Oviposition and Eclosion Periods of *Ixodes didelphidis* Fonseca and Aragão, 1951 (Acari: Ixodidae) under Laboratory Conditions

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Oviposition and eclosion periods for Ixodes didelphidis *were observed under two temperatures (25ºC and 27ºC) and 90-95% humidity. Although there was a significant increase in the eclosion period ($p<0.05$) and a tendency to increase the oviposition period at 25ºC, there was neither significant differences in the interval (days), until maximum peak of eclosion nor in the number of emerging larvae during the peak nor the total number of emerged larvae. These temperature values are not critical for embryological development of the species. Because at 27ºC and under high humidity the oviposition and eclosion periods are shorter, and the percentage of emerged larvae is higher, we consider this to be the ideal temperature for laboratory studies.*

Key words: *Ixodes didelphidis* - oviposition - eclosion - laboratory conditions

The most recent studies on Brazilian species of the genus *Ixodes*, were carried out by Labruna et al. (1997), on the weight of eggs from six ixodid species from Brazil; Barros-Battesti (1998), on the parasitism of *Ixodes didelphidis* and *I. loricatus* on small mammals; Barros-Battesti and Knysak (1999), on the geographical distribution of Brazilian *Ixodes* species; Arzua and Barros-Battesti (1999), on the relationship between *I.* (*Multidentatus*) *auritulus* and hosts; and Faccini et al. (1999), on the biological characteristics of the nonparasitic phases of *I. amarali*. Nevertheless, the biology of these species is still unknown.

I. didelphidis Fonseca and Aragão, 1951 is a tick species endemic to Brazil, with registered occurrences in the Southern, Southeast and Central-West regions (Barros-Battesti & Knysak 1999). Both sexes are found parasiting marsupials, while immature stages feed on small wild rodents. *I. didelphidis* is a species closely related to *I. loricatus*, but they differ in the morphology of the spiracular plate. Although Morel and Perez (1978) synonymized *I. didelphidis* and *I. loricatus* Neumann, and Camicas et al. (1998) included the species in the *Amerixodes* subgenus, we opted to maintain them as separate species, in *Ixodes* subgenus, until more conclusive studies have been carried out. In addition, the difficulty in maintaining colonies in order to study each development stage is due to the lack of knowledge of physical (temperature and humidity) and biological (feeding adaptation on laboratory hosts) aspects.

In Itapevi county, State of São Paulo, where Lyme-like disease cases are recognized, *I. didelphidis* and *I. loricatus* were found naturally infected by spirochetes and seem to play an important role in their enzootic transmission cycle (Yoshinari et al. 1997, Barros-Battesti 1998, Yoshinari et al. 1999).

The aim of this study was to obtain data on the oviposition period of females and daily eclosion rate of *I. didelphidis* larvae under controlled conditions of temperature and humidity. This would allow an evaluation of the effects of temperature variations (25ºC and 27ºC) on oviposition and eclosion periods, total number of eggs, daily eclosion rate for each oviposition, in order to maintain colonies in laboratory.

MATERIALS AND METHODS

During the first six months of 1999, females of *I. didelphidis* were collected from *Didelphis aurita* Wide-Neuwied (Didelphimorphia: Didelphidae). These were captured monthly from the woods and surrounding areas of a residential condominium in Itapevi county, SP (23º32'45''S and 46º56" 05''W). The area is composed of fragments of disturbed Atlantic Forest. Altitude varies from 715 m

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to 900 m (Ponçano et al. 1981), and the climate is type Cwb (mesothermic with dry winter and mild summer), with an annual rainfall ranging from 1300 to 1500 mm and annual mean temperature ranging from 20ºC to 22ºC.

Seven engorged females of *I. didelphidis*, weighing 350 mg with an average of 1.2 mm length and 0.8 mm width, were identified according to Aragão and Fonseca (1961). These females were kept in transparent vials covered with cloth and maintained in BOD (Biological Oxygen Demand) incubation chamber under high humidity (85-95%), scotophase. Three females and their eggs were exposed to a temperature of 27ºC. The remaining four females and their eggs were kept at 25ºC.

Females were examined daily. Following oviposition, all females were treated according to the technique described by Takada et al. (1994). They were then dissected in sterilized conditions, using tweezers and scalpels, in petri dishes containing 1ml of BSK medium (Barbour 1984) and 3 µl of Kanamicin antibiotic. The material was inoculated into 5 ml of BSK medium and 15 µl of Kanamicin. Cultures were maintained at 33ºC and examined weekly under dark field microscope. This procedure was necessary to exclude the possibility of colonies contaminated with spirochetes by transovarial route.

The oviposition period, total number of eggs, daily number of emerged larvae, interval (days) until maximum eclosion peak was reached, and the total eclosion period obtained in each oviposition, were recorded.

Data were analyzed by means of Mann-Whitney (U) test that used to compare independent samples. Statistical analysis was performed using the SPSS statistical program package.

RESULTS

All females used during the present study were not infected by spirochetes.

The total number of eggs, daily number of emerged larvae, the total eclosion period, and the maximum number of larvae obtained in the peak of eclosion, for each female, are shown on Table I.

The oviposition period (mean ± SD, range), the interval (days) until maximum eclosion peak, and the eclosion period (mean ± SD, range), registered for females exposed at temperature of 27ºC and 25ºC under high humidity, are shown on Table II.

The average percentage of eclosion was 96% (± 4.51) at 27ºC. At the temperature of 25ºC, the average percentage eclosion was 93% (± 1.07). On the first day of oviposition, the number of eggs varied between one and 20. The average number of emerged larvae until the maximum peak was 878 (± 199.10), at 27ºC, and 894.25 (± 187.04), at 25ºC. At both temperatures, it was observed that at 25ºC there was a tendency to increase the oviposition period. Consequently, there was an increase significant in the eclosion periods ($U<0.001$; $N=7$; $P=0.029$) under the same conditions, when they were analyzed by the Mann-Whitney statistical test (Table III).

DISCUSSION

There are evidences that in the environment, ticks remain in the host's burrow after detachment (unpublished data). It is probable that temperature and humidity are relatively constant and higher in these microhabitats. Since nothing is known about ideal conditions for colony maintenance of this *Ixodes* species, both temperatures were used, and the incubation chamber's humidity maintained high, taking into consideration the environmental conditions of the collection area. In a pilot test, when ticks were exposed to 27ºC and 80% humidity, the emergence percentage did not reach 30% and the eggs showed signs of dehydration.

Faccini et al. (1999) observed smaller laying and eclosion periods for an engorged *I. amarali* female weighing 433,5 mg and maintained at 27ºC and a relative humidity higher than 80%. The eclosion percentage registered for this species was of 34%. The low eclosion rate could be explained by the humidity of the incubation chamber and the dehydration of the eggs during manipulation, since these were weighed during the experiment. In the present study this kind of interference was avoided until the end of oviposition, when the females were removed without damaging the eggs. It is likely that this procedure favored the high eclosion rates for *I. didelphidis*.

The length of the oviposition period for each *I. didelphidis* egg was not measured, but both temperatures did not cause a significant difference in the number of eggs laid on the first day. Oliver (1989) related that, for *I. ricinus*, the laying of each egg can take from 3 to 12 min.

The daily eclosion rates for *I. didelphidis* larvae reached a peak between the fifth and seventh day (Table I). According to Oliver (1974) the daily peak for eggs produced by the majority of Ixodidae usually occurs between the third and sixth day, diminishing gradually. Though the daily oviposition rate for *I. didelphidis* was not registered, the daily number of emerged larvae suggests a possible relation between the daily oviposition rate and the daily eclosion rate, until the peak is reached.

Considering that high temperatures accelerate the pre-oviposition, oviposition and incubation periods of the eggs, and increases the number of eggs laid daily (Oliver 1989), it would be expected that, under different temperature conditions significant differences would occur in the oviposition and eclosion periods, in the daily eclosion rates

TABLE I

Daily emergence rates of larvae of *Ixodes didelphidis* and total number of eggs, and larvae emerged at 27ºC and 25ºC, under high humidity (85-95%)

	Temperature 27ºC			Temperature 25ºC			
Days	Female 1	Female 2	Female 3	Female 4	Female 5	Female 6	Female 7
1	15	9	12	16	18	9	1
2	30	43	18	61	49	45	35
3	40	115	85	63	195	55	37
4	177	220	326	76	231	239	89
5	**579**	81	233	**625**	**532**	**305**	123
6	238	**232**	**419**	81	255	100	207
7	195	177	134	64	147	207	**566**
8	344	156	130	63	87	234	54
9	240	95	86	43	47	181	44
10	154	83	57	43	83	189	48
11	152	40	36	89	12	100	72
12	138	94	71	31	14	202	174
13	153	68	33	4	4	89	12
14	190	86	22	33	20	102	22
15	113	19	14	43	10	64	10
16	69	21	17	13	10	49	16
17	57	13	9	12	7	74	11
18	42	11	3	23	3	50	13
19	9	4	16	7	12	127	0
20	-	-	-	12	1	68	0
21	-	-	-	9	0	85	3
22	-	-	-	6	0	39	1
23	-	-	-	2	11	44	-
24	-	-	-	11	2	50	-
25	-	-	-	3	-	69	-
26	-	-	-	4	-	42	-
27	-	-	-	15	-	15	-
28	-	-	-	3	-	13	-
29	-	-	-	-	-	11	-
30	-	-	-	-	-	20	-
31	-	-	-	-	-	5	-
Total larvae	2935	1567	1721	1475	1750	2882	1538
Total eggs laid	3229	1590	1741	1605	1870	3110	1630
% of eclosion	90.9	98.55	98.85	91.9	93.58	92.66	94.36

Maximum reached eclosion peak is represented in bold face.

TABLE II

Average oviposition period (OP), and the average interval of eclosion until eclosion peak (ID), and the emergence period average (EP) for larvae of *Ixodes didelphidis*, at 27ºC and 25ºC under high humidity (85-95%)

	Oviposition (days)		Emergence of larvae (days)		
	Mean ± SD	Range	ID	EP	Range
Temperature			Mean ± SD	Mean ± SD	
27ºC (n=3)	26,33 (± 3.51)	23-30	5,66 (± 0.58)	19	-
25ºC (n=4)	37,25 (± 4.92)	30-41	5,50 (± 1.00)	26,25 (± 4.03)	22-31

TABLE III

Comparison between oviposition and eclosion periods, number of eggs laid, number of larvae emerged, emergence in the maximum peak, and interval (days) until the eclosion peak, for *Ixodes didelphidis* at 27ºC and 25ºC, under high humidity values (85-95%), by the Mann-Witney test

Variable	N	(U)	Exact Sig. (1-tailed)	Probability
Oviposition period	7	0,500	0,057	0,057
Eclosion period	7	0,001	0,029	**0,029**
No. eggs laid	7	6,000	0,571	0,143
No. larvae emerged	7	4,000	0,314	0,114
No. larvae in the peak	7	4,000	0,314	0,114
Interval until the peak	7	4,500	0,371	0,114

Grouping variable: temperature; the significant probability is highlighted in bold face.

and also in the interval (days) until peak eclosion for *I. didelphidis* was reached. However, despite the significant increase in eclosion period ($p<0.05$) at 25°C, there was no significant increase in the interval (days) until the peak of eclosion, nor in the number of larvae emerged during the peak, nor in the total number of larvae emerged in each oviposition. There was also no difference in the number of emerged larvae until the maximum peak. Despite the tendency to increase the average oviposition period, at 25ºC (Table II), the value obtained was not significant. Maybe, the value would have been significant if the sample had been larger.

Therefore, the temperature values used in this study were not critical for the embryological development of the studied species. Since at 27ºC, at high humidity, the oviposition and eclosion periods are shorter and there is a higher percentage of emerged larvae, we consider this to be the ideal temperature for laboratory studies, but we suggest further testing of humidity variation.

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REFERENCES

Aragão HB, Fonseca F 1961. Notas de Ixodologia, VIII. Lista e chave para os representantes da fauna ixodologica brasileira. *Mem Inst Oswaldo Cruz 59*: 115-129.

Arzua M, Barros-Battesti DM 1999. Parasitism of *Ixodes* (*Multidentatus*) *auritulus* Neumann (Acari: Ixodidae) on birds from the city of Curitiba, State of Paraná, Southern Brazil. *Mem Inst Oswaldo Cruz 94*: 597-603.

Barbour AG 1984. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med 57*: 521-525.

Barros-Battesti DM 1998. *Estudos de Carrapatos e Pequenos Mamíferos Silvestres Naturalmente Infectados com Espiroquetas Semelhantes à Borrelia, no Município de Itapevi, Estado de São Paulo*, PhD Thesis, Faculdade de Saúde Pública, Universidade de São Paulo, 116 pp.

Barros-Battesti DM, Knysak I 1999. Catalogue of the Brazilian *Ixodes* (Acari: Ixodidae) material in the mite collection of the Instituto Butantan, São Paulo, Brazil. *Pap Avulsos Zool 41*: 49-57.

Camicas JL, Hervy JP, Adam F, Morel PC 1998. *Les Tiques du Monde*, Éditions de l'Orstom, Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris, 233 pp.

Faccini JLH, Prata MCA, Daemon E, Barros-Battesti DM 1999. *Ixodes amarali* (Acari: Ixodidae) em gambá (*Didelphis* sp.) no Estado do Rio de Janeiro, Brasil. Parâmetros biológicos da fase não parasitária. *Arq Bras Med Vet Zootec 51*: 267-270.

Labruna MB, Leite RC, Oliveira PR 1997. Study of the weight of eggs from six ixodid species from Brazil. *Mem Inst Oswaldo Cruz 92*: 205-207.

Morel PC, Perez C 1978. Morphologie des stages préimaginales des Ixodidae *s. str*. d'Europe occidentale. IV. Généralité sur le sous-genre *Ixodes* (*Ixodes*). *Acarologia 19*: 201-208.

Oliver Jr JH 1974. Symposium on reproduction of arthropods of medical and veterinary importance. IV. Reproduction in ticks (Ixodoidea). *J Med Entomol 11*: 26-34.

Oliver Jr JH 1989. Biology and systematics of ticks (Acari: Ixodida). *Annu Rev Ecol Syst 20*: 397-430.

Ponçano WL, Carneiro CDR, Bistrichi CA, Almeida FFM, Prandini FL 1981. *Mapa Geomorfológico do Estado de São Paulo*, Instituto de Pesquisas Tecnológicas do Estado de São Paulo, São Paulo, 94 pp.

Takada N, Ishiguro F, Iida H, Yano Y, Fujita H 1994. Prevalence of Lyme borrelia in ticks, especially *Ixodes persulcatus* (Acari: Ixodidae), in Central and Western Japan. *J Med Entomol 31*: 474-478.

Yoshinari NH, Barros PJL, Bonoldi VLN, Ishikawa M, Barros-Battesti DM, Pirana S, Fonseca AH, Schumaker TTS 1997. Perfil da borreliose de Lyme no Brasil. *Rev Hosp Clín Fac Med S Paulo 52*: 111-117.

Yoshinari NH, Bonoldi VLN, Barros-Battesti DM, Schumaker TTS 1999. Doença de Lyme-símile. *Rev Brasil Reumatol 39*: 57-58.