

# THE EFFECT OF INCUBATION AND PREFEEDING OF INFECTED *RHIPICEPHALUS SIMUS* NYMPHAE AND ADULTS ON THE TRANSMISSION OF *ANAPLASMA MARGINALE*

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## ABSTRACT

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Two batches of unfed *Rhipicephalus simus* nymphae carrying *Anaplasma marginale* were incubated for 72 h and 96 h respectively at 37 °C. Fifty ticks were triturated at a time and the homogenates were used to infect susceptible cattle. Others were prefed on a bovine host for 72 h before tick suspensions were prepared. The same procedure was followed, using a single batch of infected adult ticks.

Incubation had no effect on the infectivity of the nymphal homogenates, but prefeeding of nymphae reduced the prepatent period by 8 days in 1 of the 2 attempts. In the case of the adult ticks both incubation and prefeeding reduced prepatent periods by 5 and 8 days respectively.

## INTRODUCTION

The 3-host tick, *Rhipicephalus simus*, has been shown to transmit *Anaplasma marginale* transstadially in the laboratory (Potgieter & Van Rensburg 1980; Potgieter, 1981). It has also been demonstrated that fresh tick suspensions and frozen stabilates, prepared from adult male ticks infected as nymphae, produced infections when inoculated into susceptible cattle (Potgieter & Van Rensburg, 1980).

Recent studies have demonstrated that homogenates of incubated, unfed, adult *Dermacentor andersoni*, infected with *A. marginale* as nymphae, produced infection with shorter prepatent periods than those of unincubated controls (Leatch & Stiller, 1979, cited by Kocan, Hair, Ewing & Stratton, 1981). These observations were confirmed by Kocan *et al.* (1981), also with the use of adult *D. andersoni*.

The objectives of the present study were to determine the effect of prefeeding and incubation of *R. simus* nymphae and of adults infected as larvae and nymphae, respectively, on the transstadial transmission of *A. marginale*, as determined by injection of triturated ticks.

## MATERIALS AND METHODS

All the cattle used were fully susceptible, splenectomized oxen of mixed *Bos taurus* breeds. Daily routine rectal temperatures of these animals were taken and blood smears examined. After subinoculation of infected tick-derived material or tick infestations, the experimental animals were closely observed for a period of 90 days before being regarded as negative.

A field collection of *R. simus*, originating from the Louis Trichardt district in the northern Transvaal, subsequently maintained in the laboratory and designated the LT-strain, was used exclusively in these trials. The non-parasitic stages of the ticks were maintained at 25 °C and ± 85% R.H. The ticks were fed on the ears of the oxen as described by Neitz, Boughton & Walters (1971).

A blood stabilate of the BW-strain of *A. marginale* (Potgieter, Sutherland & Biggs, 1981) was used to infect the experimental cattle by intravenous inoculation. Tick suspensions were prepared as described by Potgieter & Van Rensburg (1980). Fifty ticks were triturated at a time and 5 ml (10 ticks/ml) of the freshly prepared homogenate was injected intravenously into the oxen.

### Experiment 1

#### Infection of larvae

A blood stabilate was used to infect Ox 2226 with *A. marginale*. Approximately 5 000 *R. simus* larvae, 8 weeks old, were used to infest the ears of this animal on Day 0. During the period the larvae took to engorge, the

parasitaemia ranged from 25-73%. Approximately 800 engorged larvae were collected on Days 4 and 5 and placed in the acaridarium to moult. The ensuing nymphae were used as described below.

#### Incubation of unfed nymphae

Fifty 2-week-old, unfed nymphae were incubated for 72 h at 27 °C, after which they were triturated and then injected into Ox 2694 as described above.

#### Prefeeding of nymphae

Three hundred of the nymphae were used concurrently to infest the left ear of Ox 2876. Fifty partially fed nymphae were removed by hand 72 h later and these were triturated for the preparation of a homogenate to infect Ox 2507. Thirty-six of the remaining nymphae were left to engorge and were collected on Days 4 and 5 after infestation.

#### Unfed nymphae

Fifty unfed nymphae, taken directly from the acaridarium as a control, were used to prepare a homogenate to infect Ox 1660 as above.

### Experiment 2

Experiment 1 was repeated with variations in the procedure (see Tables 1 & 2).

TABLE 1 Prepatent periods of *A. marginale* infections artificially induced in 9 oxen by the intravenous inoculation of homogenates of nymphae and adults of *R. simus*

Homogenates	Prepatent period (days)		
	Incubated 37 °C	Prefed 72 h	Unfed
50 nymphae/5 ml (Exp. 1)	29*	29	28
50 nymphae/5 ml (Exp. 2)	27**	21	28
47 adults/4 ml (Exp. 3)	19**	16	24

\* Incubated for 72 h

\*\* Incubated for 96 h

TABLE 2 Transstadial transmission of *A. marginale* by nymphae and adults of *R. simus*

Experimental animals	Number of ticks			Prepatent period (days)
	Infestation	Removed after 72 h	Engorged	
Ox 2876 (Exp. 1)	300 nymphae	50	36	—
Ox 3953 (Exp. 2)	100 nymphae	50	16	—
Ox 3711 (Exp. 2)	50 nymphae	—	30	29
Ox 9123 (Exp. 3)	120 adults	47*	—	28
Ox 9097 (Exp. 3)	100 adults	—	54**	25

\* Only the 47 ticks that had attached were removed; the rest were destroyed

\*\* 42 females and 12 males

### Infection of larvae

Ox 2666, infected with *A. marginale*, was infested with 13-week-old *R. simus* larvae from the same batch as was used in Experiment 1. On Day 0 the animal had a parasitaemia of 10% which increased to 83% during the time it took for most of the larvae to engorge. On Day 5 a total of approximately 800 engorged larvae were collected, transferred to the acaridarium, allowed to moult to nymphae, and applied as described below.

### Incubation of unfed nymphae

Fifty 10-week-old, unfed nymphae were incubated for 96 h at 37 °C. A tick suspension was prepared from the incubated nymphae and used to infect Ox 2638 as described above.

### Prefeeding of nymphae

One hundred nymphae were used to infest the right ear of Ox 3953. After 72 h 50 partially fed ticks were removed by hand and processed as described above to infect Ox 2493. Only 16 of the remaining ticks were collected as fully engorged specimens on Days 4 and 5 after infestation.

### Unfed nymphae

As in the first experiment, 50 unfed nymphae were taken directly from the acaridarium, triturated and injected intravenously into Ox 3455.

### Natural nymphal transmission

To demonstrate natural transstadial transmission of the infection by the nymphae, the left ear of Ox 3711 was infested with 50 nymphae whose larval stage was spent on B2666 (see above). A total of 28 engorged nymphae were collected from Ox 3711 by Day 5.

## Experiment 3

### Infection of nymphae

A lyophilized blood stabilate (Potgieter & Bester, 1981) was used to infect Ox 9119 with *A. marginale*. Approximately 1 000 *R. simus* nymphae which had fed as larvae on rabbits were used to infest the ears of this animal. A total of 360 engorged nymphae were collected by Day 7 and placed in the acaridarium to moult. The parasitaemia during engorgement of the nymphae in this experiment did not exceed 1%. The ensuing batch of adult ticks were applied as described below.

### Incubation of unfed adults

Ninety 7-week-old, unfed adult ticks were incubated for 96 h at 37 °C. Fifty of these ticks were used to prepare a homogenate with which Ox 9099 was injected as described above.

### Prefeeding of adults

Approximately 120 adult ticks were used to infest Ox 9123. Seventy-two hours after infestation 47 ticks were detached by hand and used in a homogenate to infect Ox 9068. The rest of the ticks were destroyed by washing the animal's ears in water soluble pyrethrins.\*

### Unfed adults

Fifty unfed adult ticks were used to prepare a homogenate and to infect Ox 9072 as described above.

### Natural adult transmission

To test the ability of the above-mentioned batch of adult ticks to transmit the infection naturally, Ox 9097 was infested with approximately 100 of the adults. The

first engorged females dropped on Day 8 and a total of 42 were collected by Day 11. Twelve males were removed by hand and the rest destroyed as described above.

## RESULTS

The results of the 3 experiments are summarized in Tables 1 & 2.

No obvious difference was observed between the prepatent period of the infection induced by inoculation of unfed, non-incubated nymphae and incubated ones (Table 1). However, in the case of the adult ticks, the prepatent period induced by inoculation with the homogenate prepared from incubated ticks was 5 days shorter than that of the non-incubated controls (Table 1).

It was possible also to demonstrate a reduced prepatent period of 21 days, compared to the average 28 days, in 1 of 2 experiments in which nymphal ticks were prefed before being used in a homogenate to infect the animal (Table 1). In a similar, single attempt with prefed adult ticks, the prepatent period was 16 days with prefed ticks compared to 24 days with the controls (Table 1).

Transstadial transmission of *A. marginale* from larva to nymph could only be demonstrated in 1 of 3 attempts through feeding nymphae on susceptible cattle (Table 2). The homogenates prepared from the 2 batches of prefed nymphae, however, proved to be infective.

On the other hand transstadial transmission from nymph to adult was successfully demonstrated in both attempts where adult ticks were fed on susceptible cattle (Table 2). It also became apparent that transmission took place effectively in the case of Ox 9123 within 72 h after infestation (Table 2).

## DISCUSSION

The observation by Kocan *et al.* (1981) that homogenates prepared from unfed adult *D. andersoni* showed longer prepatent periods than those of incubated ticks could be confirmed only with *R. simus* adults but not with the nymphae.

It was only possible to demonstrate a markedly shorter prepatent period in 1 of 2 attempts in which the nymphae were prefed before being used in a homogenate to infect the oxen.

Although the results of the present study suggest that the feeding stimulus may have a greater influence than incubation at 37 °C on the development of the parasite in *R. simus* nymphae and adults, confirmation is necessary.

There is no satisfactory explanation why 2 oxen on which nymphae were fed did not contract *A. marginale*, whereas homogenates prepared from the nymphae prefed on them for 72 h were infective. Both these animals were subsequently used in other experiments where they were shown to be susceptible to *A. marginale* (Potgieter, unpublished observations, 1981).

It would appear that the ticks that were exposed to infection as nymphae are more efficient vectors than those exposed as larvae. The larvae engorged on extremely high parasitaemias, whereas the nymphae fed on an animal which showed a parasitaemia of only 1%, yet the prepatent periods of the infections resulting from the homogenates of adult ticks were shorter than those of the nymphae. In addition, 2 out of 3 animals on which these nymphae were fed failed to contract the infection, whereas both attempts with adult ticks were successful.

We conclude that incubation at 37 °C as well as prefeeding of *R. simus* apparently influence the development of the infective agent in the tick.

\* AVI C & B concentrate special, AVIMA

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