TRANSMISSION OF *ANAPLASMA MARGINALE* WITH ADULT *BOOPHILUS MICROPLUS* TICKS FED AS NYMPHS ON CALVES WITH DIFFERENT LEVELS OF RICKETTSÆMIA

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Summary :

Nine splenectomised calves were infested with dissimilar numbers of adult *Boophilus microplus* ticks 72 h after collection as engorged nymphs from three non splenectomised calves with different levels of *Anaplasma marginale* rickettsæmia. Successful transmission of *A. marginale* appeared to be more dependent on the level of rickettsæmia of the donor calves than on the number of ticks attaching to the splenectomised calves, since infection was transmitted only when the rickettsæmia was 0.3 % or greater. Field transmission would thus depend on the rate of tick migration amongst susceptible hosts and the rickettsæmia level of cattle on which the ticks fed previously.

KEY WORDS : *Anaplasma marginale*, rickettsæmia level, *Boophilus microplus*, transtadial transmission.

MOTS CLES : *Anaplasma marginale*, taux de rickettsémie, *Boophilus microplus*, transmission transstadiale.

Résumé : TRANSMISSION DE *ANAPLASMA MARGINALE* PAR *BOOPHILUS MICROPLUS* (TIQUE-IXODIDÉS) RÉCOLTÉES AU STADE DE NYMPHES, SUR DES VEAUX PRÉSENTANT DIFFÉRENTS TAUX DE RICKETTSÉMIE

Neuf veaux splénectomisés ont été infestés par des *Boophilus microplus* adultes récoltés 72 h auparavant au stade de nymphes, gorgées sur trois veaux donneurs non splénectomisés présentant différents taux de rickettsémie dues à *Anaplasma marginale*. Le succès de la transmission de la rickettsiose dépend plus de la rickettsémie des veaux donneurs que du nombre de tiques fixées sur les veaux receveurs splénectomisés, puisque *A. marginale* n'était transmis seulement lorsque les veaux donneurs présentaient des rickettsémies égales ou supérieures à 0,3 %. La transmission naturelle semble donc dépendre du flux de migration des *B. microplus* entre hôtes réceptifs et du taux de rickettsémie des bovins donneurs infestés.

INTRODUCTION

Anaplasma marginale is widespread in cattle of tropical and subtropical environments infested with *Boophilus microplus* ticks, long incriminated as one of its main biological vectors. However, a study from the area of Argentina infested with this boophilid showed no correlation between *B. microplus* infestation and *A. marginale* infection (Rios, Aguirre and Gaido, 1990). This fact might be expected since *B. microplus* is one-host-tick (Hoogstraal and Aeschlimann, 1982) and transovarial transmission of *A. marginale* appears to be a rare event (Uilenberg, 1968, 1970, 1973; Potgieter, 1979). Therefore tick and rickettsial cycles are not synchronised.

On the other hand, intra and transtadial transmission of *A. marginale* by *B. microplus* have been described (Connell and Hall, 1972; Connell, 1974, Thompson and Roa, 1978) but the importance of this date under different field conditions remains unknown. Migration

of *B. microplus* between cattle has been documented (Mason and Norval, 1981). However the proportion of adult *B. microplus* migrating amongst cattle under natural field conditions was recorded as 0.01 % over a period of eight days in northwestern Argentina (Guglielmone and Mangold, in press). Consequently the infection rate of *B. microplus* with *A. marginale* is presumed to be high in order to become a relevant transmitter of this organism. The authors tested this hypothesis by transferring different numbers of *B. microplus* ticks from calves with three different levels of *A. marginale* rickettsæmia to susceptible splenectomised calves.

MATERIALS AND METHODS

The study was carried out in the Instituto Nacional de Tecnología Agropecuaria, Salta, Argentina, using 2-3-months-old Holstein calves purchased from an area free of *B. microplus*. They were negative for the presence of antibodies to *A. marginale* as determined by the card agglutination test (CAT) (Amerault and Roby, 1968). All calves were maintained tick and fly free in isolation boxes.

The *B. microplus* colony named I88, free of *Babesia* and *Anaplasma* infection and maintained on calves also free of these organisms, and the cryopreserved S1P strain of *A. marginale* with no appendages (Rios *et al.*, 1988) were used throughout.

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<i>A. marginale</i> rickettsaemia (%) in donor calves infested with <i>B. microplus</i> larvae to obtain engorged nymphs			Recipient splenectomised calves infested with adult ticks moulted from nymphs fed on the calves infected with <i>A. marginale</i>				
Donor Calf	Day of infestation	Day of nymphal collection	Recipient Calf	N° of ticks applied	N° of ticks attached	<i>A. marginale</i> maximum rickettsaemia	Prepatent period (days)
A	8.0	0.3	A1	80	17	8	27
			A2	40	3	12	35
			A3	20	0	non infected	
B	12.0	1.0	B1	40	20	42	22
			B2	20	8	30	25
			B3	10	4	25	25
A	< 0.01	0.02	C1	20	8	non infected	
			C2	10	9	non infected	
			C3	2	2	non infected	

Table I. – Transtadial transmission of *Anaplasma marginale* to splenectomised calves by *Boophilus microplus* adult ticks fed to nymphal engorgement on infected calves with different rickettsaemia levels.

Three calves A, B and C, were successively inoculated intravenously with 200 million S1P *A. marginale* organisms after thawing aliquots in water at 37°C. On days 14 and 20 after *A. marginale* inoculation each calf was infested with 10 000, 188 *B. microplus* larvae 30-days-old. Fifteen days after the first infestation with ticks, 300 engorged nymphs were taken from each calf and conditioned in darkness at $27 \pm 1^\circ\text{C}$, 83-86 % of relative humidity for 72 h to allow them to moult through the adult stage. Adult ticks (sex ratio = 1) were transferred to shaven areas, under cotton patches on the neck, of nine splenectomised calves which were divided into three groups. Three calves A1, A2 and A3; B1, B2 and B3; C1, C2 and C3 were infested with ticks from donor calf A, B or C, respectively. Within each group, each of the three calves received a different number of ticks as detailed in table I, to obtain an estimation of the minimum number of ticks required to transmit S1P *A. marginale*.

The number of ticks (females and males) attaching, rectal temperature and rickettsaemia by observation of Giemsa stained thin smears prepared daily from all calves were recorded. Haematocrit index using the micro haematocrit technique was evaluated three times weekly and the CAT was performed on days 33, 70 and 100 after tick infestation.

RESULTS

The main results are presented in table I. The proportion of *B. microplus* adults attaching to the splenectomised calves varied from 0 % to 100 %. All calves infested with ticks fed as nymphs on donor calves with at least 0.3 % of erythrocytes infected with *A. marginale* on the day of nymphal collection became infected even when only three

adult ticks attached (calf A2). However nine adult ticks fed as nymphs on calf C with 0.02 % rickettsaemia on the day of nymphal collection were unable to infect splenectomised calf C2.

The mean pre-patent period (interval from the time of adult tick attachment to first detection of *A. marginale* in the blood) for the five splenectomised calves which developed infection was 26.8 ± 4.91 days. The minimum haematocrit index varied from 0.11 to 0.14 and the maximal temperature ranged from 39.8 to 40.8°C.

Splenectomised calves A2 and B3 died of acute anaplasmosis without seroconverting, while calves B1 and B2 had seroconverted by day 33 from infestation and calf A1 was found positive to CAT on day 70. Splenectomised calves A3, C1, C2 and C3 remained uninfected as assessed by negative blood smears and the persistent absence of antibodies to *A. marginale*.

DISCUSSION

Previous studies of transtadial transmission of *A. marginale* by *B. microplus* ticks were carried out with donor calves in which rickettsaemias were higher than 10 % on the day of tick collection (Connell and Hall, 1972; Connell, 1974; Thompson and Roa, 1978) and the number of ticks attaching to the recipient calves used to test the transmission of infection were not specified. On the other hand Samish, Pipano and Hadani (1993), using *Boophilus annulatus*, were able to transmit *A. marginale* by transferring 220 engorged nymphs from a splenectomised calf with 4 % rickettsaemia when the ticks were collected, to another splenectomised calf, where only two engorged female ticks were recovered (number of males attached not stated).

The present study showed that as few as three *B. microplus* ticks fed on a calf with a 0.3% rickettsæmia on the day of collection were able to transmit the infection. Nevertheless it is uncertain when the nymphs obtained the infection. The maximum *A. marginale* rickettsæmia of calf C (fed ticks were unable to transmit the infection) was 0.02 % of the erythrocytes, indicating that this level of infection could be too low to infect the *B. microplus* nymphs.

A. marginale was viable for 72 h in *B. microplus* ticks held off the host during moulting from nymphs to adults while Samish, Pipano and Hadani (1993) transmitted the *A. marginale* with *B. annulatus* maintained 4 days off the hosts. Dalglish and Stewart (1982) succeeded in the transmission of *A. marginale* with extracts of *B. microplus* maintained 2, 7, 11 and 14 days at 37, 27, 14 and 4°C, respectively. This can be taken as evidence that boophilid ticks can act as biological vectors of *A. marginale* (Samish, Pipano and Hadani, 1993). Nevertheless it is also possible that *Boophilus* ticks operate as mechanically support to maintain *A. marginale* for a relatively brief period of time as obtained with short term *in vitro* culture of this rickettsia (Davis, Talmadge, Parish, Johnson and Vibber, 1978).

Aguirre, Bermúdez, Mangold and Guglielmone (1988) working with 7-19 months-old heifers in enzootic conditions for *A. marginale* found that more than 95 % of the positive blood smears showed a rickettsæmia of 0.02 % or lower while rickettsæmia greater than 0.3 % of the erythrocytes were commonly found by Madruga, Kessler, Gomes, Schenk and Andrade (1985) and Ríos, Aguirre and Gaido (1990) dealing with younger cattle. If migration of *B. microplus* occurs in sufficient numbers amongst field cattle its relevance for *A. marginale* transmission will vary according to the rickettsæmia of cattle where the ticks fed previously. Quantitative studies with migrating *B. microplus* ticks under known natural conditions are needed to further understand the importance of this tick species in the epidemiology of anaplasmosis.

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