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Communication

[*Comunicação*]

Rabbits (*Oryctolagus cuniculus*) as experimental hosts for *Amblyomma dubitatum* Neumann (Acari: Ixodidae)

[*Coelhos* (Oryctolagus cuniculus) *como hospedeiros experimentais de* Amblyomma dubitatum *Neumann (Acari: Ixodidae)*]

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A recent publication by Guglielmone et al. (2003) listed 57 described species of the genus *Amblyomma* Koch endemic to the Neotropics. In this paper the authors have followed Guglielmone et al. (2003) for nomenclature purpose. Except for those species primarily associated with domestic animals and few recently reared species from wildlife (e.g. Aguirre et al., 1999; Labruna et al., 2002, 2003), data on biology and ecology for most of described Neotropical species of *Amblyomma* are unknown.

The scientific interest in species of *Amblyomma* has recently increased due to the potential role played by some of them in transmission of bioagents to humans and domestic animals (Aguirre et al., 1999). Regarding *Amblyomma dubitatum* Neumann (= *A. cooperi)*, the scientific interest has increased in Brazil since the isolation of spotted fever group rickettsiae from this species (Lemos et al., 1996). Its potential role in natural transmission of these bacteria among capybaras, geographic distribution and host relationships have recently been summarized by Labruna et al. (2004 a, b). Labruna et al. (2004a) evaluated the life cycle of *A. dubitatum* in the laboratory using capybaras (*Hydrochaeris hydrochaeris*) as hosts, adding detailed information to the paper of Almeida et al. (2001).

It is notorious that successful laboratory rearing of a given vector species is paramount in studies on transmission of bioagents. Regarding tick laboratory rearing, the domestic rabbit (*Oryctolagus cuniculus*) has been used as experimental host for several species of ticks for a long time (Kohls, 1937; Chacon et al., 2004). These animals are easily handled and non-laborious to maintain. The aim of this paper was to evaluate the usefulness of the domestic rabbit as experimental host for *A. dubitatum*.

All the used rabbits were tick naive California x New Zealand crossbred, reared for commercial purpose. Rabbits were two to three month-old, weighting 1.5 to 2.1kg. During the experiment, they were kept in individual wired cages in a ventilated room under natural light regime, fed with commercial food pellets and water *ad libitum.* Each rabbit was used for a single infestation. Infestations with larvae and nymphs were carried out on the rabbit's ears according to Neitz (1971). Briefly, ticks were released inside muslin sleeves glued to the ear base with non-toxic glue (Ulna past). Infestations with adult ticks were carried out inside muslin sleeves glued with Ulna past to the rabbit's back according to Pinter et al. (2002). One sleeve per host was used in all infestations.

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Ticks used in this study were obtained from the progenies of three engorged females from the laboratory colony of *A. dubitatum* used by Labruna et al. (2004a). Eggs collected from these three females at three day-intervals after starting of oviposition were pooled in batches of 200mg, transferred into 10ml plastic vials plugged with cotton and incubated at 27±1°C, 80±10% RH and 24h darkness. This incubator condition was used for all free-living stages of the tick during the present study. Naturally detached engorged ticks were daily collected from the infested hosts.

Twelve rabbits were infested with approximately 1,845 larvae each, 20 to 30 day-old. Detached engorged larvae were weighed in pools of 50 larvae each. Seven rabbits were infested with 260 nymphs each, aging 25 to 35 day-old. Detached engorged nymphs were individually weighed. Six rabbits were infested with 15 adult couples each, aging 45 day-old. Engorged females were individually weighed. Difference in host number for tick stages was due to the availability of rabbits. Tick biological parameters for larvae, nymphs, adult females and eggs (Tables 1 and 2) were recorded as described by Labruna et al. (2004a). The biological data of *A. dubitatum* using rabbits as hosts were compared with those obtained by Labruna et al. (2004a) using capybaras for the same purpose.

Results on the life cycle of A. *dubitatum* on rabbits are presented in two ways: those similar to the data obtained by Labruna et al. (2004a) for capybaras are shown in Tables 1 and 2. Some of these data have already been published by Chacon et al. (2004). They are cited herein for the sake of comparison with the data on capybaras (Labruna et al. 2004a). Additional results of the life cycle are presented in Tables 3 and 4. Data of Table 3 shows the results of daily drop-off percentages of engorged larvae, nymphs, and females, whereas daily percentages of ecdisis of immatures are shown in Table 4.

Table 1. Biological data of larvae and nymphs of *Amblyomma dubitatum* experimentally fed on rabbits (present study) and capybaras[1]

Biological parameter	Rabbit		Capybara	
	Larvae	Nymphs	Larvae	Nymphs
Exposed ticks (n)	22140 (12)[2]	1820 (7)[2]	4000 (2)	100 (2)
Engorged ticks (%)	10036 (45.3)[2]	1136 (62.4)[2]	2545 (63.6)	48 (48)
Feeding period (days)[3]	5.7±1.1 (4-8)[2]	6.7±1.5 (4-10)[2]	5.7±1 (4-11)	6.4±1 (4-9)
Molted ticks (%)	6523 (65)	1013 (89.2)	1272 (50)	45 (93.7)
Weight of engorged ticks (mg)[3,4]	45.5±3.9 (34.7-51.8)	13.7±6.1 (2.1-29.5)	13.3±0.78 (12.2-14.5)	23.1±6.4 (9.2-32.1)
Premolt period (days)[3]	17.9±2.1 (13-25)	18.6±1.6 (15-23)	18.3±2.8 (14-19)	16.5±1.2 (14-19)

n = number of individual hosts; [1]Labruna et al. (2004a); [2]Chacon et al. (2004); [3]Values presented as mean±SE (range in parentheses); [4]Larvae weighed in pools of 50 when fed on rabbits and in pools of 10 when fed on capybaras. Nymphs weighed individually. Free-living phases were observed under 27 ± 1°C, 80 ± 10% relatively humidity and scotophase

Table 2. Biological data of females of *Amblyomma dubitatum* experimentally fed on rabbits (present study) and capybaras[1].

Biological parameter	Rabbit	Capybara
Exposed females (n)[2]	90 (6)	21 (3)
Engorged females (%)[2]	12 (13.3)	17 (80.9)
Feeding period (days)[2,3]	21.7±2.6 (18-24)	8.9±1.7 (6-14)
Weight of engorged females (mg)[2,3]	142.6±76.7 (18.3 – 315.4)	1174±224.8 (626-1520)
Pre-oviposition period (days)[3]	13.4±4.4 (10-23)	6.7±1.2 (5-9)
Egg mass weight per female (mg)[3]	62.7±48.6 (19.9–141.5)	584.1±148.7 (291-806)
Egg incubation period (days)[3]	32.7±0.6 (32-33)	36.9±2.2 (32-40)
Egg mass hatching (%)[3]	<1%	40.9±29.6 (0-90)
Egg production efficiency[3]	28.8±9.8 (19.8-44.9)	49.9±8.6 (23.4-57.6)

n = number of individual hosts; [1]Labruna et al. (2004a); [2]Chacon et al. (2004); [3]Values presented as mean±SE (range in parentheses).
Free-living phases were observed under 27±1°C, 80±10% relatively humidity and scotophase

Table 3. Daily drop-off percentage of engorged larvae, nymphs and females of *Amblyomma dubitatum* experimentally fed on rabbits

Tick stage	Feeding period (days)						
	4	5	6	7	8	9	10
Detached larvae (n=12)	14	36	26	17	8	0	0
Detached nymphs (n=7)	4	20	34	18	11	9	8
	Feeding period (days)						
	18	19	20	21	22	23	24
Detached females (n=6)	8	8	8	42	8	8	16

n = number of individual hosts

Table 4. Daily percentage of ecdisis of nymphs and females of *Amblyomma dubitatum* experimentally fed on rabbits and kept at 27±1°C and 80±10% relatively humidity

Tick stage	Premolt period (days)												
	13	14	15	16	17	18	19	20	21	22	23	24	25
Nymphs	1	2	7	17	23	24	13	9	4	2	1	1	1
Females	0	0	3	7	17	24	21	2	8	2	1	0	0

The results clearly show that naive rabbits were quite suitable experimental hosts for the immature stages of *A. dubitatum* considering the infestation conditions adopted in the present study (ticks released inside muslin sleeves glued to the ears) (Tables 1 and 2). Labruna et al. (2004a) reported lower performance for *A. dubitatum* larvae and nymphs fed on chickens, guinea pigs and a wild rodent (*Calomys callosus*), however ticks were freely released on the back of these hosts (i.e. no sleeves were used to avoid ticks from escaping). The results presented in Table 2 clearly show that rabbits were not a suitable host for females of *A. dubitatum,* in contrast to capybaras. So far, a suitable laboratory host for the adult stage of *A. dubitatum* has not been reported.

The daily drop-off percentages of larvae and nymphs feeding on rabbits were quite similar. Peak drop-off concentrated on days 4 – 7 and 5 – 8, respectively, with approximately 93% of recovery for larvae and 83% for nymphs (Table 3). Chacon et al. (2004) have found an increase in daily drop-off pattern in older unfed larvae which were 35 to 45 days. The daily percentage of ecdisis for nymphs and females concentrated on days 16 to 19 and 17 to 21, respectively, and was higher than 70% for both stages. Although the recovery of approximately 82% of females was also similar to the immature, the daily drop-off concentrated on the 7th day post infestation (Table 4). Shortening of daily drop-off is an important feature in colony maintenance for cutting labor efforts and costs. Contrary to the daily drop-off percentage, an increase in the daily ecdisis percentages were seen in larvae as in nymphs.

The total average life cycle of *A. dubitatum* fed on capybara [189.4 days, according to Labruna et al. (2004a)] was slightly shorter when rabbits were used as hosts (216.7 days). Labruna at al. (2004a) have shown that *A. dubitatum* engorged nymphs secreted blackish drops through their cuticle during the premolt period. Interestingly, these blackish drops were not seen in the present study. The reasons for such differences are unknown. Engorged larvae and nymphs fed on rabbits weighed 30 to 40% less than those fed on their natural host, capybara (Labruna et al., 2004a) (Table 1). Engorged females fed on capybaras (Labruna et al., 2004a) were almost 10 times heavier than those fed on rabbits. These differences reflect the strong tick-host interaction that exists between *A. dubitatum* and its natural host, capybaras. On the other hand, even though rabbits are not natural hosts for *A. dubitatum,* it was verified showed that they could be successfully used as laboratory hosts for infestation with immature ticks providing that infestations are conducted inside muslin sleeves as it has done. The role of *A. dubitatum* in the transmission of rickettsioses to humans has recently increased in Brazil (Lemos et al. 1996, Labruna et al., 2004b); the present work indicates that rabbits can be adopted as a laboratory host for the study of the *A. dubitatum*-

Rickettsia interaction, considering the tick immature stages.

Keywords**:** rabbit, *Amblyomma dubitatum*, Ixodidae, Acari, life cycle, experimental host

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RESUMO

O ciclo biológico do Amblyomma dubitatum, *que se alimentava experimentalmente em coelhos domésticos, foi avaliado em condições laboratoriais e comparado com dados recentemente obtidos de infestações experimentais em capivaras* (Hydrochaeris hydrochaeris)*, hospedeiros naturais para essa espécie. Os coelhos foram considerados hospedeiros experimentais adequados para larvas e ninfas porque quatro dos cinco parâmetros avaliados (número de carrapatos que ingurgitaram, período de alimentação, percentagem de carrapatos que realizaram muda e período de pré-muda) foram semelhantes aos resultados obtidos com capivaras. As percentagens diárias de desprendimento e de muda das larvas e ninfas confirmam os coelhos como hospedeiros experimentais adequados para esses estágios de* A. dubitatum. *Os resultados do experimento indicam que os coelhos são hospedeiros inadequados para os adultos.*

Palavra-chave: coelho, Amblyomma dubitatum, *Ixodidae, Acari, ciclo biológico, hospedeiro experimental*

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