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PROOF OF TRANSOVARIAL TRANSMISSION OF *COWDRIA RUMINANTIUM* BY *AMBLYOMMA HEBRAEUM*

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ABSTRACT

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Transovarial transmission of *Cowdria ruminantium* by *Amblyomma hebraeum* does occur in certain instances. Both the transovarial and the filial infection rates appear to be very low. The infection may reappear only in the adults or nymphae, or in all 3 stages of the tick's life cycle.

INTRODUCTION

Heartwater has been transmitted experimentally by 11 species of *Amblyomma* ticks (Uilenberg, 1983). Complete transtadial transmission was reported in 5 of these species, namely *A. hebraeum* (Lounsbury, 1900; Alexander, 1931; Neitz, 1947); *A. variegatum* (Daubney, 1930; Neitz, 1956; Ilemobade & Leeflang, 1977); *A. tholloni* (Mackenzie & Norval, 1980); *A. cohaerens* (Uilenberg, unpublished data, 1982); and *A. maculatum* (Uilenberg, 1982). In these species heartwater was transmitted from larvae to nymphae and from nymphae to adults, as well as from larvae through nymphae to adults.

In the case of *A. pomposum* (Neitz, 1947) and *A. lepidum* (Karrar, 1966), transmission was demonstrated only from larvae to nymphae and nymphae to adults. Experiments with *A. sparsum* (Norval & MacKenzie, 1981); *A. astrion* (Uilenberg & Niewold, 1981) and *A. cajennense* (Uilenberg, unpublished data, 1982) revealed that these species were poor vectors and that transmission took place only from larvae to nymphae. Lewis (1949) found *A. gemma* to be a vector of heartwater. However, he did not mention which stages of the tick's life cycle were involved.

All attempts in the past to demonstrate transovarial transmission have failed. This mode of transmission has been investigated in *A. hebraeum* (Lounsbury, 1900); *A. variegatum* (Ilemobade & Leeflang, 1977), and *A. tholloni* (MacKenzie & Norval, 1980), as well as in *A. maculatum* and *A. cohaerens* (Uilenberg, 1982; and unpublished data, 1982).

The other species mentioned in Uilenberg's review were either found to be poor vectors or were not tested for transovarial transmission. Apart from the published reports of unsuccessful attempts, laboratory experience has not supported such a mode of transmission.

We were of the same opinion, and in fact often used the progeny of heartwater-infected *A. hebraeum* females as non-infective control ticks in experiments. However, an unexpected case of heartwater in a sheep infested with nymphae from such a control group of ticks indicated that transovarial transmission of *C. ruminantium* might take place occasionally (Bezuidenhout, unpublished data, 1979). We therefore conducted an experiment to investigate this possibility and found that transovarial transmission does indeed occur in certain instances. Our findings became known and have sometimes been referred to in the literature (Camus & Barre, 1982; Uilenberg, 1982; 1983).

Although we fully realize the preliminary nature of these investigations, we have nevertheless decided to make our results known officially.

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MATERIALS AND METHODS

Amblyomma hebraeum larvae were infected with *C. ruminantium* as follows:

A susceptible Merino sheep was inoculated intravenously (i.v.) with 5 mℓ of heartwater blood vaccine as issued by the Veterinary Research Institute, Onderstepoort. Four days later the wool on the sheep's back was shaved with an electric hair clipper and then with a safety razor to ensure a smooth hairless surface.

Three calico bags, each with a leather ring as a base, were glued to the skin by means of Genkem contact adhesive. Batches of ± 15 000 *A. hebraeum* larvae were confined in separate bags on the 5th, 6th and 7th day after the sheep had been inoculated with infected blood. This method ensured that most larvae dropped at the height of the febrile reaction. Only those larvae that dropped when the sheep's temperature was above 40 °C were used in further experiments.

About 3 weeks after they had moulted, samples of nymphae and adults which had developed from these larvae were fed on susceptible sheep to determine their infectivity. All ticks in the nonparasitic phases of their life cycle were kept in an incubator at 27 °C and 80 % R.H.

To determine whether transovarial transmission can take place, the engorged females were divided into 5 groups, each containing 8 females. The progeny of the females in each group were fed on separate heartwater-susceptible sheep at each stage of their life cycle, as follows:

Three weeks after hatching the larvae from each of the 5 tick groups were placed on separate sheep. Two weeks after moulting, roughly 2 000 nymphae from each group were then placed separately on a further 5 sheep. Finally, the adults were placed on the last 5 sheep 14 days (120 males/sheep) and 18 days (120 females/sheep) respectively after moulting (Table 1).

All ticks in this experiment were confined in calico bags on the backs of the sheep.

The experiment was carried out in tick-free stables and the rectal temperatures of all the sheep were taken daily. An autopsy was performed on Sheep 8776 which died during the experiment.

Brain smears were stained with Giemsa and examined with a research microscope. Surviving sheep were challenged by i.v. injection of 5 mℓ of heartwater infected blood vaccine 32–76 days after they had been infected with ticks (Table 1). After inoculation of the blood, their temperatures were taken daily for another 25 days. Sheep that reacted during challenge, as well as Sheep 7986 which showed a primary reaction, were treated intramuscularly with oxytetracycline at a rate of 8 mg/kg for 1 or more days.

TABLE 1 Experiment to investigate the possible transovarial transmission of *Cowdria ruminantium* by *Amblyomma hebraeum*, Onderstepoort, 1979

Tick group*	Tick stage	Sheep No.	Result	Incubation period (days)	Immunity Test		
					Interval (days after tick feeding)	Result	Immune state
1	Larvae	8776	RD	9	—	—	—
	Nymphae	7986	RT	12	40	NR	I
	Adults	8687	R	16	37	NR	I
2	Larvae	8759	NR	—	76	RT	S
	Nymphae	7991	NR	—	41	RD	S
	Adults	8713	NR	—	37	R	S
3	Larvae	8770	NR	—	40	RT	S
	Nymphae	8715	NR	—	43	RT	S
	Adults	166	NR	—	32	RT	S
4	Larvae	8774	NR	—	40	RT	S
	Nymphae	8689	NR	—	43	RT	S
	Adults	167	R	19	32	NR	I
5	Larvae	8762	NR	—	43	RT	S
	Nymphae	8380	NR	—	43	RT	S
	Adults	169	NR	—	43	NR	I

R = reacted NR = no reaction RT = reacted and treated RD = reacted and died I = immune
S = susceptible
* = Each group contained the progeny of 8 female *A. hebraeum*

RESULTS

The successful infection of the original larvae was shown by the fact that the subsequent nymphal and adult stages, when fed, caused heartwater in sheep.

Sheep 8776, which received the larvae from Group 1, died after contracting typical heartwater (Table 1). Giemsa-stained hypocampal smears from this sheep showed colonies of intracellular organisms indistinguishable from *Cowdria ruminantium*. Nymphae and adult ticks from the same group also caused heartwater reactions in Sheep 7986 and 8687 respectively. Sheep 7986 was treated while Sheep 8687 recovered spontaneously. Both animals were later found to be immune when challenged with infected blood.

None of the sheep that received nymphal and adult ticks from Groups 2, 3 and 5 showed any symptoms of disease. However, Sheep 8770 and 8762 from Groups 3 and 5 showed early temperature reactions (40 °C), starting 4 days after their infestation with larvae. These temperature reactions lasted for about 3 days and reached 41 °C. When the sheep were challenged later, however, they were found to be susceptible; these early temperature reactions are, therefore, not regarded as heartwater reactions. All other non-reacting sheep except Sheep 169 were also found to be susceptible when challenged. Sheep 169 did not show a primary reaction but was found to be immune when challenged. None of the animals which received larvae or nymphae from Group 4 showed any reaction. However, Sheep 167, which showed a temperature reaction starting 19 days after its infestation with male ticks and then recoverd spontaneously, was found to be immune against heartwater during challenge. In this case the primary reaction is therefore regarded as a heartwater reaction.

DISCUSSION

The preliminary nature of this experiment is well realized. However, the fact that larval ticks from Group 1 were capable of causing heartwater when fed on susceptible sheep is the 1 evidence that *C. ruminantium* can sometimes be carried transovarially. It was also found that, despite the fact that in some groups no disease was caused by the previous stage, heartwater was produced once by nymphae (Bezuidenhout, unpublished data, 1979) and once by adults (Table 1). However, where the disease was transmitted by the larvae it was also caused by the successive stage of the same group of ticks (Table 1).

It is difficult to determine why our studies were successful and other similar investigations failed to demonstrate transovarial transmission of heartwater organisms. Comparison of results is not always possible, though, in that in most cases different *Amblyomma* species were used, the methods of infecting ticks and the number of ticks tested, as well as the strains of *Cowdria* used, either were not the same or were not reported.

There are, however, certain factors that may play an important role in successful transovarial transmission and, where possible, these factors will be compared with regard to the investigations conducted up to date.

According to Burgdorfer & Varma (1967), transovarial development produces 2 distinct infection rates which may not bear any relation to each other. Firstly, there is the transovarial infection rate which determines the percentage of females that pass organisms to their progeny and, secondly, there is the filial infection rate which determines the percentage of infected progeny derived from an infected female.

It is not known how many of the original 40 female ticks used for the experiment were, in fact, infected with *C. ruminantium*. This, together with the fact that their progeny were also fed in large groups on susceptible sheep, makes the estimation of either of the above-mentioned infection rates impossible. When taking the work of Lounsbury (1900) and that of Ilemobade & Leeflang (1977) into account, it would appear that the transovarial infection rate is very low. The fact that the infection appeared in certain instances only in the nymphal or only in the adult stage might be regarded as an indication of a low filial infection rate.

Rehacek (1965) thought that the efficacy of transstadial and transovarial transmission of rickettsiae and viruses of ticks depends on the arthropod species and the amount of organisms taken in originally by the vector. Apart from the fact that a minimum infective dose is necessary to infect ticks with rickettsiae, it is also thought that the higher the infective dose the better the chances for the development of a permanent generalized

infection; this could also facilitate transovarial transmission (Burgdorfer & Varma, 1967). The infective dose received by the larvae might therefore play a very important role in the successful transovarial transmission of *Cowdria*. Whether this aspect could explain why Lounsbury (1900) and Ilemobade & Leeflang (1977) failed to show transovarial transmission is not known. Alexander (1931) showed that there is a great variation in the infectivity of blood, not only of different sheep, but also at different times during the course of the disease. He found that during almost the whole incubation period of the disease the blood was not infective: even the subinoculation of large amounts did not set up a reaction. The peripheral blood was first shown to be infective approximately 24 hours before the initial rise in temperature.

Ilemobade & Leeflang (1977) placed larvae on infected animals injected with a stabilate (D225) on the same or the previous day. The mean feeding time of *A. variegatum* larvae is 6,32 days (Ilemobade & Mohammed, 1976). The incubation periods of animals injected with stabilate D225 were 8–17 days (Ilemobade, 1976). It would therefore appear that the larvae had only a limited opportunity to become infected, and that the infective dose they took in could have been lower than it would have been if they had dropped a few days later. The exact method of infecting larvae followed by Lounsbury (1900); Uilenberg, (1982) and MacKenzie & Norval (1980) is not known.

A further important difference between our experiment and those of the other authors is that we tested a large number of ticks. We therefore had a better chance to show up infection in the progeny, especially if the filial infection rate proved to be very low.

An aspect worth mentioning, although it is not completely relevant, is the fact that in addition to feeding larvae on susceptible sheep, Ilemobade & Leeflang (1977) injected newly hatched larvae intravenously into sheep in an attempt to prove the absence of *C. ruminantium* in the strain of ticks they used.

At Onderstepoort, however, it was found that the i.v. injection of even 75 engorged, and presumably infected larvae did not produce the disease in sheep (Bezuidenhout, unpublished data, 1979). In experiments by Du Toit described by Alexander (1931), similar negative results were obtained after injecting 5 larvae i.v. into sheep, 14 and 28 days after they had been fed on a reacting ox. If presumably infected larvae from a batch of ticks which subsequently proved to be infective in the nymphal and adult stage are unable to produce the disease after i.v. injection into sheep, then the chances of unfed larvae causing the disease would probably be nil. This method is therefore unsuitable for testing the infectivity of a strain. Since the number of *Cowdria* organism which larvae might receive from infected females is possibly low, it is doubtful whether even the feeding of such larvae will always produce the disease.

After *A. hebraeum* larvae have become infected with *C. ruminantium*, there is an increase in infectivity, probably as a result of multiplication during feeding of the nymphae and adults (Bezuidenhout, unpublished data, 1979). This amplification of the organisms, especially in the nymphal stage, might lead to a more generalized infection of the tick's tissues including the genital system. We therefore believe that transovarial transmission is more likely to take place in the progeny of ticks that became infected as larvae than in those that picked up the infection in the nymphal or adult stage.

In the case of *A. tholloni*, the larvae and nymphae tested for transovarian transmission were infected during the nymphal and not the larval stage of the previous generation (MacKenzie & Norval, 1980).

Cowdry (1925) only observed organisms in unfed nymphae 40 days and, in another series, 61 days after they had become infected as larvae. In experiments by Du Toit, described by Alexander (1931), it was possible to produce a positive heartwater reaction in sheep only 84 days after the *A. hebraeum* nymphae that were used had become infected as larvae. Whether these findings indicate that a minimum period is necessary for the multiplication of *Cowdria* in the tick, or for the infection of certain tissues, is not known. Furthermore, the development of the organisms in the tick might be temperature-dependent, in which case the incubation temperature of ticks may be important.

After consideration of the above aspects we feel that, although proof now exists that *C. ruminantium* can be transmitted transovarially, this is probably the exception rather than the rule. It is doubtful whether transovarial transmission plays an important role, if any, in the epizootiology of heartwater.

This finding is nevertheless important in that the possibility of transovarial transmission must now be a major consideration when comparative biological and epizootiological studies with *A. hebraeum* are being conducted.

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