

**DIFFERENCES IN THE ACQUIRED RESISTANCE OF DOGS, HAMSTERS, AND GUINEA PIGS TO  
REPEATED INFESTATIONS WITH ADULT TICKS *Rhipicephalus*  
*sanguineus* (ACARI: IXODIDAE)**

**DIFERENÇAS NA RESISTÊNCIA ADQUIRIDA DE CÃES, HAMSTERS E COBAIAS A  
INFESTAÇÕES REPETIDAS POR CARRAPATOS *Rhipicephalus*  
*sanguineus* (ACARI:IXODIDAE) ADULTOS**

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**SUMMARY**

Tick-bite naive experimental groups of dogs, hamsters and guinea pigs were infested three times with adult ticks *Rhipicephalus sanguineus* and the acquired resistance, based on the variation of some biological parameters of the female tick was compared. The results showed that hamsters and mainly guinea pigs develop a very efficient immunity to this tick species as demonstrated by a very significant drop in the efficiency rate of female ticks in converting their food reservoir to eggs and larvae from the first to the second and third infestations. At the same time, dogs were unable to display such a resistance. Female tick performance was similar throughout the infestations in this host; there was even a tendency of improvement of the performance with the succession of infestations. These results underline the need of comparative studies on the acquired resistance to ticks involving natural and unnatural hosts as a way of putting in evidence defence mechanisms which might be altered or hidden in natural host - parasite relationships.

**UNITERMS:** *Rhipicephalus sanguineus*; Resistance; Dogs; Hamsters; Guinea pigs; Metastigmata

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**INTRODUCTION**

Ticks have long been regarded as constraints to human welfare and that of domesticated animals.

Apart from their capacity of blood-sucking and damaging tissues they attach to, ticks are vectors of many important diseases such as piroplasmosis, east coast fever, Lyme disease and many others. So it would be most desirable to effectively control this arthropode. Currently this is being done with the aid of acaricides, but due to their toxicity, high cost and appearance of drug resistant parasite strains, alternative control measures are needed.

Induction of resistance to ticks in hosts seems to be a promising alternative and has been widely studied, mainly in the second half of this century. But, even though most studies demonstrated that there is a naturally acquired resistance in many hosts (TRAGER<sup>10</sup>,1939; RIEK<sup>16</sup>, 1962; GEORGE et al.<sup>11</sup>, 1985) this is far from being a rule. A good example of that is the dog tick *Rhipicephalus sanguineus* natural relationship, which seems to be characterized by failure of the host in developing resistance to that parasite (CHABAUD<sup>6</sup>,

1950; GARIN; GRABAREV<sup>10</sup>, 1972). Similar situation was observed by RANDOLPH<sup>13</sup> (1979) involving *Ixodes trianguliceps* and its natural host, the field mice *Apodemus sylvaticus*. These observations put in evidence parasite specificity for hosts which involves a very effective host resistance manipulation. Whithout any doubt, it is an interesting balance of power between the host and its parasite and may the knowledge of the involved elements coul allow us to use them the other way round, and raise an effective resistance against ticks.

Therefore the aim of this investigation was to, as a basic step for further studies, observe the development of resistance to the tick *Rhipicephalus sanguineus* in hamsters and guinea pigs and compare it to that of dogs.

**MATERIAL AND METHOD**

**Ticks:** a *Rhipicephalus sanguineus* tick colony was set up in the laboratory in order to supply the experiments with infed adult ticks. Initially, engorged females were collected from dogs of the Veterinary School Hospital in Jaboticabal, São Paulo, Brasil. Once identified, they were kept under constant

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temperature and relative humidity conditions of 29°C and 80%, respectively.

Continuous tick supply was then given by feeding each stage on tick-bite naive guinea pigs.

**Hosts:** three animal species were used in these experiments: three mongrel dogs (*Canis familiares*); a group of five hamsters (*Mesocricetus auratus*) and five guinea pigs (*Cavia cutleri*). The first one was used for being the natural host of *Rhipicephalus sanguineus* ticks and the other two for being quite commonly used laboratory animals. Every animal was tick-bite naive. For this purpose we had to breed dogs in a tick-free room. Guinea pigs weighed about 500 g and hamsters about 120 g at the beginning of the experiments. All rodents were female. One female and two male dogs of about 6 kg and five month old each were used. Water and food were given at "ad libitum" consumption.

**Infestations:** each animal group was subjected to three successive infestations using unfed adult *Rhipicephalus sanguineus* ticks. Every infestation, one month apart, consisted of four female and five male ticks in the case of rodents and 25 females and 30 males on every dog. Ticks were placed inside a feeding chamber consisting of a plastic tube with 2.5 cm of diameter and 3.0 cm of height, glued, on the previous day, to the shaved back of the hosts. Chambers placed on dogs had twice this diameter. Neck collars were also used to prevent grooming. In order to avoid the escape of ticks during the experiments, hosts were kept in cages placed in trays surrounded by a gutter filled with water and oil. Daily observations were performed on some biological parameters of the female ticks.

**Biological parameters:** the following biological parameters, related to female tick feeding and reproductive performance, were observed during each infestation: engorged female (FW) and egg mass (EW) weights, engorging, pre-oviposition and incubation periods, larval hatchability rates (LH) and efficiency rates of female ticks in converting their food reservoir to eggs (ERCE) and larvae (ERCL). Female weight was measured immediately after tick detachment. Egg mass was weighed 15 days after tick detachment, as some preliminary observations demonstrated that there was no significant increase in this parameter after such a period of time. The engorging period was assumed to be the time that elapsed since the liberation of ticks on the hosts till their detachment, partially or fully engorged; pre-oviposition, the time from detachment until beginning of oviposition and incubation period the time from beginning of oviposition till the beginning of hatching of larvae. The larval hatching rate for each tick was obtained by the mean value of visual evaluation performed by three persons separately. The effi-

ciency rates of conversion to eggs and to larvae were calculated as follows:

$$ERCE = \frac{EW \times 100}{FW}$$

$$ERCL = \frac{EW \times LH}{FW}$$

**Statistics:** means of the biological parameters for the three groups were compared by the Tukey's test ( $P < 0.05$ ).

## RESULTS

**Tick colony:** Every stage of the tick *Rhipicephalus sanguineus* attached readily to tick-bite naive guinea pigs inside the feeding chamber, detaching as soon as they were fully engorged. This way it was possible to produce a large amount of unfed adult ticks.

**Infestations:** The results of the infestations are summarized on Fig. 1 to 3. The statistical analysis was performed to compare differences between data obtained from ticks fed on dogs and the ones fed on rodents in each infestation.

The mean engorged weight of female ticks of the first infestation (Fig. 1a) was not significantly different among the three host species. But from the second infestation on, a very significant drop in the weight of female ticks fed on hamsters and guinea pigs was noticed, if compared to data obtained from the dogs. In hamsters, the weight of the female ticks was very close in infestations two and three. Ticks fed on guinea pigs, on the other hand, had another drop in the weight from infestation two to three. At the same time, ticks fed on dogs maintained their weights throughout the experiment, and there even was a slight increase in this parameter in infestations two and three.

The mean egg mass weights of female ticks (Fig. 1b) had a variation similar to that observed for engorged female weight. However, immediately in the first infestation, there was a significant difference between egg mass weights of ticks fed on dogs and guinea pigs, where egg masses of ticks from guinea pigs showed less weight.

Ticks fed on dogs engorged in six days in all infestations (Fig. 2a) but there was a slight and continuous decrease of some hours in the mean engorging period from the first to the third infestation. Ticks obtained from guinea pigs, on the contrary, took, already in the first infestation, almost two days more than dogs to engorge, and double of the time in second and third infestations. Hamsters had an initial engorging time of

### Engorged female weight

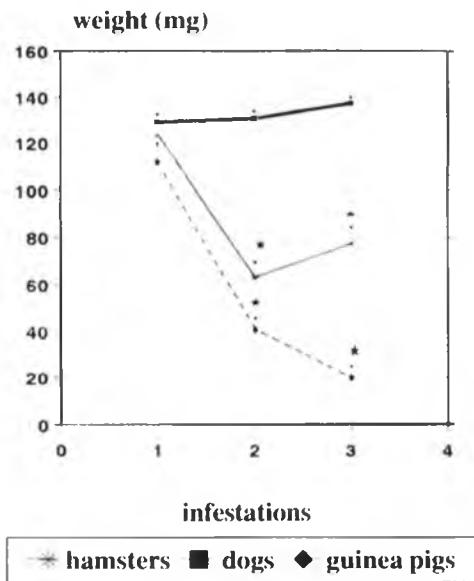


FIGURE 1a

Effect of three consecutive infestations on engorged female weights of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

### Engorging period

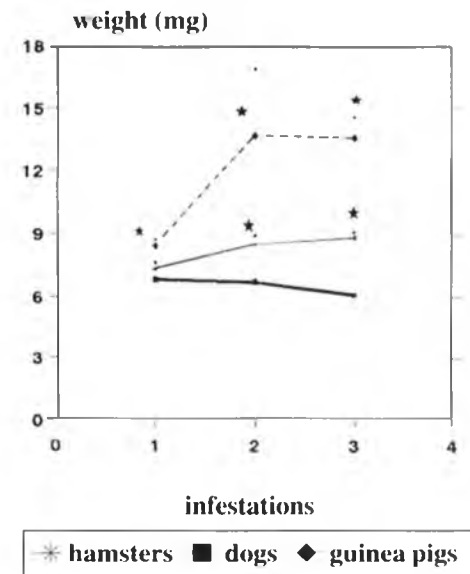


FIGURE 2a

Effect of three consecutive infestations on engorging period of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

### Egg mass weight

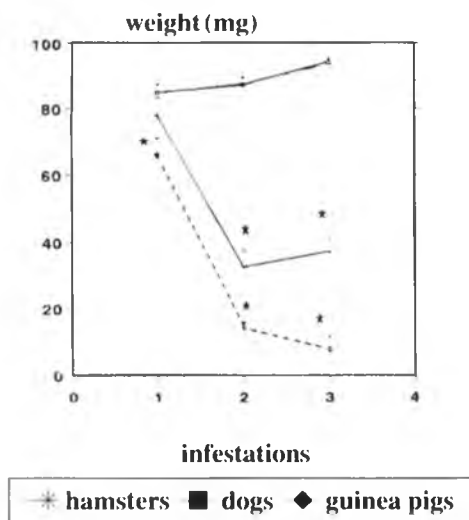


FIGURE 1b

Effect of three consecutive infestations on the egg mass weights of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

seven days increasing to eight and a half in the second and third infestations. Apart from the first infestation of hamsters, every result was considered significantly different from data obtained from dogs.

The mean pre-oviposition periods of ticks fed on the three host species (Fig. 2b) were quite similar and constant, except for the third infestation in guinea pigs, when ticks took 50% more time to start oviposition. In spite of the similarity of most results, they were considered significantly different from the statistic view.

Likewise in the last parameter, incubation periods (Fig. 2c) were very uniform throughout the experiments, with only a small increase (10%) during third infestation of guinea pigs, if compared to dogs' data, but again all results were considered significantly different.

Larval hatchability rate (Fig. 3a), again displayed similar and constant results in ticks from the three host species except for data from the third infestation of guinea pigs when this parameter represented only 67% of the mean obtained from ticks of dogs during third infestation.

Efficiency rates of female ticks in converting their food reservoir to eggs or larvae (Fig. 3b, 3c) showed very similar patterns throughout all infestations. Ticks from dogs did not

### Pre - oviposition period

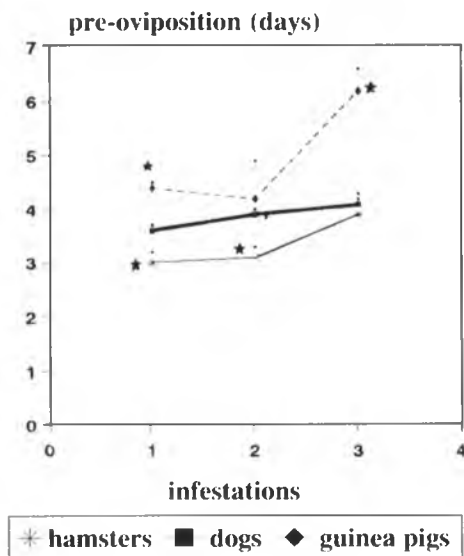


FIGURE 2b

Effect of three consecutive infestations on pre-oviposition period of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

### Larval hatchability rate

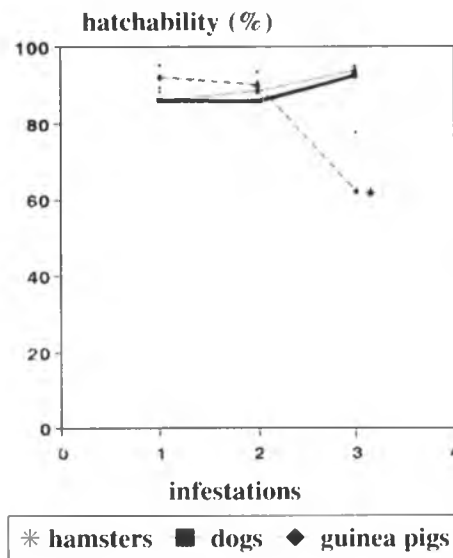


FIGURE 3a

Effect of three consecutive infestations on larval hatchability rate of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

### Incubation period

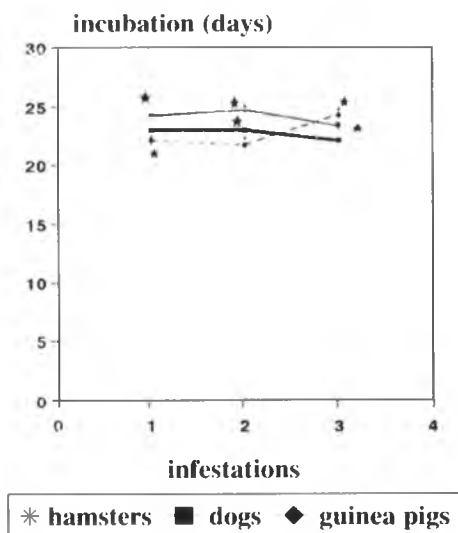


FIGURE 2c

Effect of three consecutive infestations on incubation period of the tick *Rhipicephalus sanguineus* fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \*statistically different ( $p < 0.05$ ).

### ERCE (%)

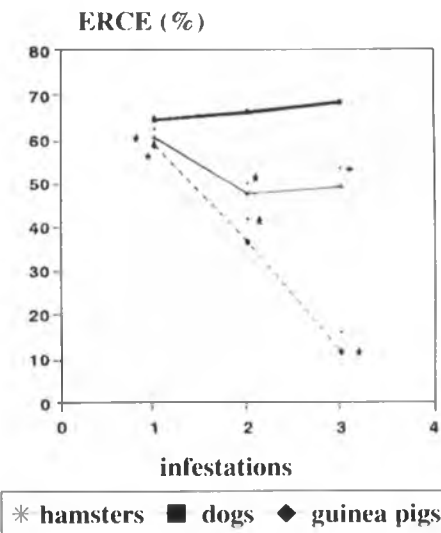


FIGURE 3b

Effect of three consecutive infestations on efficiency rate of female *Rhipicephalus sanguineus* ticks in converting their food reservoir to eggs when fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \* statistically different ( $p < 0.05$ ).

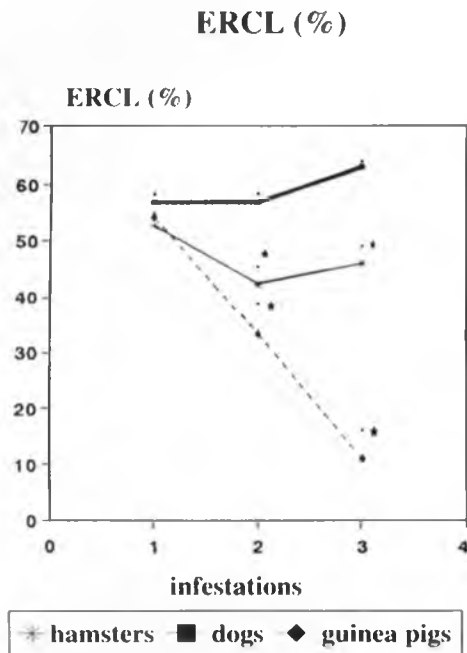


FIGURE 3c

Effect of three consecutive infestations on efficiency rate of female *Rhipicephalus sanguineus* ticks in converting their food reservoir to larvae when fed on hamsters, dogs or guinea pigs. Results are expressed as means  $\pm$  s.e.m. \* statistically different ( $p < 0.05$ ).

alter these parameters with the succession of infestations, although a tendency to increase was detected.

Ticks from guinea pigs, on the contrary, had an intense and continuous decrease in these parameters, displaying a difference of 83% in both, when compared to data obtained from dogs in the third infestation. Ticks from hamsters, at the same time, had a less intense drop in these values, which were only 28% smaller than to that of dogs following the third infestation.

Apart from the results described above, some other interesting observations were made. Ticks, once inside the feeding chamber, attached in a few hours to dogs and hamsters and engorged normally. The loss of ticks, in these cases, was mainly related to accidental escape (Tab. 1). Ticks put on guinea pigs for the second and third infestations, however, showed an unwillingness to attach and to feed; many died without oviposition, and some of them only engorged partially, others engorged partially, detached and became dark before death. There were ticks which would not attach at all, wandering around inside the feeding chamber. The ones which did not attach in ten days, during the second infestation, were put on a tick-bite naive guinea pig. In that situation they attached immediately, engorged and achieved weights comparable to those seen during the first infestation.

Tick-bite naive guinea pigs and hamsters were used as control during infestations two and three. These rodents produced ticks with parameters comparable to that of the first infestation.

Another interesting feature was related to hosts' behaviour. While dogs and hamsters did not seem to be bothered by ticks in any infestation, guinea pigs reacted violently to them during second and third infestations; they constantly tried to take off the feeding chamber, and when, after the detachment of the last female, the chamber was taken off, guinea pigs would furiously bite the area where ticks had been or where some males were still attached, causing considerable self-damage.

In dogs, skin was not much affected by ticks. A little oedema could be seen underneath ticks when many attached together. Hamsters had only mild skin reaction to ticks along the infestations, characterized by oedema and a little exudation. Guinea pigs, on the other hand, developed quite strong skin reactions during infestations two and three, with erythema, oedema, exudation and sometimes necrosis.

During the third infestation, hamsters developed a pneumoenteritis and lost weight recovering later on.

TABLE 1

Total number of *Rhipicephalus sanguineus* ticks set free and recovered from hamsters, dogs and guinea-pigs during each infestation. Jaboticabal - SP, 1991.

Host	dogs			hamsters			guinea pigs		
	1	2	3	1	2	3	1	2	3
infestation n°									
n° of ticks									
- set free	87	72	87	21	20	20	20	20	16
engorged*	71	63	70	21	19	18	16	4	12
- laid eggs	70	62	69	21	19	18	16	4	6
recovered**	80	86	81	100	95	90	80	20	37

\* fully or partially engorged

\*\* ticks were considered as recovered if they attached, engorged and laid eggs

## DISCUSSION

Confirming earlier observations by CHABAUD<sup>6</sup> (1950); GARIN; GRABAREV<sup>10</sup> (1972); THEIS; BUDWISER<sup>18</sup> (1974), our results indicated that dogs do not develop a perceptible resistance to ticks, *Rhipicephalus sanguineus*. At the same time guinea pigs developed a very effective resistance. Hamsters were not as effective as guinea pigs in

expressing resistance, although they developed a partial one. The less evident differences were observed during the first infestation. Better performance of ticks in tick-bite naive hosts is a quite well known phenomenon (TRAGER<sup>19</sup>, 1939; WIKEL; ALLEN<sup>24</sup>, 1976; ABDUL-AMIR<sup>1</sup>, 1987), even though some minor differences could already be seen in this infestation, as for example, significantly lower weights of the egg mass of ticks fed on guinea pigs. These initial differences are rather difficult to explain and probably involve hosts' features such as skin and hair coat types, sebaceous gland and mast cell density and other factors which are characteristic to each species of host and which were defined by BROWN<sup>3</sup> (1988) as being innate resistance attributes to ticks.

From the second infestation on, differences related to tick performance fed on each host species were much more evident. This, of course, can be explained by the presence, on resistant animals (guinea pigs and hamsters), of much more effectively damaging elements as a result of a more specific reaction modulated by the immune system. The manifestation of this resistance was similar to the ones described previously: CHIERA et al.<sup>7</sup> (1985) observed a decrease in females' and egg mass weights of ticks *Rhipicephalus appendiculatus* fed on resistant bovines; BROWN et al.<sup>5</sup> (1984) described that ticks *Amblyomma americanum* became darker and died when fed on pre-sensitized bovines, and BROWN; ASKENASE<sup>4</sup> (1981) reported that although ticks *Rhipicephalus sanguineus* and *Amblyomma americanum* could engorge on previously infested guinea pigs they weighed less, had altered colours and the mean engorging period had enlarged. These types of alterations reflect damage to female digestive and reproductive systems but they might also reflect deleterious action upon male ticks. According to BROWN et al.<sup>5</sup>(1984) impaired fertilization and fecundity of females may as well be consequence of males being also adversely affected by resistance. THEIS; BUDWISER<sup>18</sup> (1974) suggest that extended engorging periods can be caused by failure in female's fertilization.

Another important expression of resistance was given by efficiency rates of female ticks in converting their food reservoir to eggs. Not only did female ticks from resistant hamsters and mainly guinea pigs weigh less but also put proportionally less egg mass per weight. These rodents displayed great efficiency in reducing tick's reproductive capacity.

During third infestation ticks from hamsters had similar performance to those of the second one. Although this situation has already been described by WHELEN et al.<sup>21</sup>, (1986) who observed that female weights of ticks *Dermacentor andersoni* were similar during infestations two, three and four but lower than from the first, in our case the pneumoenteritis developed by the hamsters during third

infestation could have impaired immunity resulting in a better performance of ticks. According to LATA et al.<sup>12</sup>, (1986) female ticks *Boophilus microplus* inoculate antigens, toxins and toxic metabolites while engorging, and WEBSTER; MITCHELL<sup>20</sup> (1989) suggest that ticks may vector piogenic bacteria. Both could well have impaired hamsters' immunity. This is true mainly if we consider this host's lower body weight and number of ticks engorging on them. Guinea pigs' death when too many larvae or nymphs were feeding on them (personal observation) could be explained in the same way. Guinea pigs, on the other hand, had an improvement in the resistance from infestation two to three too.

Dogs, at the same time, were not able to develop an effective resistance throughout the infestations. Biological parameter data were characterized by high repetitivity in and among infestations. Although failure in the development of resistance against ticks is not commonly described in literature, similar results, involving dogs and *Rhipicephalus sanguineus* ticks, were obtained by CHABAUD<sup>6</sup> (1950); GARIN; GRABAREV<sup>10</sup>(1972); and by RANDOLPH<sup>13</sup> (1979) who infested *Apodemus sylvaticus* field mice with its usual tick, the *Ixodes trianguliceps*.

Not only ticks from dogs have any drop in their engorged and egg mass weights in infestations two and three but they also had an increase in these parameters. Although these increases were slight, we must keep them in mind as they might indicate a facilitating effect on tick feeding from one infestation to another. This becomes even more intriguing if compared to results obtained from guinea pigs and hamsters, where drastic drops occurred.

A doubt that may arise is whether the number of ticks used on dogs would be sufficient to elicit an immune response. ASKENASE et al.<sup>2</sup> (1982) observed that a single female tick *Ixodes holocyclus* fed on a guinea pig for just four hours was able to induce some resistance to subsequent infestations. In our case 60 to 70 ticks (male and females) attached and inoculated their secretion products for about six days. We considered this enough for an antigenic stimulation.

There are many experimental evidences which might give a clue to dogs' failure in eliciting resistance to this tick; THEIS<sup>17</sup> (1968) observed that this tick species has a very superficial attachment, its hypostome does not reach deeper layers of the skin, which might induce less intense reactions. Another interesting feature in the presence of a histamine blocking agent in the *Rhipicephalus sanguineus* tick's salivary gland (CHINERY; AYITEY<sup>8</sup>, 1977) which might be involved in the dog's tolerance, but its exact role is still unknown. It also seems that tick saliva may have an antiinflammatory effect; RIBEIRO et al.<sup>14</sup> (1985) and RIBEIRO; SPIELMAN<sup>15</sup> (1986) observed that the saliva of the tick

*Ixodes dammini* can rapidly inactivate inflammatory mediators such as bradykinin and anafilatoxins. But maybe the most studied feature involving resistance is the immunosuppression induced by ticks. WIKEL<sup>22</sup> (1982) noticed that lymphocytes of guinea pigs infested with ticks *Dermacentor andersoni* showed in vitro significantly less responsiveness to the T-lymphocyte mitogens concanavalin A and phytohaemagglutinin, but stimulation by the B-lymphocyte mitogen *Escherichia coli* lipopolysaccharide was unimpaired. The same author (WIKEL<sup>23</sup>, 1985) demonstrated that guinea pigs' responsiveness to a thymic dependent antigen, sheep red blood cell, was reduced as a result of *Dermacentor andersoni* tick infestation, and FIVAZ<sup>9</sup> (1989) observed that immunosuppression in rabbits infested with the brown ear tick *Rhipicephalus appendiculatus* was caused by putative lymphocytotoxic factor(s) in tick salivary secretions, as evidenced by in vitro lymphocytotoxicity assays.

In spite of these evidences, our results involving guinea pigs and hamsters prove that *Rhipicephalus sanguineus* tick is able to induce a very strong resistance. The absence of an effective resistance in dogs must be centered on this host's particular characteristics or in the evolution of highly specific immunosuppression or evasion mechanisms in ticks *Rhipicephalus sanguineus* to dogs.

Thus, the biological parameters of the ticks obtained after infestations are truly the manifestation of the resultant coming out from the interaction of evasion and immunosuppressive elements of the parasite and reaction of the host. For a full understanding of these elements, separated analysis of each is desirable and a first step was, precisely, making comparative infestations using natural and natural hosts. The unnatural were to put in evidence mechanisms which are suppressed or altered in the natural host-parasite relationship. These differences between a natural and unnatural host became very evident in our results. Further comparative studies involving

tick attachment-site histopathology, resistance induction by the inoculation of tick antigens, immuno-characterization of tick antigens, tick antigen target search using immunohistochemical techniques and lesions on the arthropodes fed on resistant hosts will certainly contribute to a better understanding of mechanisms involved in this host-parasite relationship.

## CONCLUSIONS

1. Dogs seem not to develop resistance to adult tick *Rhipicephalus sanguineus* during repeated infestations; contrarily, guinea pigs develop a very strong resistance and hamsters acquire only a partial resistance to the same tick species on repeated infestations;
2. Resistance to these ticks was characterized by drop in engorged female and egg mass weights, prolonged engorging period, lower efficiency rate of conversion of the food reservoir to eggs and larvae, unwillingness of female ticks to feed and death of ticks before oviposition;
3. The tick *Rhipicephalus sanguineus* is very well adapted to its host, the dog, and it must have highly specific evasion and/or immunosuppressive mechanisms concerning its host.

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## SUMÁRIO

Grupos experimentais de cães, hamsters e cobaias sem contato anterior com carrapatos, sofreram três infestações consecutivas por carrapatos *Rhipicephalus sanguineus* adultos e a resistência adquirida comparada com base na variação de alguns dos parâmetros biológicos da fêmea do carrapato. Os resultados mostraram que hamsters e cobaias principalmente, desenvolvem uma reação imune muito eficiente a esta espécie de carrapato como demonstrado por uma queda altamente significativa na taxa de eficiência da fêmea em converter sua reserva alimentar em ovos e larvas da primeira para as segunda e terceira infestações. Já cães foram incapazes de desenvolver tal resistência. Neste hospedeiro a performance das fêmeas dos carrapatos foi similar durante todas as infestações, havendo até uma tendência de melhora com a sucessão das infestações. Estes resultados reforçam a necessidade de estudos comparativos sobre a resistência adquirida a carrapatos, envolvendo hospedeiros naturais e não naturais, como forma de colocar em evidência mecanismos de defesa que possam estar alterados ou ocultos em relações parasita-hospedeiro naturais.

UNITERMOS: *Rhipicephalus sanguineus*; Resistência; Cães; Hamsters; Cobaias; Carrapatos

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