruptedly fed for 12, 24, and 48 hours and groups fed 2 and 6 h a day, for a period of 8 days. Subsequently, ticks were exposed to rabbits for complementary feeding and their biological parameters were analyzed. TIcks were capable of feeding, showing rounded idiosoma, visible even to naked eyes, following the feeding period. The groups fed for 24 hours, 2 hours/day for eight consecutive days or 6h/day for eight consecutive days presented greater weight gain, without statistically significant differences. These results suggested that 24 hours of artificial feeding were enough for fasting females to increase weight by 2.43 mg. Artificial feeding through capillaries did not interfere with parasitic and non-parasitic phases of *A. cajennense* females.

KEY WORDS**:** Ticks, *in vitro* feeding, micropipettes.

RESUMO

O presente trabalho teve como objetivos adaptar a técnica de alimentação artificial por meio de tubos capilares para fêmeas de *Amblyomma cajennense* e verificar a sua influência sobre os parâmetros biológicos da espécie. Fêmeas em jejum por 45 dias foram alimentadas com sangue bovino citratado usando tubos capilares por diferentes períodos de tempo: 12, 24 e 48 horas, 2 horas/dia e 6 horas/dia por 8 dias consecutivos, sendo que cada grupo foi composto por 20 carrapatos. Em seguida, os carrapatos foram expostos a coelhos para completar a alimentação e seus parâmetros biológicos foram analisados. Após alimentação artificial, os carrapatos apresentaram idiossoma arredondado mesmo à vista desarmada. Os grupos 24 horas, 8 dias/2 horas/dia e 8 dias/6 horas/dia tiveram maior ganho de peso, não havendo diferença estatística entre eles. Desta forma, nas condições do presente trabalho, 24 horas de alimentação artificial foram suficientes para que fêmeas em jejum aumentassem seu peso em 2,43mg. A alimentação artificial utilizando tubos capilares não interferiu nos parâmetros relativos às fases parasitária e não-parasitária de fêmeas *A. cajennense*.

PALAVRAS-CHAVE: Carrapatos, alimentação *in vitro*, tubos capilares.

INTRODUCTION

Ticks are vectors of a wide variety of pathogens, including virus, bacteria and protozoa, which infect human beings and

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[1] Laboratório Multidisciplinar de Biologia, Centro Universitário de Lavras, Rua Padre José Poggel, 506, Centenário, Lavras, MG 37200-000, Brasil. E-mail: isisabel@unilavras.edu.br

[2] Curso de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária (IV), Universidade Federal Rural do Rio de janeiro (UFRRJ), Km 7 da BR 465, Seropédica, RJ 23890-000, Brasil. Bolsistas CAPES, CNPq e FAPERJ.

[3] Departamento de Epidemiologia e Saúde Pública, IV, UFRRJ, Cx. Postal 74548, Seropédica, RJ 23890-970, Brasil. E-mail: adivaldo@ufrrj.br. Bolsista CNPq.

other vertebrate animals (JASINSKAS et al., 2000). The study of these pathogens in laboratory requires the maintenance of animals and ticks, in costly and time-consuming routines. Besides, moreover, is often necessary to infect healthy animals in order to determine an experimental model. Another common difficulty in the researches on pathogens transmission is the fact the acquisition of their feeding has to coincide with the period of increase or of having a maximum level of parasitemia in the vertebrate host, which is not easy to accomplish, since the host's pre-patent period varies widely (HOWARTH; HOKAMA, 1983). Therefore, considering this context, the alternative of feeding ticks using artificial methods offers many advantages when they were compared to feed on vertebrates.

Artificial feeding methods have already been thoroughly studied in relation to arthropods that feed quickly, particularly solenophages. For the Ixodidae family, however, these methods are more difficult to put on practice, since it is necessary to create a feeding site, to secrete cement, and to fix their mouthparts before a long feeding period (BROADWATER et al., 2002). Apart from this, their complementary feeding must be fresh and palatable during this period.

The method that uses capillaries for artificial feeding, although it does not allow total engorgement of specimens and it presents some differences regarding natural feeding, does in fact reduce the amount of variables to be considered during the feeding process. At the same time, it is advantageous when compared to other methods, because it permits experimental manipulations. Apart from this, it is possible to infect the ticks with clones of pathogens or with different strains at the same time (FINGERLE et al., 2002).

The cayenne tick, *Amblyomma cajennense* (Fabricius, 1787), is an America's autochthonous ixodid tick. Its low parasitic specificity leads to a high commitment level regarding the transmission of zoonotic agents already established in Brazil, as well as of pathogens that cause emergent zoonoses (BARROS-BATTESTI, 1998; RIBEIRO; GUIMARÃES, 1998; DE LEMOS et al., 2001). In order to maintain this specimen in laboratory, rabbits are used to feed ticks in initial stages, and equines are the best choice to feed adults (PRATA, 2002), which makes the studies rather difficult to conduct. Rabbits can also be used to feed adults (CHACÓN et al., 2004); however, tick feeding can cause serious injuries to the animal skin.

The possibility of establishing an experimental model, through which it could be possible to study the relationship *Amblyomma*-pathogen in host absence, may contribute considerably to understand the transmission routes of viruses, bacteria and protozoa that it might transmit. In this context, the present work aimed to adjust the artificial feeding system through capillaries, and to verify the influence of this method over the biological parameters of *A. cajennense* starving females when artificially feding.

MATERIALS AND METHODS

A hundred *A. cajennense* females that have been starving for 45 days were weighed and fixed on EPS plate (19cm x 10cm), with double face tape. Then, capillaries for micro-hematocrit determination (75mmx1.0mmx1.5mm) without anticoagulant, full of citrated bovine blood, were placed over ticks' mouthparts, forming an approximately 30°-angle.

Females were divided in five experimental groups with 20 individuals each and fed as follows: groups uninterruptedly fed for 12, 24, and 48 hours and groups fed 2 and 6 h a day, respectively, for 8 days. Artificial feeding was conducted at 27ºC and in a relative humidity over 80%. Capillaries used in groups fed for 24 hours and 48 hours were replaced every 12 hours. After the referred exposure periods, the females were washed and weighed for verifying blood ingestion. After that, they were placed on rabbits' back together with 20 males in order to complement the feeding process. Ticks of the control group were fed directly on the rabbits, according to methodology described by Abel (2004). From the eighth post-infestation day until the end of infestation, ticks that detached spontaneously were collected once a day, cleaned, weighed and fixed on Petri dishes. Then, ticks were maintained at 27ˆC and 85% humidity, when their biological parameters were analyzed.

The biological parameters analyzed were those related to parasitic phase: number of recovered females/number of exposed females, period of feeding and weight of recovered females; parameters related to non-parasitic phase: pre-oviposition and oviposition periods; and parameters related to oviposition: total weight, egg production index (EPI = (weight of eggs/initial weight of engorged tick) x 100), females' weight loss and nutrient index (NI =[weight of eggs/(initial weight.engorged tick-residual weight. tick)] x 100) (BENNETT, 1974).

For results analysis, the biological parameters were examined independently. The statistical non-parametrical tests Kruskal-Wallis and Dunn were used, with 5% significance level.

RESULTS AND DISCUSSION

After artificial feeding, it was possible to observe blood ingestion even at naked eyes and before the weighing procedure, since the ticks presented rounded idiosoma (Figure 1).

The use of weighing to verify weight gain was appropriate, since it turned the method more practical and feasible, with equipment easily found in any laboratory room. In general, there are more sophisticated techniques being used for confirming ingestion after exposure to capillaries, such as radioactive markers, xenodiagnosis and molecular techniques (BURGDORFER, 1957; JOYNER et al., 1972; PURNELL et al., 1972; RECHAV et al., 1999; JASINSKAS et al., 2000; BROADWATER et al., 2002). On the other hand, some authors, such as Lösel et al. (1993), Humphery-Smith et al. (1993) and De La Vega et al. (2000), preferred the weighing method and were successful in their experiments. In spite of their proven efficiency, sophisticated techniques are more expensive and turn the whole process of artificial feeding through capillaries quite slow.

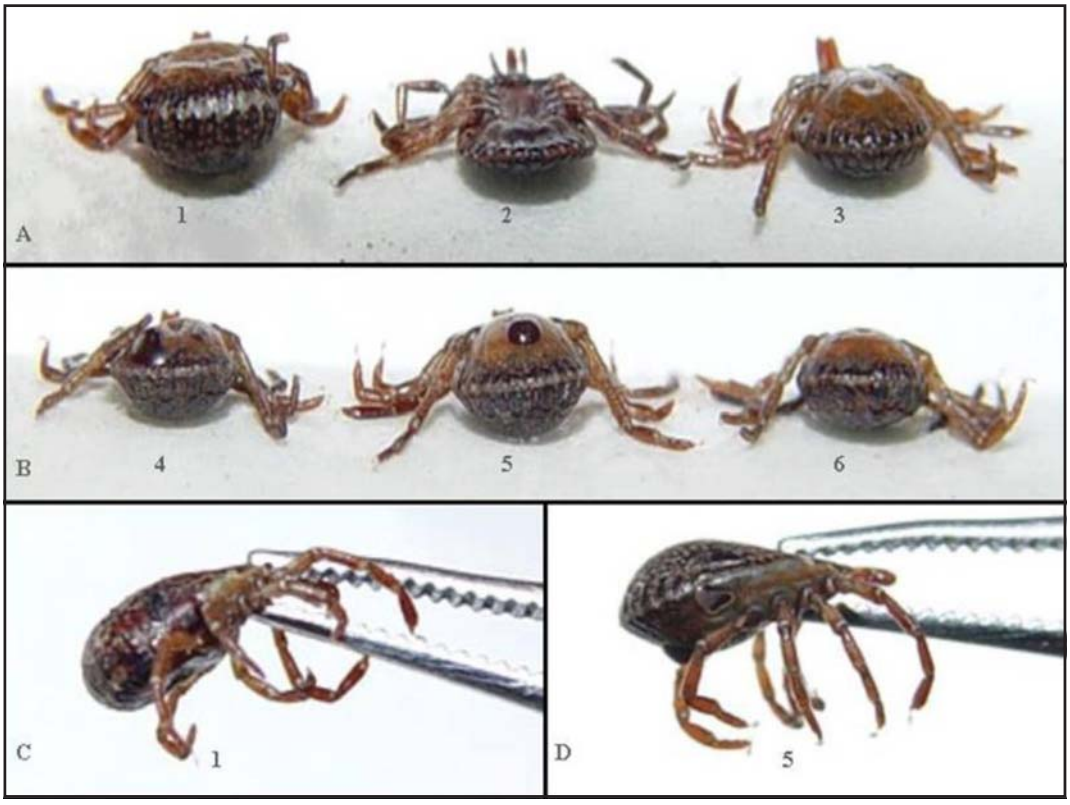

Figure 1. *Amblyomma cajennense* females after artificial feeding through capillaries, subsequent to a fasting period of 45 days. A and B, females after eight days of artificial feeding, for 6 hrs/day; C and D, females 1 and 5, respectively, after eight days of artificial feeding, for 6 hrs/day, lateral view.

Table 1. *Amblyomma cajennense* females after artificial feeding through capillaries, subsequent to a fasting period of 45 days, kept at 27ºC and in a relative humidity over 80%.

Artificial feeding Groups	n	Weight artificial feeding (mg) before	Weight artificial feeding (mg) after	Acquired weight (mg)
12 hours	19	5.87±0.96[a]	6.62±1.34 [a]	0.75±0.68[a]
24 hours	20	6.51±1.11[a]	8.94±2.02 [ab]	2.43±1.14[bc]
48 hours	14	5.63±2.02[a]	7.17±3.05 [ab]	1.54±1.36[ab]
8days 2hours/day	11	5.99±1.75[a]	8.09±2.61 [ab]	2.10±1.28[bc]
8days 6hours/day	20	6.31±1.23[a]	9.56±2.79 [b]	3.25±2.05[c]

Values followed by different letters in a same column are significantly different at a level of 5%, according to the Kruskal-Wallis and Dunn's tests.

Table 2. Parameters related to the parasitic phase of *Amblyomma cajennense* females fed on rabbits after artificial feeding through capillaries, subsequent to a fasting period of 45 days, kept at 27ºC and in a relative humidity over 80%.

Groups	n	Recovered females (%)	Engorged females weight (mg)*	Feeding period (days)**
12 hours	19	95.00	449.80±119.42[a]	10.88±1.71[ab]
24 hours	20	100.00	525.48±198.77[a]	8.94±0.77[b]
48 hours	14	70.00	483.88±203.07[a]	12.08±2.36[a]
8days 2hours/day	11	55.00	504.75±102.40[a]	9.55±1.75[ab]
8days 6hours/day	20	100.00	452.81±160.79[a]	10.05±2.44[ab]
Control	36	90.00	436.59±148.60[a]	10.69±2.05[ab]

Average values followed by different letters in a same column are significantly different at a level of 5%, according to the Kruskal-Wallis (*) or Kruskal-Wallis and Dunn's tests (**).

Observing the excretion process after feeding period is another way of verifying whether or not the ingestion was successful. This process was consider so important, that use it as the only method for evaluating their results (INDEST et al., 2001; FINGERLE et al. 2002). In the present work, ticks excreted a whitish or sometimes reddish substance through the anal opening. This observation has also indicated that the ticks managed to achieve the phase of slow feeding, described by Sonenshine (1991). The reddish excreta observed (Figure 1) were probably composed of great amount of hematin, with non-digested hemoglobin, besides other nutrients and guanine,

Table 3. Parameters related to the non-parasitic phase of *Amblyomma cajennense* females fed on rabbits after artificial feeding through capillaries, subsequent to a fasting period of 45 days, kept at 27ºC and in a relative humidity over 80%.

Groups	n	Pre-oviposition period (days)	Oviposition period (days)
12 hours	19	7.79±1.58 [a]	27.58±9.29 [a]
24 hours	20	7.55±0.94 [a]	26.65±7.67 [ab]
48 hours	14	5.50±1.51 [b]	18.79±4.16 [b]
8days 2hours/day	11	7.82±0.98 [a]	31.73±6.29 [a]
8days 6hours/day	20	8.20±1.44 [a]	28.50±9.23 [a]
Control	36	7.47±1.95 [a]	26.31±8.36 [ab]

Average values followed by different letters in a same column are significantly different at a level of 5%, according to the Kruskal-Wallis and Dunn's tests.

Table 4. Parameters related to the oviposition of *Amblyomma cajennense* females fed on rabbits after artificial feeding through capillaries, subsequent to a fasting period of 45 days, kept at 27ºC and in a relative humidity over 80%.

Groups	n	Total egg weight (mg)	EPI (%)	Females' weight loss (mg)	NI (%)
12 hours	19	200.16±81.16	43.86±12.55	255.36±104.67	79.63±17.80
24 hours	20	248.32±128.28	46.23±12.27	335.19±134.98	71.50±14.17
48 hours	14	215.51±124.27	42.03±10.84	325.33±151.87	63.94±14.45
8days 2hours/day	11	217.10±76.63	43.04±11.77	292.63±99.30	73.79±8.62
8days 6hours/day	20	175.05±68.41	40.69±14.81	239.59±88.99	73.18±11.16
Control	36	196.69±95.01	43.20±10.82	261.51±113.65	77.75±28.02

EPI=egg production index; NI=nutrient index
Average values observed in a same column do not differ significantly at a level of 5%, according to Kruskal-Wallis test.

a typical composition for this feeding phase, according to Sonenshine (1991).

The average weight of *A. cajennense* females, before and after artificial feeding through capillaries, is presented in Table 1. It was observed that, considering the experimental groups, the weight gain was significantly more evident from the twenty fourth hour after exposure to capillaries ($p<0.05$). After 24 hours of feeding, a direct relationship failed to be observed between the feeding period and acquired weight, since there was no statistically significant difference between the groups in terms of feeding period. However, the group fed for 48 hours acquired less weight as compared to the group fed for 24 hours. For ticks, blood uptake is characterized by blood uptake periods inserted between rest periods (SONENSHINE, 1991). It is possible that along 48 hours of feeding the rest period occurred, leading the tick to spend energy without blood uptake. So the ticks fed for 48 hours did not acquire weight, while in the other groups it could not be observed probably because there was a better mimicry of the natural feeding between them. In this case, ticks had the opportunity to take small blood meals during a bigger period of time.

After artificial feeding, females were exposed to rabbits in order to complete the feeding process. The capillaries did not affect the females' ability to feed on rabbits; in fact, it was rather evident that some ticks were really eager for food after artificial feeding. A few days later, the fully engorged females presented indexes of recovering ranging from 55% to 100% (Table 2). When compared with results obtained by Prata (2002) in horses (47.03%), these results become more important. It is interesting to note that females from different experimental groups and quite different initial weight presented similar weight after feeding complementation. This observation can be justified when the feeding period is analysed. This parameter ranged from 8.94 to 12.08 days. It seems that in order to compensate a low initial weight some females took more days to complete feeding and detach naturally from the rabbits' back, though no significant difference among these values was verified. (Table 2).

The parameters related to the non-parasitic phase are available in Table 3. The pre-oviposition and oviposition periods of all experimental groups did not differ significantly from the same period of the control group, except for the group exposed for 48 hours that spent 5.50 days to begin oviposition. Therefore, the oviposition period did not differ significantly between the experimental groups and control.

The parameters related to ovipostion are available in Table 4. All experimental groups presented statistically similar values to those presented by the control group ($p>0.05$) for the following parameters: total egg weight, EPI, females' weight loss and NI.

The analysis indicates that artificial feeding did not interfere on ticks' biology, as it has been already observed by De La Vega et al. (2000), when they worked with *Rhipicephalus* (*Boophilus) microplus.*

In the experimental context of the present work, the artificial feeding technique through capillaries was proven satisfactory for feeding *A. cajennense* females that were starved for a 24-hour period, because it is fast, easy and inexpensive. After the artificial feeding, females presented biologic parameters compatible to those presented by the control group.

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