RELATIONSHIP BETWEEN WEIGHT AND NUMBER OF ENGORGED *AMBLYOMMA CAJENNENSE* LARVAE AND NYMPHS (FABRICIUS, 1787) (ACARI: IXODIDAE) IN EXPERIMENTAL INFESTATIONS ON RABBITS.

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SUMMARY: With the aim to determinate the correlation between the weight and number of *Amblyomma cajennense* larvae and engorged nymphs, experimental infestations were carried out on rabbits. Engorged individuals were collected daily, and for each rabbit 20 groups of 50 engorged larvae and 13 groups of 50 engorged nymphs were sorted and weighed. The mean weight of 50 engorged larvae was 38.2 ± 1.5 mg. There were no significant differences at the 5% level among the mean weights of the groups of engorged larvae in relation to the rabbit used for engorging. However, the differences related to day of collection were significant ($p \leq 0.05$), decreasing from the third to the sixth day after infestation. The mean weight of 50 engorged nymphs was 677.2 ± 54.9 mg. There were significant differences among the means according to host and day of collection ($p \leq 0.05$), the heavier mean weight being found on the fourth day, when compared to the third and fifth days post-infestation. The nymphs that developed to males had a shorter engorging period and lighter weight than the nymphs that developed to females.

KEY WORDS: *Amblyomma cajennense*, weight x number of larvae and nymphs, rabbits.

INTRODUCTION

In studies on biological cycles of heteroxenic ticks, it is common to carry out infestations in animals with larvae, nymphs and adults, obtained under laboratory conditions, aiming to determine the various parameters related to the parasitic and non-parasitic phases of the different stages.

In Brazil, the species *Amblyomma cajennense* (Fabricius, 1787) has been studied by various researchers, due to its ability to cause damage to domestic animals and to its importance in Public Health. TRAVASSOS & VALLEJO-FREIRE (1944) established colonies of *A. cajennense* in laboratory conditions for the preparation of the vaccine against the Rocky Mountain spotted fever. CUNHA (1978) experimentally analyzed the toxic effect of the different stages of the biological cycle of this tick species. OLIVIERI & SERRA-FREIRE (1984a,b) studied the larval and nymphal stages of the biological cycle of *A. cajennense*. The effect of different temperatures on the larval (DAEMON & ISHIZUKA, 1992) and nymphal (DAEMON & ISHIZUKA, 1995) ecdyses of this ixodid were also evaluated. PRATA *et alii* (1995a) determined the number of eggs in one gram of oviposition (EPG). PRATA *et alii* (1995b) and PRATA *et alii* (1996) carried out studies on the biological parameters of the larval and nymphal phases of the biological cycle of *A. cajennense*, respectively.

In these experiments, the approximate number of larvae used in each infestation is obtained through simple arithmetic calculations, since the number of eggs present in one gram of oviposition (EPG) (PRATA *et alii*, 1995a) and the percentage eclosion are known. However, when the engorged specimens are collected, the lack of a parameter that allows the conversion of the weight in number of engorged larvae or nymphs forces the researcher to count the individuals one by one, what turns out to be a tiring and time-consuming process, because of the large number of larvae and nymphs used in the infestations. In the present work the objective was to determine the relationship between the weight and the number of engorged larvae and nymphs of *A. cajennense*, allowing the conversion of the weight in number, using simple arithmetic calculations.

MATERIALS AND METHODS

The experiment was carried out in the W.O. Neitz Parasitological Research Station, of the Department of Animal Parasitology, Universidade Federal Rural do Rio de Janeiro, located in Seropédica, State of Rio de Janeiro, from January through April, 1997. Ten *A. cajennense* engorged females were collected from naturally infested horses kept in an area nearby UFRRJ. The females were taken to the laboratory, where they were cleaned, weighed and kept in a BOD incubator at 27ºC, with relative humidity higher than 70% and scotophase, conditions usually used for biological studies on tropical ixodids. The oviposition of the females was pooled, and 100mg aliquots were transferred to previously prepared plastic syringes, also kept in BOD, where the eclosion of larvae took place.

In order to determine the relationship between weight x number of engorged larvae, infestations were carried out in six adult New Zealand cross California rabbits (*Oryctolagus cuniculi*), being three males and three females. Each animal was infested with 200 mg or approximately 3280 larvae (PRATA *et alii*, 1995a), fasted for 15 days. Larvae were then deposited on the external ear of the rabbits, where a fabric bag had been previously adapted, according to the method of NEITZ *et alii* (1971). The bags were opened daily, and the detached engorged larvae were collected. Twenty groups of 50 engorged larvae were formed for each rabbit, which were weighed in an analytical scale. After weighing, the engorged larvae were placed in previously prepared plastic syringes, kept in BOD, under the same conditions described previously.

After the larval moulting, infestations with nymphs of *A. cajennense* were carried out in another six rabbits, which had the same characteristics of those used in the infestations with larvae, with the objective of determining the relationship between weight x number of engorged nymphs. Each rabbit was infested with approximately 1100 nymphs fasted for 15 days, which were deposited on the external ear (NEITZ, 1971). The detached engorged nymphs were collected daily, being formed 13 groups of 50 nymphs for each rabbit, which were weighed in an analytical scale. After weighing, the nymphs were placed in syringes and kept in BOD, under the conditions described above.

The analysis of variance (ANOVA) and the Tukey test were performed at the 5% significance level, to verify the presence of significant differences related to the weight of engorged larvae and nymphs among the rabbits and the collection days.

RESULTS AND DISCUSSION

Weight x number of engorged larvae relationship: Engorged *A. cajennense* larvae were collected between the third and the sixth days after infestation. The mean larval engorging period was 4.08 ± 0.62 days. This period was similar to the ones registered in the literature (ROHR, 1909; HOOKER *et alii*, 1912; OLIVIERI & SERRA-FREIRE, 1984a; PRATA *et alii*, 1995b). The percentage recovery of engorged larvae varied between 46.03 and 72.07%, with a mean of 57.90 ± 8.93%. From the 20 groups of 50 engorged larvae of each rabbit, five were formed on the third day of infestation, 10 on the fourth day and 5 on the fifth day. On the sixth day engorged larvae could not be collected in sufficient numbers to form groups. The mean weights of the groups of 50 *A. cajennense* engorged larvae of each rabbit are shown in Table 1. The analysis of variance showed no significant differences among the mean weights of the groups of engorged larvae according to the rabbit used for engorging. There were significant differences, however, among the days of collection, related the mean weights of the groups of engorged larvae (ANOVA, followed by the Tukey test), weights decreasing as the period after infestation increased (Figure 1). Then, it is possible to state that, in experimental infestations on rabbits, the mean weight of 50 engorged larvae was 38.2 mg (general mean), or 39.2 mg on the third day, 38.3 mg on the fourth day and 37.0 mg on the fifth day after infestation. One *A. cajennense* engorged larvae weighed approximately 0.76 mg (general mean), or 0.78 mg on the third day, 0.77 mg on the fourth day and 0.74 mg on the fifth day after infestation. Then, using simple arithmetic calculations it is possible to convert the weight of engorged *A. cajennense* larvae to their number, without the need to count them.

The larval pre-ecdysis period was 10.80 ± 0.84 days; the larval ecdysis took place in 4.40 ± 0.55 days. OLIVIERI & SERRA-FREIRE (1984a) studied the larval stage of *A. cajennense* life cycle under the same condutions of the present work, found a pre-ecdysis period of 10.91 ± 0.04 days and a ecdysis period of 5.59 ± 0.03 days. DAEMON & ISHIZUKA

Table 1 - Mean weights of 20 batches of 50 *A. cajennense* larvae each. Larvae was obtained by experimental infestation of rabbits.

Rabbit	Mean weight of 50 engorged larvae (mg) Mean ± Standard Deviation
1	37.6 ± 0.8
2	38.6 ± 1.1
3	38.3 ± 2.1
4	38.0 ± 1.6
5	38.2 ± 1.0
6	38.4 ± 2.0
Mean	38.2 ± 1.5

Differences between means were not statistically significant (α=5%-ANOVA).

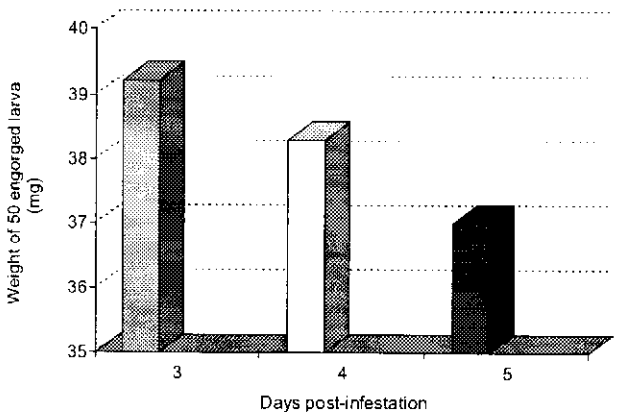

Fig. 1 - Mean weight of groups with 50 *A. cajennense* engorged larvae, obtained from experimental infestations on rabbits (*Oryctolagus cuniculi*), by day of detachment. There was statiscally significant difference between means (a=5% for ANOVA and Tukey test).

(1992), evaluating the effect of different temperatures on the larval ecdysis of *A. cajennense*, observed that the pre-ecdysis period was 11.31 ± 1.19 days, while the ecdysis period was approximately five days, values which were similar to those of the present work. Of the total engorged larvae obtained, 97% moulted to nymphs, a value higher than the 91.67% obtained by DAEMON & ISHIZUKA (1992), at 27°C.

The similarity of results obtained in experiments carried out from 1984 through 1997 indicates a stability in the populations of the tick *A. cajennense*, which makes the data obtained in the present work even more reliable.

Weight x number of engorged nymphs: Engorged *A. cajennense* nymphs were collected between the third and seventh days after infestation. The mean nymphal engorging period was 4.23 ± 0.82 days. The percentage recovery of engorged nymphs varied between 68.73 and 84.36%, with a mean of 75.54 ± 5.86%. OLIVIERI & SERRA-FREIRE (1984b) determined a mean nymphal engorging period of 5.31 ± 0.18 days. PRATA *et alii* (1996) observed that the nymphal engorging took place between three and five days, with a larger quantity of nymphs taking five days for engorging and obtained a percentage recovery of engorged nymphs of 53.44%. In spite that these experiments used similar methodology, they were carried out in distinct times and with different nymphal fasting periods, what may explain the variation among the results.

From the 13 groups of 50 engorged nymphs of each rabbit, two were formed on the third day, eight on the fourth day, and three on the fifth day after infestation. On the sixth and seventh days engorged nymphs could not be recovered in sufficient numbers that allowed the formation of groups. The mean weights of the groups of 50 engorged *A. cajennense* nymphs from each rabbit are presented in Table 2. The mean of the weight of the groups of 50 engorged nymphs among each rabbit was 677.2 mg (or 13.5 mg, for one nymph). In two rabbits the means were higher than the others (ANOVA, followed by the Tukey test). This difference cannot be related to sex or the color of the fur of the host, because the highest means occurred in one male and one female rabbit, and all rabbits had white fur. Further studies become necessary to determine the factors involved in the process. In the same way as for larvae, the means of the weights of the groups of 50 nymphs were different according to the day of collection. (Figure 2), however there was not a decreasing order of weights, as for larvae. The highest mean was observed on the fourth day after infestation (698.8 mg), followed by the fifth day (651.5 mg), and the third (628.9 mg).

Table 2 - Mean weights of 13 batches of 50 *A. cajennense* engorged nymphs in each experimentally infested rabbit.

Rabbit	Mean weight of 50 engorged nymphs (mg) Mean ± Standard Deviation
1	656.4 ± 53.5 b
2	735.6 ± 45.4 a
3	665.6 ± 32.9 b
4	640.9 ± 30.5 b
5	644.3 ± 40.8 b
6	720.1 ± 42.4 a
Mean	677.2 ± 54.9

Means followed by the same letter showed no statistically significant differences (α=5% for ANOVA and Tukey test).

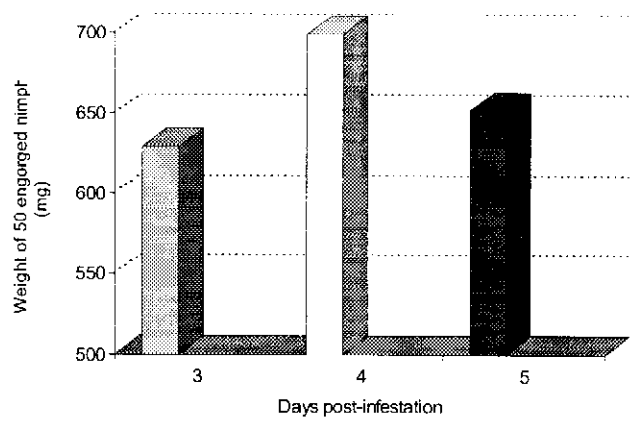

Fig. 2 - Mean weight of groups with 50 *A. cajennense* engorged nymphs, obtained from experimental infestations on rabbits (*Oryctolagus cuniculi*), by day of deatachment. There was statiscally significant difference between means (a=5% for ANOVA and Tukey test).

Due to the differences obtained among rabbits, the conversion weight x number of engorged *A. cajennense* nymphs should be carefully used, just to get a primary idea of the number of engorged nymphs obtained. When the experiment demands a higher precision of the data, it is recommended to count them.

The nymphal pre-ecdysis period was 13.33 ± 0.58 days; the nymphal ecdysis took place in 5.67 ± 0.58 days. OLIVIERI & SERRA-FREIRE (1984b), in an experiment with similar methodology, obtained a pre-ecdysis period of 15.66 ± 0.15 days and ecdysis period of 3.91 ± 0.11 days. DAEMON & ISHIZUKA (1995) observed that, at 27°C, the pre-ecdysis period was 15.33 days and the ecdysis period varied between one and seven days. The different nymph fasting periods used in the various studies might be responsible for the variation in the results.

From the total recovered engorged nymphs (4986 nymphs), approximately 98% moulted to adults. OLIVIERI & SERRA-FREIRE (1984b) observed that, for each group with 10 engorged metanymphs, 81.8 % moulted. DAEMON & ISHIZUKA (1995) and PRATA *et alii* (1996) obtained percentages close to the ones of the present work (95.83 and 95%, respectively).

The proportion of males and females obtained from the engorged nymphs in each collection day, although not included in the objectives of the experiment, is noteworthy. From the total engorged nymphs collected on the third day after infestation (793 nymphs), 75.03% moulted to males, 24.47 moulted to females and 0.50% died before moulting to adults; from the nymphs collected on the fourth day, (2669 nymphs), 49.01% moulted to males, 49.49% moulted to females and 1.50% died; on the fifth day, from the 1144 engorged nymphs recovered, 29.98% moulted to males, 65.04% moulted to females and 4.98% died. The engorged nymphs collected on the sixth and seventh days post-infestation (347 and 33 nymphs, respectively) were discarded, due to the insufficient number to form groups. These results make evident that the nymphs that originated males had a shorter engorging period (and, according to Figure 2, lighter weight) than the nymphs which originated females. These results are in agreement with those obtained by other researchers. KNITH *et alii* (1978), in studies on the biological cycle of *Hyalomma marginatum rufipes* and RECHAV & KNITH (1981), analysing the biology of *Rhipicephalus glabroscutatum*, also observed that the nymphs which originated males had a shorter engorging period and lighter weight than the ones which originated females. Although in the present work this analysis was performed in a preliminary way, requiring further studies to be confirmed, the comparison between the results of the present experiment and from previous data indicates similar behavior among nymphs of different tick species.

SUMÁRIO

Com o objetivo de determinar a relação entre o peso e o número de larvas e ninfas ingurgitadas de *Amblyomma cajennense*, foram realizadas infestações experimentais em coelhos. Diariamente foram recolhidos os exemplares ingurgitados, separando-se e pesando-se 20 grupos de 50 larvas ingurgitadas e 13 grupos de 50 ninfas ingurgitadas para cada coelho. O peso médio de 50 larvas ingurgitadas foi de 38,2 ± 1,5 mg. Não houve diferenças significativas a nível de 5% entre os pesos médios dos grupos de larvas ingurgitadas de acordo com o coelho utilizado para ingurgitamento. Entretanto, as diferenças com relação ao dia de coleta foram significativas ($p \leq 0,05$), decrescendo do terceiro para o quinto dia após a infestação. O peso médio de 50 ninfas ingurgitadas foi de 677,2 ± 54,9 mg. Houve diferenças significativas entre as médias de acordo com o hospedeiro e com o dia de coleta ($p \leq 0,05$), sendo o quarto dia o de maior peso médio, quando comparado com o terceiro e quinto dias pós-infestação. As ninfas que originaram machos tiveram período de ingurgitamento mais curto e peso menor que as ninfas que originaram fêmeas.

PALAVRAS-CHAVE: *Amblyomma cajennense*, peso x número de larvas e ninfas, coelhos.

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