OVIPOSITION IN *BOOPHILUS MICROPLUS* INFECTED ARTIFICIALLY WITH *BABESIA BOVIS* AND NATURALLY WITH *B. BOVIS* AND *BABESIA BIGEMINA*

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SUMMARY

Ten *Boophilus microplus* engorged females (GI) (average weight 327.7 mg), with induced *Babesia bovis* infection (0.2 to 20.5 kinetes per microscope field of haemolymph on day 5 from detachment) were monitored for egg production. Egg output was also measured in 20 engorged females ticks (GII) (average weight 327.2 mg) free of *B. bovis* and in 11 *B. microplus* (GIII) (average weight 211.5 mg) found naturally infected with *B. bovis* and *Babesia bigemina* (0.1 to 14.9 kinetes per microscope field of haemolymph) on day 5 from collection. All ticks were held in darkness at $27 \pm 1^\circ \text{C}$, 83-86 % relative humidity.

The pre-oviposition period in GI was 2.4 ± 0.52 days and

3.1 ± 0.37 days in GII ($P < 0.01$, « *t* » test). The Reproductive Efficiency Index (REI) (number of eggs laid/tick weight) was 5.75 ± 2.239 and 12.86 ± 1.340 for GI and GII, respectively ($P < 0.01$). No evident harmful effects of babesial infection on egg output of ticks from GIII were found; pre-oviposition period and REI were 2.8 ± 0.40 days and 11.05 ± 1.521 , respectively. The correlation coefficient between REI and number of kinetes per microscope field of haemolymph was -0.3669 in GI ($P > 0.05$) and -0.0855 ($P > 0.05$) in GIII. Adaptive tolerance between *B. microplus* and *Babesia*, probably occurs under natural conditions.

RÉSUMÉ : Ponte des femelles de *Boophilus microplus* artificiellement infectées par *Babesia bovis* et infectées naturellement par *B. bovis* et *Babesia bigemina*.

On a mesuré la ponte chez dix femelles gorgées de *B. microplus* (GI) (poids moyen 327,7 mg) infectées artificiellement par *B. bovis* (0,2 à 20,5 kinètes par champ microscopique d'hémolymphe le 5^e jour après le détachement); chez vingt femelles gorgées (GII) (poids moyen 327,2 mg) sans infection par *B. bovis* et chez onze femelles gorgées (GIII) (poids moyen 211,5 mg) trouvées infectées naturellement par *B. bovis* et *Babesia bigemina* (0,1 à 14,9 kinètes par champ microscopique d'hémolymphe) aussi le 5^e jour après le détachement. Toutes les tiques sont conservées à l'obscurité à $27 \pm 1^\circ \text{C}$ et à 83-86 % d'humidité relative.

La période de pré-ponte dans GI a été $2,4 \pm 0,52$ jours et

$3,1 \pm 0,37$ jours en GII ($P < 0,01$, « *t* » test). L'Indice d'Efficacité Reproductive (IER) (nombre d'œufs déposés/poids de la tique) dans GI et GII a été respectivement $5,75 \pm 2,239$ et $12,86 \pm 1,340$ ($P < 0,01$). On n'a pas constaté des effets notables de l'infection par *Babesia* sur le taux de ponte des tiques dans GIII; la période de pré-ponte et l'IER ont été respectivement $2,8 \pm 0,40$ jours et $11,05 \pm 1,521$. Le coefficient de corrélation entre le IER et le nombre de kinètes par champ microscopique d'hémolymphe a été $-0,3669$ dans GI ($P > 0,05$) et $-0,855$ ($P > 0,05$) en GII. Il est probable qu'existe une tolérance adaptative entre *B. microplus* et *Babesia* dans des conditions naturelles.

INTRODUCTION

Induced infections with *Babesia bovis* and *Babesia bigemina* are deleterious to the reproductive efficiency of *Boophilus microplus* engorged females derived from colonies previously free of infection with these protozoan (Riek, 1964, 1966; Davey, 1981; Guglielmone Mangold, Bermúdez

and Hadani, 1985). However, adaptive tolerance between *Babesia* and *B. microplus* presumably occurs under field conditions since no harmful effects were found in ticks carrying high level of babesial infection naturally acquired (Guglielmone, Mangold, Aguirre, Gaido and Olsen, 1989).

This article provides further evidences of adaptive tolerance between cattle, *Babesia* and its South American vector, that arise from the evaluation of the oviposition in *B. microplus* carrying induced *B. bovis* infection and in ticks with babesial infection contained in the field.

MATERIALS AND METHODS

A colony of *B. microplus* free of *Babesia* spp. was used to infest three 4-6 months old Holstein Friesian calves, negative to immunofluorescent antibody tests for *B. bovis* and *B. bigemina*.

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Each calf was infested with 2,500 30-days-old *B. microplus* larvae on days 0 and 7. Two of these calves were inoculated subcutaneously with 5 ml of a stabilate of a pathogenic *B. bovis* strain nominated S2P on day 11 from first infestation. The stabilate was obtained by grinding (at 4° C in RPMI 1,640 with 3.4 % of bovin serum albumin) *B. microplus* infected larvae (derived from engorged female ticks infected with *B. bovis* S2P by feeding on a splenectomised calf infected with this strain) fed on a calf free of *Babesia* since infective *B. bovis* stage to cattle develops in larvae while feeding (Riek, 1966). The stabilate was conserved at -196° C using dimethyl sulfoxide as crioprotector and thawed by immersion in water at 37° C before use.

Three hundred and fifty engorged female ticks weighing 280-380 mg, detached from days 21 to 25 from the calves infected with S2P. During this period, parasitaemia of calves (evaluated by daily inspection of 25,000 red cells from blood obtained by puncture of tip of the tails) ranged from 0.01 to 0.02 %. The ticks were maintained in darkness at 27 ± 1° C, 83-86 % relative humidity and inspected daily to record the preoviposition period. On day 5 from detachment a haemolymph sample was obtained from each tick to detect and estimate the infection with *B. bovis* by counting the number of kinetes per microscope field of haemolymph following Guglielmone *et al.* (1989). A group of 10 ticks (GI) with an average weight of 327.7 ± 33.04 mg and 0.2; 0.3; 0.3; 1.1; 1.3; 6.2; 7.4; 18.1 and 20.5 kinetes per microscope field of haemolymph, respectively, was selected to evaluate the number of eggs laid. A group of 20 ticks (GII) from the calf free of *B. bovis*, with an average weight of 327.2 ± 31.90 mg, was treated as those ticks from GI to also measure egg production.

Additionally 245 *B. microplus* engorged females were taken off cattle from a dairy farm located, approximately, at 24° 55'S 65° 29'W and treated as ticks from GI and GII. A group of 11 ticks (GIII) with an average weight of 211.5 ± 47.90 mg was found infected with *B. bovis* and *B. bigemina* on day 5 from collection. The number of kinetes per microscope field of haemolymph were as follows: 0.1; 0.1; 0.1; 0.2; 0.2; 0.3; 0.4; 0.4; 1.9; 2.3 and 14.9, respectively.

The Reproductive Efficiency Index (REI) of Drummond and Whetstone (1970) (number of eggs laid/tick weight) was obtained for each *B. microplus* engorged female. The « *t* » test was used to detect significant differences ($P \leq 0.05$) between the preoviposition period and REI of ticks from GI and GII. The correlation coefficient (*r*) between REI and number of kinetes per microscope field of haemolymph was obtained for GI and GIII.

RESULTS AND DISCUSSION

The mean pre-oviposition period and REI with their standard deviations and ranges for GI, GII and GIII are presented in Table I.

A harmful effect on the oviposition of *B. microplus* engorged females was caused by induced *B. bovis* infection. Infected ticks produced fewer eggs per mg of weight than ticks free of this protozoan, which is in accordance with the results presented by Davey (1981). However, this author found no difference in the pre-oviposition period of infected and non-infected *B. microplus* engorged females, while infected ticks from this study (GI) had a shorter pre-oviposition period than those of GII (non-infected). This may indicate a mechanism to avoid the effects of the infection by starting oviposition earlier than non-infected ticks.

TABLE I. — Mean, standard deviation and range of pre-oviposition period (PO) and Reproductive Efficiency Index (REI) of *Boophilus microplus* engorged females with induced *Babesia bovis* infection (GI), free of *Babesia* spp. (GII) or with naturally acquired babesial infection (GIII).

	GI	GII	GIII*
N° of ticks	10	20	11
PO (days)	2.4 a** ± 0.52	3.1 b ± 0.37	2.8 ± 0.40
range	2-3	3-4	2-3
REI	5.75 a ± 2.239	12.86 b ± 1.340	11.05 ± 1.521
range	3.0-10.2	10.2-15.9	8.7-13.1

Statistical analysis performed after logarithmic transformation of individual data. The results shown are non-transformed means.

* Not included in the statistical analysis.

** Numbers in row not sharing letters are significantly different ($P < 0.01$).

Data from GIII indicated a small, if any, effect of naturally acquired babesial infection on the *B. microplus* ovipositional success although the engorgement weight was lower than expected. Tick removal before natural detachment may explain the light weight of the naturally infected *B. microplus*. Similar weights were found previously in *B. microplus* carrying natural babesial infection (mean 224.7 ± 45.96 mg) or non-infected (223.7 ± 51.38 mg) removed by hand from cattle in other region of Argentina (Guglielmone *et al.*, 1989); the REI of ticks from GIII (11.05) was also similar to those REI found in this previous study (11.36 ± 1.525 for infected *B. microplus* and 11.15 ± 1.287 for non-infected ticks). These REI values appear to be a result of adaptive tolerance between *Boophilus* and *Babesia* under natural conditions. On the contrary, the strong adverse effect of induced babesial infection on *B. microplus* oviposition may be a consequence of inadequate adaptation between ticks and protozoan used in the laboratory. Continuous breeding of *B. microplus* on calves infected with strains of *Babesia* originally pathogenic to ticks can provide additional information to understand this relationship.

As expected, the *r* between REI and number of kinetes in the haemolymph of ticks with naturally acquired infections (GIII) was not statistically significant ($r = -0.0854$, $P > 0.05$). The effect of haemolymph infection on the oviposition of *B. microplus* engorged females carrying induced infection with *B. bovis* (GI) was not linear ($r = -0.3669$, $P > 0.05$). This suggests that the susceptibility of *B. microplus* to *B. bovis* infection has a genetic basis or that the level of haemolymph infection is not a reliable indicator of the severity of the infection.

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