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The Role of Males of the Bont Tick (*Amblyomma hebraeum*) in the Transmission of *Cowdria ruminantium* (Heartwater)

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ABSTRACT

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The role of males of the bont tick (*Amblyomma hebraeum*) in the transmission of *Cowdria ruminantium* (heartwater) was investigated. Transstadial (nymph to adult) and intrastadial transmission were demonstrated. Males transferred from live or dead hosts to live hosts were shown to transmit *C. ruminantium* repeatedly. It was concluded that male transmission is of importance in the epidemiology of heartwater.

INTRODUCTION

It is well established that *Cowdria ruminantium*, the cause of heartwater disease in ruminants, is transmitted transstadially from larva to nymph and nymph to adult by its *Amblyomma* tick vectors (Uilenberg, 1983). Occasionally it may also be transmitted transovarially (Bezuidenhout and Jacobsz, 1986). Male ticks have been shown to be capable of transmitting *C. ruminantium* (Alexander, 1931), but were regarded as poor vectors (Ilemobade and Leeflang, 1977). However, the role of males in the epidemiology of the disease has not been studied. Of particular interest is the possible occurrence of host-to-host transfer, as has been recorded in *Boophilus microplus* (Mason and Norval, 1981) and *Dermacentor andersoni* (Coan and Stiller, 1986). Intrastadial transmission of *Babesia bigemina* (Dalgliesh et al., 1978) and *Anaplasma mar-*

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ginale (Potgieter, 1981; Stiller et al., 1983; Zaugg et al., 1986) by male ticks has also been demonstrated.

This paper reports studies on the transmission of *C. ruminantium* by males of *A. hebraeum* and host-to-host transfer of these males during feeding. For comparison, an experiment on transmission by females was also carried out.

MATERIALS AND METHODS

Transmission by males and females

Merino sheep and Boer goats, born and raised in non-heartwater areas in the highveld of Zimbabwe (Norval, 1973), were used in the experiments. The ticks were of the Malipati stock, established at the Veterinary Research Laboratory in 1978, and were maintained free of *C. ruminantium* by feeding on uninfected sheep, goats and cattle. The ticks were confined in cloth bags which were glued with contact adhesive to shaved areas on the backs of the sheep and goats. Brain biopsies were taken according to a variation on the method of Synge (1978). Brain smears from brain biopsies and from post-mortem material were stained with Giemsa and examined for the presence of colonies of *C. ruminantium*.

Transstadial transmission by males

The first experiment was to determine whether or not males of *A. hebraeum* infected as nymphs would transmit *C. ruminantium* transstadially and then intrastadially. One hundred and fifteen males, which had fed as nymphs during the febrile reaction of a sheep infected by blood inoculation with a Lemco (Zimbabwe) isolate of *C. ruminantium*, were placed on sheep No. 497. This sheep contracted heartwater after an incubation period of 13 days. It was treated intravenously with oxytetracycline hydrochloride (8 mg kg^{-1}) on Day 16 and, just prior to treatment, 40 males were removed. These were allocated to two batches of 20 and placed in bags on separate sheep (Nos. 052 and 053), ~4 h after removal. The sheep were then monitored for febrile reactions by daily recording of rectal temperatures. Both sheep reacted after 12 days and brain biopsies were taken on the third day of fever, after which the animals were treated. Just prior to treatment 10 males from Sheep 052 were removed and placed on Sheep 490 and 10 males from Sheep 053 were removed and placed on Sheep 1462. Again, temperature reactions were monitored.

Intrastadial transmission

The second experiment was to determine whether or not uninfected males could pick up *C. ruminantium* from infected sheep and then transmit it intrastadially to susceptible sheep. Sheep 093 was inoculated with 5 ml of fresh blood from Sheep 59 reacting to a Nyatsanga (Zimbabwe) isolate of

C.ruminantium. Nine days after inoculation, at the beginning of the febrile reaction, 100 unfed, uninfected males of *A. hebraeum* were placed on the sheep. Eight days later, 50 of the males were placed on Sheep 1146, which was monitored for a febrile reaction. When this occurred, a brain biopsy was taken, which proved positive for *C. ruminantium*. Thirty of the males were removed and placed on Sheep 031, which was monitored for a febrile reaction.

Transstadial transmission by females

The third experiment was to determine the ability of females to transmit *C. ruminantium*. Ten females collected as engorged nymphs from cattle in a heartwater area were placed on two Boer goats. An ether extract of fed males of *A. hebraeum* containing the aggregation–attachment pheromone (AAP) (Rechav et al., 1977) was applied to stimulate the attachment of females in the absence of males. The goats were monitored for a febrile reaction.

Movement of males

On live hosts

Yearling Friesland×Hereford heifers raised at the Veterinary Research Laboratory Field Station at Mazowe, in an area of Zimbabwe free of *A. hebraeum* (Norval, 1973), were used. The heifers were housed in tick-proof stalls at the Field Station for the duration of the experiment. Two pairs of heifers were placed in separate stalls. Ether extract of fed males of *A. hebraeum* containing AAP was applied onto the udder and escutcheon of these heifers prior to the release of 50 unfed males. Twenty-four hours was allowed for attachment after which tick counts were made. The stalls were then cleared of bedding and cleaned. At this point, two tick-free heifers were introduced into each stall with the other animals. Thereafter, the ticks were counted at weekly intervals and their positions of attachment were mapped. Mapping, which was carried out by the same person on each occasion, consisted of noting on all animals the number of ticks in each of the following areas: right and left front and back feet; right and left front and back legs; right and left axillae; abdomen; each teat of the udder; perineum; escutcheon; tail; neck; abdomen; sides.

After 6 weeks, 50 unfed females were placed on one of the heifers in each group on which males had originally been placed. Cattle were examined daily and fully engorged females were removed. Within 17 days, all the females had been removed. Weekly counts and mapping of the males were continued for 7 weeks after the females had been introduced.

From dead to live hosts

Ruminants that die from heartwater may be infested with numerous male bont ticks. Two experiments were performed to determine whether males detach after death of a host and transfer to another host.

Seventy uninfected *A. hebraeum* males were placed within a cloth bag on the back of a goat. The goat was infected with the Lemco (Zimbabwe) isolate of *C. ruminantium* and subsequently was allowed to die in a stall shared with a tick-free Merino sheep. At death, the cloth bag was removed from the goat and the ticks were allowed to 'escape' into the environment. Ticks from the goat were monitored for detachment, movement and transfer to the live sheep for 48 h. Similarly, in a second experiment, 70 uninfected male ticks were allowed to attach in a cloth bag on the back of a sheep infected with the Ball strain of *C. ruminantium*. When the sheep died 7 days after the attachment of the ticks, it was placed in a stall with a tethered Jersey weaner steer. The cloth bag was removed from the sheep and ticks were monitored for detachment, movement and transfer to the steer for 48 h. At all times, the minimum distance between the carcass and the steer was 0.5 m.

RESULTS

Transmission

Transstadial transmission by males (Table 1)

Sheep 052 and 053 both developed febrile reactions typical of heartwater, and brain biopsies were taken. Sheep 052 was negative by brain biopsy smear but its blood was found to be highly infective upon subinoculation to sheep, goats and cattle. It was not treated, but recovered spontaneously. Sheep 053 was shown to be positive by brain biopsy and was treated with oxytetracycline.

TABLE 1

Transmission of heartwater by males of *A. hebraeum*

Sheep No.	No. males	Source sheep No.	Days from placement to first day of temperature	Brain biopsy	Post-mortem brain smear	Outcome
(1) Transstadial and intrastadial transmission						
052	20	497	14	Negative	N/A[1]	Not treated, recovered
053	20	497	12	Positive	N/A[1]	Treated, recovered
490[2]	9	052	18	Positive	Positive	Treated, died
1462	10	053	15	Positive	Positive	Not treated, died
(2) Intrastadial transmission						
1146	50	093	22	N/A	Positive	Not treated, died
031	30	1146	14	Positive	Positive	Treated, died

[1]N/A = not applicable.

[2]In this case the ticks had remained on Sheep 052 for 6 weeks after it had reacted. In all other cases, the ticks were removed at the time of reaction.

Sheep 1462 died of heartwater 18 days after 10 males removed from Sheep 053 were placed on it. Six weeks after Sheep 052 had reacted, the nine surviving males were removed and placed on Sheep 490. Only three of these attached. However, 18 days later the sheep reacted and died of heartwater.

Intrastadial transmission (Table 1)

Sheep 1146 reacted 22 days after attachment of the male ticks and died of heartwater. Thirty ticks were removed from it and placed on Sheep 031, which also reacted and died of heartwater.

Transstadial transmission by females

Both Boer goats infested solely with females of *A. hebraeum* died of heartwater.

Movement of males

On live hosts

Three periods of time were considered (Table 2): (i) the period before introduction of female ticks; (ii) the period during presence of female ticks; (iii) the period after detachment of female ticks.

The number of males on the first day after infestation was 135. Over the 13-week period of study, there was a continual loss of males into the stalls and only 25 remained on cattle at the end of the experiment. However, very little movement of male ticks was noted during the first 6 weeks.

Most of the female ticks placed on the heifers attached in the immediate vicinity of previously attached males. Of the females that fell onto the bedding at the time of release, some were recovered on the other male-infested heifers

TABLE 2

Movement of males of *A. hebraeum* on heifers

	No. males at beginning of period	No. males at end of period	No. males at new positions on originally infested heifers[1]	No. males at new positions on originally uninfested heifers[1]
Weeks 1–6 pre-female period	135	126	0 (0%)	1 (0.74%)
Weeks 7–10 female period	126	99	8 (5.9%)	2 (1.48%)
Weeks 11–13 post-female period	99	25	5 (3.7%)	2 (1.48%)
Total period	135	25	13 (9.6%)	5 (3.7%)

[1]Percentages calculated with respect to original 135 ticks.

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TABLE 3

Male and female tick count 1 week after placement of females on heifers
Stall 1. Fifty female *A. hebraeum* ticks placed on heifer No. 1325 (1325 and 1315 were originally infested with males)

	Heifer number			
	1325	1315	1331	1323
Number of males	25	19	1	0
Number of females	21	6	1	0

Stall 2. Fifty female *A. hebraeum* ticks placed on heifer No. 1347 (1347 and 1346 were originally infested with males)

	Heifer number			
	1347	1346	1349	241
Number of males	30	24	0	0
Number of females	17	6	0	0

TABLE 4

Numbers of males detaching from carcass and reattaching on a new live host

	Number detaching	Number reattaching
Dead goat/live sheep		
24 h	3	0
48 h	0	0
Dead sheep/live steer		
24 h	20	2
48 h	52	15

(Table 3) where, in all but one case, the females paired with previously attached males. The lone female tick was found on the back left hoof of Heifer 1346 and the nearest male was on the brisket.

From dead to live hosts (Table 4)

Tick-infested dead goat in a stall with a live sheep. After 24 h, three ticks had detached from the goat, but were in a weak state and died while still on the goat. No further detachment by the ticks was noted in the next 24 h.

Tick-infested dead sheep in a stall with a live steer. In contrast to the previous

experiment, ticks on the dead sheep began to detach 12 h after death. At 24 h after death, two males had attached to the feet of the steer and at 48 h, 15 males had attached to its feet. Twenty-eight days later the steer developed a febrile reaction. Five days later the steer showed terminal nervous signs typical of heartwater and was killed. The post-mortem brain smear was positive for heartwater.

DISCUSSION

The report by Alexander (1931) that males of *A. hebraeum* can transmit *C. ruminantium* when infected as nymphs (transstadially) has been confirmed. However, our finding that intrastadial transmission by male ticks does occur, contrasts with his observation that 'Male *A. hebraeum*, transferred from a sick to a healthy animal, appear to be innocuous'. The repeatability and low number of ticks required for intrastadial transmission by males indicates that this mechanism could be of importance in *C. ruminantium* transmission in the field. The finding that batches of males can repeatedly transmit *C. ruminantium* is of epidemiological significance as is the observation that the males that fed on a reacting sheep and remained on it for 6 weeks, were still infective. Although Alexander (1931) stated that adults of *A. hebraeum* are vectors of heartwater, he did not test the vectoring capacity of females alone. Ilemobade and Leeflang (1977) showed that one female of *Amblyomma variegatum* is capable of transmitting *C. ruminantium*. We now have shown that females of *A. hebraeum* are also vectors.

Movements of male ticks (probably in response to a sex pheromone) increased markedly once females were introduced onto the hosts. Female-produced sex pheromones have been shown to exist in both *A. hebraeum* and *A. variegatum* (the tropical bont tick and principal vector of heartwater in Central, North and West Africa) and to stimulate the movement of males over short distances (Rechav et al., 1982). Feeding females of other tick species such as *Amblyomma americanum* (Gladney and Drummond, 1970), *Rhipicephalus appendiculatus* (Leahy et al., 1976), *Dermacentor variabilis* and *D. andersoni* (Homsher and Sonenshine, 1976) also produce sex pheromones. In the field, the attachment of new females on a host could be expected to cause males in the vicinity to detach and migrate to female attachment sites.

During the concluding weeks of the study of the movement of males, it was noted that the lesions at the sites of male attachment were becoming abscess-like and this appeared to cause some males to detach and reattach within a radius of a few centimetres. Often, these detached ticks went unaccounted for, possibly having fallen from the host after detachment.

Males that move to female attachment sites may be dislodged by the host's activities and any of these males could attach on the same or a different host. Similarly, attached males (and possibly females) could be removed by groom-

ing and re-attach to hosts. It has previously been observed that cattle in the field spend considerable time grooming to remove adults of *A. hebraeum* (Norval et al., 1989b) and that grooming is possibly the only mechanism by which cattle are able to express resistance to adults of *A. hebraeum* (Norval et al., 1988).

Eighteen (13.3%) of the original 135 attached males were observed to have moved to new positions. Of these, 13 (9.6%) were on hosts previously infested and five (3.7%) were on previously uninfested hosts. These figures are considerably lower than the 31% transfer of *D. andersoni* males reported by Coan and Stiller (1986), but are nevertheless epidemiologically significant.

It is known that the male aggregation–attachment pheromone is an important trigger in the attraction of unfed adults of *A. hebraeum* to hosts (Norval et al., 1989a). Only 3.7% of the ticks in the experiment on the movement of males attached to tick-free hosts. It is thought that the presence of the male-produced aggregation–attachment pheromone on the tick-infested cattle may have attracted the detached ticks to tick sites on the cattle that were originally infested. Uninfested cattle which lacked this pheromone may not have been as attractive to the detached ticks.

Semi-engorged nymphs of *R. appendiculatus* infected with *Theileria parva*, the causative organism of East Coast Fever (ECF), are known to be able to detach from a carcass of a steer which has died from ECF and reattach and complete engorgement on a carnivore which has eaten the carcass (Purnell et al., 1970). After moulting, the resultant adult ticks are able to transmit ECF to susceptible steers. Our findings that males of *A. hebraeum* on carcasses of animals which die of heartwater can transfer to live hosts in stall confinement and transmit *C. ruminantium* is an indication that this type of transmission may also occur in the field.

The transfer of *C. ruminantium*-infected males of *A. hebraeum* from live or dead hosts to other live hosts and the repeated transmission of *C. ruminantium* by males could therefore be of importance in the epidemiology of heartwater.

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