RHIPICEPHALUS SANGUINEUS TICK IN BRAZIL: FEEDING AND REPRODUCTIVE ASPECTS UNDER LABORATORIAL CONDITIONS.

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SUMMARY: A *Rhipicephalus sanguineus* tick colony was established at the Department of Animal Pathology of the São Paulo State University-UNESP, Jaboticabal, Brazil. Feeding and reproductive parameters of adult female *R. sanguineus* ticks fed on four different hosts - domestic dog (*Canis familiaris*), crab-eating-fox (*Cerdocyon thous*), hamster (*Mesocricetus auratus*) and guinea pig (*Cavia cutleri*) - are displayed. Data on some biological parameters of immature forms fed on tick-bite naive guinea pigs is also shown. It was concluded that, under laboratorial conditions (29°C, 80% relative humidity, 12 h photoperiod), the *R. sanguineus* tick life cycle, Brazilian strain, may be completed in approximately 90 days.

KEY WORDS: tick, *Rhipicephalus sanguineus*, dog, crab-eating-fox, hamster, guinea pig, biological parameters.

INTRODUCTION

The hard tick *Rhipicephalus sanguineus*, commonly referred to as the "kennel tick" or "brown dog tick", is a cosmopolitan parasite and probably the most prevalent of ixodid species (PEGRAM *et alii*, 1987; SWEATMAN, 1967). *R. sanguineus* is widely distributed in the Americas, Europe, Africa, Asia and Australia. In Africa it is predominantly a parasite of carnivores but may also infest man and, rarely, domestic livestock. Elsewhere, it is confined almost entirely to domestic dogs (PEGRAM *et alii*, 1987). In fact, the dog is considered this tick species' natural host (HOOGSTRAAL, 1956; THEIS & BUDWISER, 1974).

The tick *R. sanguineus* has been linked to many diseases (HOOGSTRAAL, 1956; SWEATMAN, 1967; PARKER & WILSON, 1979; STEPHEN & ACHYUTHARAO, 1980; PÉTER *et alii*, 1984; FOX & SYKES, 1985) and apparently does not induce resistance in its host, the dog, even after repeated feeding (CHABAUD, 1950; GARIN & GRABAREV, 1972; THEIS & BUDWISER, 1974, SZABÓ *et alii*, 1995).

Domestic dogs in São Paulo State, Brazil, suffer heavy *R. sanguineus* infestations (SZABÓ, personal observation), but not much is known about this tick species' biology in Brazil. In 1989, a *R. sanguineus* tick colony was established at the Department of Animal Pathology of The São Paulo State University-UNESP, Jaboticabal, Brazil, to supply tick-host resistance experiments with unfed adult ticks.

The present work shows some data about biological parameters of the *R. sanguineus* ticks from this colony, under laboratory conditions. The aim of the work is to display some basic information about the biology of the *R. sanguineus* tick in Brazil.

MATERIALS AND METHODS

All observations below were taken from the tick colony itself or from control animals of various experiments performed since 1989 (BECHARA *et alii*, 1994; SZABÓ *et alii*, 1995; FERREIRA & BECHARA, 1995). Control animals refer to those that had not been vaccinated with tick extracts and were not treated with acaricides nor immunomodulators.

Ticks

A *R. sanguineus* tick colony was established by collecting ticks from healthy dogs of the Veterinary College Hospital in Jaboticabal, Brazil. Once identified, they were

kept under a constant temperature of 29°C±2, a relative humidity of 80±5% and a 12:12 (L:D) photoperiod. Continuous tick supply was then given by feeding nymphs and larvae on tick-bite naive guinea pigs and adults on dogs or tick-bite naive guinea pigs.

Tick identification

Adult *R. sanguineus* ticks were identified according to THEIS (1968) and FREITAS *et alii* (1978) as follows: ticks of this species are brown, the stigma plate is comma-shaped in both sexes; the base of the rostrum (capitulum) has an hexagonal appearance when viewed from the dorsal side; ventrally, coxa I is split by a long cleft; the anal groove is distinct and curves around the anus posteriorly. Males have an adanal shield on either side of the anus, and on either side of the adanal shield there may be a bluntly pointed adanal shield; scutum extends over the entire dorsal surface. In females there are no adanal shields and dorsally there is scutum only over the anterior portion of the tick.

Hosts

Four animal species were used: a) five male and seven female mongrel dogs (*Canis familiares*); b) four male and one female crab-eating-foxes (*Cerdocyon thous*); c) five female hamsters (*Mesocricetus auratus*) and d) 46 female albino guinea pigs (*Cavia cutleri*). Dog was used for being considered *R. sanguineus* tick's natural host, crab-eating-fox for being a wild Brazilian carnivore, close relative of the domestic dog, and the rodents for being commonly used laboratory animals in tick-host resistance experiments. Rodents and three dogs were tick-bite naive at the beginning of experiments.

Dogs weighed six to 20 kilograms and were five months to two years old. Foxes weighed four to eight kilograms and were of unknown age. Guinea pigs weighed about 500g and hamsters about 120g at the beginning of the experiments. Water and commercial food were given "ad libitum" to each animal. Crab-eating foxes also received fruits, vegetables, eggs and meat once a day. Guinea pigs and hamsters were supplied by The São Paulo State University's Central Bioterium (Botucatu, SP) and dogs were brought up locally, or kindly lent by owners. Crab-eating-foxes were kindly lent by Leme's and Sorocaba's zoos (São Paulo, Brazil).

Infestations

Animals were subjected to either one or three successive infestations using unfed adult *R. sanguineus* ticks. Every infestation, one month apart, consisted of 4 female and 5 male ticks in the case of rodents and 25 females and 30 males on every dog or fox. Ticks were placed inside a feeding chamber consisting of a plastic tube (2.5 cm of diameter and 3 cm of height) glued, on the previous day, with an atoxic and non-lesive preparation (Britannia Adhesives-Unit 4), to the shaved back of the hosts. Tubes placed on dogs and foxes had twice this diameter. When needed, Elizabethan neck collars were also used on animals to prevent grooming.

In order to avoid the escape of ticks during experiments, hosts were kept in cages placed in trays surrounded by a gutter filled with water and oil. Special cages were used for the handling of foxes.

Tick-bite naive guinea pigs (n=14) were also infested with batches of nymphs (one egg mass derived) or larvae (14 guinea pigs). Daily observations were performed on some biological parameters of the ticks.

Biological parameters

The following biological parameters, related to female tick feeding and reproductive performances, were observed during each infestation: engorged female and egg mass weights, engorgement, pre-oviposition and incubation periods and larval hatchability rates.

Female weight was measured as soon as ticks detached, partially or fully engorged. Egg mass was weighed 15 days after tick detachment as some preliminary observations at our laboratory demonstrated that there was no significant increase in this parameter after that period of time.

The engorgement period was assumed to be the time that elapsed since the release of ticks on the hosts till their detachment partially or fully engorged; pre-oviposition, the time that passed by from detachment of the female tick until beginning of oviposition and the incubation period the time from the 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.

Engorged nymphs and larvae were counted and then weighed in batches and engorgement and moulting periods observed. Engorgement period of nymphs and larvae was assumed to be the time that elapsed since the release of unfed ticks on the hosts till the detachment of most of them and the moulting period the time needed for moulting of the first tick since detachment of the first tick.

RESULTS

Tick colony

Nymphs and larvae of the tick *R. sanguineus* attached readily to tick-bite naive guinea pigs inside the feeding chamber, detaching fully engorged. Adults engorged on both dogs and tick-bite naive guinea pigs, but attached to dogs faster. This way it was possible to produce a large amount of larvae, nymphs and adults of *R. sanguineus* ticks.

Infestations

Ticks, once inside the feeding chamber, attached in a few hours to dogs, foxes and hamsters and engorged normally. A tendency of ticks to fix and feed close to each other, in group, was observed. Ticks put on guinea-pigs for the second and third infestations, however, showed an unwillingness to attach and to feed; many died without ovipositing, 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.

In one experiment, ticks which did not attach in ten days, during the second infestation, were put on a tick-bite naive guinea pig. In this situation they attached immediately, engorged and achieved weights comparable to those seen during the first infestation.

Biological parameters

Biological parameters of adult female *R. sanguineus* ticks fed on dogs, crab-eating-foxes, guinea pigs and hamsters are shown on Tables 1 and 2.

As previous experiments at our laboratory demonstrated that female *R. sanguineus* ticks develop similarly on tick-bite naive or on infested dogs (SZABÓ *et alii*, 1995) and crab-eating-foxes (FERREIRA & BECHARA, 1995), data from all infestations on these carnivores are shown together.

As guinea pigs and hamsters develop resistance to ticks following a single infestation (SZABÓ *et alii*, 1995), data from tick bite-naive rodents, under first infestation, are shown separately.

Biological parameters of *R. sanguineus* larvae and nymphs fed on tick-bite naive guinea pigs are shown on Table 3.

A total of 26 infestations on dogs and 13 on foxes were performed. Recovery rate of female ticks from dogs was of 82.8% (in average 20.7 engorged female ticks out of 25 unfed per infestation) and from foxes of 78.8% (in average 19.7 engorged female ticks out of 25 unfed per infestation). Some tick loss occurred due to accidental escape. A mean of 2.3 and 1.5 female ticks from dogs and foxes, respectively, died without ovipositing per infestation. These ticks

Table 1 - Biological parameters of *R. sanguineus* ticks fed on domestic dogs and crab-eating-foxes

	dog (n=12)	fox (=5)
FW (mg)	126 ± 27 (538)	128 ± 25 (256)
EMW (mg)	76 ± 27 (478)	81 ± 30 (236)
EP (days)	7.3 ± 1.7 (538)	8.7 ± 1.6 (253)
POP (days)	4.2 ± 1.4 (432)	4.3 ± 2.1 (238)
IP (days)	22.9 ± 3 (404)	24 ± 2.2 (224)
LH (%)	90.2 ± 13.5 (359)	83.7 ± 31.2 (235)

FW-engorged female weight; EMW-egg mass weight; EP-engorgement period; POP-pre-oviposition period; IP-egg incubation period; LH-larval hatchability. Results are shown as mean ± standard deviation. Number of repetitions are shown between brackets below x±sd.

Table 2 - Biological parameters of *R. sanguineus* ticks fed on tick-bite naive or previously infested guinea-pigs and hamsters.

	naive guinea pig (n=18)	Infested guinea pig (n=12)	naive hamster (n=5)	Infested hamster (n-5)
FW (mg)	102 ± 3 (60)	26 ± 17 (26)	123 ± 27 (19)	71 ± 28 (41)
EMW (mg)	55 ± 21 (58)	6.6 ± 8 (20)	78 ± 24 (19)	37 ± 21 (41)
EP (days)	9.4 ± 1.8 (60)	13 ± 3.4 (26)	7.3 ± 1.2 (19)	8.8 ± 1.4 (41)
POP (days)	4.5 ± 1.8 (58)	6.8 ± 2 (18)	3.1 ± 0.8 (19)	3.3 ± 1.4 (41)
IP (days)	22 ± 1.6 (58)	21 ± 3.6 (18)	—*	24 ± 1.7 (41)
LH (%)	92.3 ± 12.5 (58)	73.2 ± 31.8 (10)	81.1 ± 23.7 (19)	91.4 ± 8.3 (41)

FW-engorged female weight; EMW-egg mass weight; EP-engorgement period; POP-pre-oviposition period; IP-egg incubation period; LH-larval hatchabilty. Results are shown as mean ± standard deviation. Number of repetitions are shown between brackets below x̄±sd. * incubation period of ticks fed on tick-bite naive hamsters was not observed.

Table 3 - Biological parameters of *R. sanguineus* tick larvae and nymphs fed on tick-bite naive guinea-pigs.

	number of batches	ticks per batch	number of ticks	engorgement period (days)	moulting period (days)	mean weight of ticks (mg)
Larvae	14	586 ± 491	8203	5.0 ± 0.6	11.0 ± 1,5	0.27 ± 0.03
Nymphs	14	167 ± 54	2339	7.4 ± 1.0	22.9 ± 2.9	3.2 ± 0.23

Every batch was fed on one animal. Ticks per batch refer to number of larvae or nymphs that were recovered, engorged. Results are shown as mean ± standard deviation.

usually detached fully engorged but were very distended and moved with difficulty or did not move at all, and became dark and died in one or two days.

Eighteen guinea pigs and five hamsters suffered first infestation (tick-bite naive). A total of 11 and five infestations were performed on previously infested guinea pigs and hamsters, respectively. Recovery rate of female ticks were as follows: naive guinea pigs 66% (in average 3.3 engorged female ticks out of 5 unfed per infestation); naive hamsters 76% (in average 3.8 engorged female ticks out of 5 unfed per infestation); infested guinea pigs 32% (in average 1.6 out of 5) and infested hamsters 74% (in average 3.7 out of 5). Some tick loss occurred due to accidental escape. A mean of 0.1 and 0.4 engorged female ticks from naive guinea pigs and hamsters, respectively, died per infestation without ovipositing. All engorged female ticks that detached from previously infested guinea pigs and hamsters oviposited.

Engorged larvae and nymphs were dark grey. It was observed that some engorged nymphs died (10%) without moulting. These nymphs were usually darker than others.

It has to be mentioned that following moulting and hatching for approximately three days, ticks remained non-proper for infestations. Emerging ticks were light brown, moved slowly and remained at the bottom of the moulting and hatching chambers. Mature ticks, on the other hand, acquired a dark brown colour, and tended to accumulate on the top of the chambers. More adult female ticks emerged than males.

Larvae and nymphs stayed together in batches and adults kept wandering around the chambers isolatedly. Mature ticks moved fast whenever removed from the chambers. Ticks from the colony survived for 3-4 month without feeding. But tick viability for infestations decreased after 45 days.

It can be concluded that *R. sanguineus* tick life cycle may be completed, under laboratorial conditions, in 90 ± 12 (days) if data from dogs (adult ticks) and tick-bite naive guinea pigs (larvae and nymphs) are considered.

Feeding lesions

Tick attachment sites on dogs and foxes were not much damaged. A little skin thickening with oedema and some exudation could be seen underneath ticks when many attached together. Hamsters had only mild skin reaction to ticks along the infestations, characterised by oedema and a little exudation. Guinea pigs, on the other hand, developed quite strong skin reactions during infestations two and three, with erithema, oedema, exudation and sometimes necrosis.

Host reactions

Dogs and hamsters seemed to be bothered by infestations only immediately after tick release inside the feeding chamber when many ticks were moving on its hosts back. Guinea pigs, on the other hand, reacted strongly to ticks 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.

DISCUSSION

Results above demonstrated that *R. sanguineus* tick, Brazilian strain, is able to complete its life cycle in circa 90 days. It should be stressed, however that these results were obtained under laboratory conditions.

Under natural conditions, environment, host availability and behaviour, and natural enemies of ticks will, most probably, elongate the cycle. For the same reasons, tick mortality rate is also expected to be higher outside laboratory.

Guinea pig was shown to be the least suitable host for adult ticks. Already in the first infestation, females engorged on guinea pigs and their egg masses weighed less. Moreover, guinea pigs displayed a strong resistance from second infestation on, when the lowest recovery rate was observed.

Development of resistance to ticks after repeated infestations is a well known phenomenon (reviewed by BROWN, 1988 and RECHAV, 1992). Dogs and foxes, on the other hand, originated the heaviest tick and egg masses, and adult ticks seemed to prefer these hosts as attachment usually occurred sooner. Moreover, these carnivores seemed to react less to *R. sanguineus* as demonstrated by reduced skin reaction and lack of strong

pruritus or pain. These observations are in accordance with previous results by HOOGSTRAAL (1956) and THEIS & BUDWISER (1974), which assume that dogs are natural hosts of the tick *R. sanguineus*.

According to RANDOLPH (1979), lack of resistance against parasites is a common feature of natural host-parasite relationships and the few natural (original) tick host relationships studied seem to be characterised by lack of resistance of the host to the tick even after repeated infestations (CHABAUD, 1950; RANDOLPH, 1979; FIELDEN & RECHAV, 1992). Crab-cating-foxes and domestic dogs seem to share physiological features to such an extent that ticks perform similarly on them. This is not surprising once these carnivores can be considered close relatives. Thus, *R. sanguineus* is expected to adapt easily to other Brazilian carnivores such as *Dusicyon australis and Chrysocyon brachyurus*.

Larvae and nymphs were fed on tick-bite naive guinea pigs to simplify host handling in the laboratory. But, although adult *R. sanguineus* ticks feed rather on dogs, small wild vertebrates (rodents) are common hosts of immature forms of three-host ticks (AESCHLIMANN, 1991). Thus, not much interference is expected when tick-bite naive guinea pigs are used.

The cause of death of females fed on dogs and foxes without oviposition is unknown to us and its occurrence will be further investigated. Anyhow, tick recovery rate, feeding and reproductive performance of ticks fed on the carnivores were much higher than those fed on the rodents, mainly if resistance acquisition is also considered.

In summary, results presented here present some data about the biology of the tick *R. sanguineus* in Brazil, information which seems to be lacking from local literature. Such knowledge is essential for any tick control trial, such as chemical, immune or biological one. This information is relevant if the world-wide importance of this tick is considered.

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SUMÁRIO

Uma colônia do carrapato *Rhipicephalus sanguineus* foi estabelecida no Departamento de Patologia Animal da Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal-UNESP, São Paulo, Brasil. Parâmetros do desempenho reprodutivo e alimentar de carrapatas *R. sanguineus* adultas, alimentadas sobre cão doméstico (*Canis familiares*), cachorro-do-mato (*Cerdocyon thous*), hamster (*Mesocricetus auratus*) e cobaia (*Cavia cutleri*) são apresentados. Alguns parâmetros biológicos sobre formas imaturas alimentadas em cobaias sofrendo primeira infestação são também mostrados. Concluiu-se que, em condições laboratoriais (29°C, 80% de umidade relativa, 12 h de fotoperíodo), o ciclo biológico do carrapato *R. sanguineus*, amostra brasileira, pode ser completado em cerca de 90 dias.

PALAVRAS-CHAVE: carrapato, *Rhipicephalus sanguineus*, cão doméstico, cachorro-do-mato, cobaia, hamster, parâmetros biológicos.

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