

NATURAL TRANSMISSION OF HEARTWATER

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ABSTRACT

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Heartwater has been transmitted experimentally by 12 *Amblyomma* species. Their importance depends on the extent of their distribution, adaptation to domestic stock and their efficacy as vectors. Except for one report of transovarial transmission, transmission is transstadial.

Ticks may obtain the infection while feeding on reacting animals, subclinically infected hosts or perhaps on immune animals after reinfection. There is a marked increase in the infectivity of infected ticks during feeding but this decreases before and during moulting. The demonstration of *Cowdria ruminantium* in salivary glands of ticks suggests that transmission takes place via the saliva and that regurgitation from the gut may not be as important as previously thought.

Transmission takes place on the 2nd day from the time infected nymphae were placed on the animals and on the 4th day in the case of adult ticks.

HISTORICAL BACKGROUND

The suspicion that ticks, and more specifically the bont tick *Amblyomma hebraeum*, play a role in the transmission of heartwater, existed long before it was experimentally proved by Lounsbury in 1900.

In his diary on the 9th of March 1838, Louis Trichardt mentions a disease "Nintas" from which his stock began to suffer about 3 weeks after massive tick infestation. In a report of the Commission on Diseases of Cattle and Sheep, which met at Grahamstown in February 1877, Mr John Webb describes a disease "boschickness" (= heartwater) which had caused heavy mortalities among sheep in the eastern Cape. He drew a clear comparison between the disease and the presence and distribution of the "bonte tick" in the Cape of Good Hope. According to him, Mr William Bowker found the first bont tick on a cow which had been introduced from Zululand about 40 years earlier.

PROVEN VECTORS OF HEARTWATER

Lounsbury (1900), in a series of well-planned experiments, was the first to prove that the bont tick *A. hebraeum* is an efficient vector of heartwater. Since then, the disease has been transmitted experimentally by 12 species of *Amblyomma* (Table 1). A number of other tick species have also been tested for heartwater transmission but all with negative results (Table 2). Circumstantial evidence also indicates that heartwater is not transmitted by ticks other than those belonging to the genus *Amblyomma*.

Proven *Amblyomma* vectors, on the other hand, are not all of equal significance in the transmission of the disease. Their importance depends on the extent of their distribution, adaption to domestic stock and their efficacy as vectors (Uilenberg, 1983a). When these factors are taken into consideration it seems that *Amblyomma variegatum* is the most important vector, followed by *A. hebraeum*.

MODE OF TRANSMISSION

All proven vectors of heartwater are 3-host ticks. Except for 1 report on transovarial transmission (Bezuidenhout & Jacobsz, 1986) transmission is transstadial. Not all vectors possess the same ability to transmit the disease. Some show complete transstadial transmission, i.e. from larvae to nymphae, from nymphae to adults and from larvae through nymphae (even if the nymphae feed on a non-susceptible animal) to the adult stage (Table 1). For other species transmission has only been shown from the larval to nymphal stage and sometimes also from the nymphal to the adult stage. An intrastadial transmission experiment using *A. hebraeum* males gave negative results (Lounsbury, 1902).

TABLE 1 Proven experimental vectors of heartwater⁽¹⁾

<i>Amblyomma</i> spp.	Proven mode of transmission	First reference ⁽²⁾
African		
<i>A. hebraeum</i>	I, II, III	Lounsbury (1900)
<i>A. variegatum</i>	I, II, III	Daubney (1930)
<i>A. pomposum</i>	I, II	Neitz (1947)
<i>A. gemma</i>	?	Lewis (1949)
<i>A. lepidum</i>	I, II	Karrar (1966)
<i>A. tholloni</i>	I, II, III	MacKenzie & Norval (1980)
<i>A. sparsum</i>	I	Norval & MacKenzie (1981)
<i>A. astrion</i>	I, III	Uilenberg & Niewold (1981);
<i>A. cohaerens</i>	I, III	Uilenberg (1983b)
<i>A. marmoreum</i>	I, III	J. D. Bezuidenhout (unpublished data, 1986)
American		
<i>A. maculatum</i>	I, II, III	Uilenberg (1982)
<i>A. cajennense</i>	I	Uilenberg (1983b)

I from larval to nymphal stage

II from nymphal to adult stage

III from larval through nymphal to adult stage

? stage(s) not reported

(1) From Uilenberg (1983a) with some modifications

(2) Refers only to the first proven transmission of heartwater by the tick species and not to any particular mode of transmission.

TABLE 2 Experiments with ticks that failed to transmit heartwater

Tick species	Reference
<i>Amblyomma americanum</i>	Uilenberg, Camus & Barré (1985)
<i>Amblyomma neumannii</i>	Uilenberg, Camus & Barré (1985)
<i>Amblyomma imitator</i>	Uilenberg, Camus & Barré (1985)
<i>Amblyomma nuttalli</i>	Alexander (1931)
<i>Rhipicephalus evertsi evertsi</i>	Lounsbury (1902)
<i>Boophilus decoloratus</i>	Lounsbury (1902)
<i>Ornithodoros savignyi</i>	Lounsbury (1902)

ACQUISITION OF THE INFECTION

Ticks can acquire the infection while feeding on a reacting animal (Lounsbury, 1900). There is also evidence that ticks can become infected while feeding on subclinically infected hosts during the period in which a heartwater reaction or circulation of the organisms would be expected. This has been proven in the case of the blesbok (Neitz, 1937), helmeted guinea fowl and leopard tortoise (J. D. Bezuidenhout & J. A. Olivier, unpublished data, 1985). Because *Cowdria ruminantium* may

be present in blood for 60 days after spontaneous recovery, or even after treatment (Neitz, 1939; Ilemobade, 1976; 1978), it is generally accepted that such animals may serve as a source of infection for ticks and act as carriers of the disease.

The blood of immune animals can again become infected for varying periods of time after reinfection with virulent blood (Neitz, 1939; Neitz, Alexander & Adelaar, 1947). These findings led to the assumption that such animals may serve as a source of infection for ticks. Experimental proof is, however, lacking.

In an experiment involving 2 sheep, artificially infected with heartwater, and on which nymphae were placed at regular intervals, it was found that only those *A. hebraeum* nymphae that dropped during the febrile reaction of the disease as well as those that dropped up to 3 days after treatment of the disease, were able to transmit heartwater in the adult stage. None of the ticks that dropped before the start of the febrile reaction, or those that commenced feeding during the 20 days thereafter, could transmit the disease in the adult stage (J. D. Bezuidenhout & J. A. Olivier, unpublished data, 1986). Barré & Camus (unpublished data, 1986) found that the infectivity of goats to ticks was limited from 2 days before the temperature reaction to 3 days after its termination. In another experiment nymphae were unable to transmit the disease after they had fed as larvae over a period of 42 days on an immune sheep which was challenged with virulent blood (J. D. Bezuidenhout & J. V. Badenhorst, unpublished data, 1986). Unfortunately the infectivity of this sheep's blood was not determined by subinoculation.

BEHAVIOUR OF *C. RUMINANTIUM* IN THE TICK

Homogenates prepared from *A. hebraeum* larvae during and after feeding on a reacting sheep were not infective when inoculated intravenously into susceptible animals (Du Plessis, 1982). In the same way homogenates from the same group of ticks, but made after they had moulted to the nymphal stage, were not infective in the case of the Kümm strain of *C. ruminantium* (Du Plessis, 1982). They were, however, infective in the case of the Ball 3 strain but only when a high concentration (5 nymphae) was injected into susceptible sheep (J. D. Bezuidenhout, unpublished data, 1981).

Soon after flat, infected nymphae were allowed to feed, their infectivity increased markedly until they were fully engorged and dropped from the host. Infectivity persisted for most of the nymphal stage but tended to drop during the last few days before moulting to the adult stage. The infectivity of adults followed a similar pattern to that of the nymphae except that suspensions from flat adults were infective although at a low level.

Eggs that originated from infected females were not capable of producing heartwater after injection into susceptible animals (J. D. Bezuidenhout, unpublished data, 1981).

An electron microscope study of *A. variegatum* nymphae failed to demonstrate organisms for a period of 15 days after they had been allowed to feed on a reacting ox. Only after this time were organisms in colony form detectable in gut epithelial cells (Kocan, Morzaria, Voigt, Kiarie & Irvin, 1986). Cowdry (1925) only observed organisms in unfed *A. hebraeum* nymphae 40 days after they had become infected as larvae. These findings indicate that a certain period of time is necessary for the organism to infect tick tissues and for the development of detectable colonies. It is not known whether the increase in infectivity is due to multiplication of the organism or an increase in virulence.

There is no obvious difference in the number of colonies found in the gut of pre-fed adults compared to those in flat adults (J. D. Bezuidenhout, unpublished data, 1984). More work is needed to confirm this observation. An increase in infectivity of flat ticks is not brought about by incubation at 37 °C for 2–3 days (A. J. van Winkelhoff, 1979, cited by Uilenberg, 1983a). The negative results obtained by Theiler & Du Toit (1928) in this regard cannot be considered valid because the control ticks were also negative. Suspensions prepared from flat adults that have not been incubated at 37 °C can be infective (see above). Consequently the 1 successful attempt with incubated flat adults (R. H. Dwinger, 1981, cited by Uilenberg, 1983a) could have been a normal finding in that incubation may not have been responsible for the infectivity of the ticks. It seems, therefore, that feeding on the host is important in stimulating an actual increase in infectivity.

TRANSMISSION OF *C. RUMINANTIUM* BY TICKS

Cowdry (1925) was unable to demonstrate organisms in the salivary glands of infected ticks. He did, however, find organisms in the gut epithelium and sometimes in the gut lumen. He was of the opinion that infection of the host took place either by regurgitation or excretion of the organism from the alimentary canal.

Saliva collected from groups of infected *A. hebraeum* adults in some instances has been found to be infective (Bezuidenhout, 1981). Possible contamination with gut material and the low viability of the organism could have been responsible for false positive and negative results in this work and consequently it is difficult to draw any final conclusions.

Colonies of *Cowdria* have recently been demonstrated in salivary gland acini of pre-fed *A. hebraeum* nymphae that had been infected in the larval stage (Kocan, Bezuidenhout & Hart, 1987). This discovery together with the finding that salivary gland homogenates prepared from infected pre-fed adult females were infective (J. D. Bezuidenhout, unpublished data, 1985), suggests that transmission takes place through saliva and that gut regurgitation may or may not play a role.

Very little information is available on the length of the feeding time required for transmission to take place. Neitz (1968) mentions briefly that organisms are liberated within 24 h after tick attachment.

In a recent study infected nymphae and adults were allowed to feed on susceptible sheep for varying periods before the sheep were treated with an acaricide (J. D. Bezuidenhout & J. A. Olivier, unpublished data, 1986). In the case of the nymphae only those animals on which the ticks had been present for 38 h or more contracted heartwater. The animals on which the nymphae were present for 18 or 26 h did not become infected. In the case of the adult ticks, which had been infected as larvae, transmission occurred after the ticks had been present on the animals for 75 h but not for 20, 26 or 50 h.

It is difficult to determine the exact time it takes ticks to attach. Not all of them attach at the same time. In the above-mentioned experiments it took roughly 6 h before a reasonable number of ticks had attached. This period has been included in the times mentioned above.

FUTURE STUDIES

In spite of all the information available on the natural transmission of heartwater, experimental evidence is lacking on the reasons for a low or high percentage of infected ticks on a farm. In an endemic area there are few clinical cases of heartwater on which ticks can become

infected and although the blood of immune animals is infective after reinfection it has not been proved that ticks will become infected when feeding on such animals.

Through subinoculation it has been established that blood of recently recovered animals (irrespective of whether recovery was spontaneous or after treatment) is infective for a certain period of time (Ilemobade, 1978). However, ticks feeding on such animals apparently do not easily become infected, if at all (J. D. Bezuidenhout, unpublished data, 1986). The role that wild hosts may play in the maintenance of infection is perhaps more important than previously suspected and this aspect needs further investigation.

Intrastadial transmission by ticks or even mechanical transmission by insects should again be studied to determine whether they play any role in the epidemiology of the disease.

It will be interesting to know whether all *Cowdria* isolates are equally well transmitted by a specific strain of *Amblyomma* and by the different species of *Amblyomma*. Different strains of the same species of *Amblyomma* may show a difference in their rate of infectivity when infected with the same *Cowdria* isolate. These differences, if any, could be very important in the preparation of highly infective tick suspensions for the purpose of vaccination, inoculation of cultures, antigen production for serological tests and purification.

A study of the factors that may influence the success rate of transovarial transmission could prove to be a very important project, especially if the findings of Bezuidenhout & Jacobsz (1986) can be confirmed.

The effect of time and temperature of incubation of non-parasitic stages of ticks on the infectivity of these ticks needs further investigation. It has been reported (Lounsbury, 1906) that ticks kept at a low temperature (cool room) have failed to transmit heartwater whilst ticks collected from the same animals at the same times, but exposed to higher temperatures, transmitted the disease in a number of cases. This finding has been used to explain the erratic appearance and disappearance of the disease under natural conditions.

The development of an easy and practical laboratory test to determine the infectivity of individual ticks is important for many aspects of heartwater research.

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