

# Epidemiology of heartwater in Nigeria

by A. A. ILEMOBADE and P. LEEFLANG (\*)

## RÉSUMÉ

Des études épidémiologiques et essais au laboratoire ont montré qu'*Amblyomma variegatum* est un vecteur de *Cowdria ruminantium*, organisme causal de la cowdriose au Nigeria.

L'infection initiale des tiques était réalisée en utilisant des larves et des nymphes d'*A. variegatum*. La transmission trans-stadiale n'a pu être démontrée.

Bien qu'au laboratoire aucune différence n'ait été observée entre nymphes mâles et femelles quant à leur capacité relative de transmettre *C. ruminantium*, les résultats des études sur le terrain ont suggéré que, dans la nature, les femelles jouent un rôle plus important dans la transmission du pathogène que les autres stades.

Il a été démontré qu'une seule femelle d'*A. variegatum* est capable de transmettre l'infection ; les tiques mâles peuvent également transmettre le parasite, mais ils le font moins régulièrement que les femelles.

A propos d'une épidémie survenue dans un centre de recherches, le rôle que certains animaux, autres que les tiques, pourraient avoir joué dans l'épidémiologie de la cowdriose, a été discuté. Dans ce cas particulier, certains rongeurs pourraient avoir servi de réservoir d'infection.

## INTRODUCTION

The widespread distribution of heartwater (*Cowdria ruminantium* infection) in Nigeria has long been recognized (2) but its importance in the livestock development has not fully been appreciated. Recently, however, large-scale projects involving improvement of indigenous ruminant production and importation of exotic breeds of cattle and sheep, have been embarked upon by Federal and State governments and heartwater has proved to be one of the restraining factors. Consequently, efforts are being made to control the disease.

Up till now, the epidemiology of heartwater in Nigeria has received only casual attention. In order to establish a rational basis for control, therefore, an understanding of the epidemiology of the disease under Nigerian conditions is a pre-requisite.

## MATERIALS AND METHODS

The tick, *Amblyomma variegatum*, has been incriminated in the transmission of heartwater in Nigeria without substantial evidence (2). This link between *A. variegatum* and heartwater appears to be based on « guilt by association » rather than any systematic investigation. In attempting to establish the role of *A. variegatum* in the epidemiology of the disease in Nigeria, therefore, the basic criteria listed by BARNETT (3) for incriminating a specific arthropod with transmission of any causative agent of a disease were adopted. Essentially, these are :

a) biological association of *A. variegatum* infestation of, and occurrence of clinical or subclinical heartwater infection in, ruminants ;

b) demonstration of *C. ruminantium* in *A. variegatum* under natural conditions, and

c) transmission of heartwater by *A. variegatum* under controlled conditions.

(\*) Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

## Experimental animals

Initially, Yankassa sheep (10) obtained from Jibiya in Katsina province were used for the isolation of *C. ruminantium*. Later, because these sheep showed variable susceptibility to the pathogen, cross-bred Friesian-Zebu calves raised under tick-controlled conditions, and brown goats, a variety of Red Sokoto (10), obtained locally from Zaria area, were the only animals used later. Experiments have shown that the brown goats and cross-bred calves are highly and uniformly susceptible to heartwater with mortality of 100 p. 100 (6).

On arrival in the laboratory, all animals were routinely treated with a therapeutic dose of Thiabendazole (Merck Sharp and Dohme), sprayed against ticks and subsequently kept in a tick-free facility. Blood smears from all animals were routinely examined every week for the presence of blood parasites. Two to 3 weeks elapsed before the animals were used and they were in apparently healthy conditions at the start of the experiments.

## Strain of *Cowdria ruminantium*

The strain of *C. ruminantium* used in the transmission experiments was isolated from a cow showing clinical signs of heartwater. The strain, designated D225, was passaged through goats and subsequently preserved at low temperatures (7).

## Experimental procedures

### 1) *Biological association of A. variegatum infestation of, and occurrence of clinical or subclinical heartwater infection in, ruminants*

The seasonal incidence of *A. variegatum* in northern Nigeria and in particular with reference to the Zaria area has been reported by MOHAMMED (11). This information was used along with personal field observations.

The incidence of heartwater was based on laboratory examination of brain specimens from animals presented for necropsy at the department of Veterinary Pathology, Ahmadu Bello University (ABU), Zaria, during the period 1971-75 (table 1). The sample originated from local and research farms around Zaria. The problems of field diagnosis has led to restriction of data collection to these locations. In order to make interpretation of the small number of data more acceptable and minimize bias, therefore, incidence of heartwater was also based on whether or not the disease was diagnosed in a particular month per 5 year period (1971-75).

Squash smears were made from the cerebrum of brain specimens according to the method described for cerebral babesiosis by LEE-FLANG (8), stained with Giemsa solution and examined microscopically ( $\times 790$ ) for the presence of *C. ruminantium*.

In addition, a record was kept during the years 1974 and 1975 of those animals which

TABLE 1 Seasonal incidence of heartwater based on brain specimens presented for laboratory diagnosis.

Month	1971	1972	1973	1974	1975	Total for 5-year period	Monthly diagnosis of heartwater per 5-year period.
January	0	0	6	0	0	6	1/5
February	0	0	3	0	0	3	1/5
March	0	0	0	0	1	1	1/5
April	0	0	0	1	0	1	1/5
May	0	0	0	5	1	6	2/5
June	0	1	0	7	1	9	3/5
July	2	1	1	3	2	9	5/5
August	1	0	1	0	1	3	3/5
September	0	1	0	0	2	3	2/5
October	2	0	0	0	2	4	2/5
November	0	2	0	0	2	4	2/5
December	1	0	0	3	1	5	3/5

died of heartwater and the proportion of those infested with adult *A. variegatum* was recorded (table 2).

TABLE 2 The relationship of animals which died of heartwater and the proportion infested with adult *Amblyomma variegatum* (1974-1975)

Month	N° of animals which died of heartwater	N° infested with adult <i>A. variegatum</i>
January	0	0
February	0	0
March	1	0
April	1	1
May	6	5
June	8	6
July	5	5
August	1	0
September	2	0
October	2	0
November	2	0
December	4	0

## 2) Demonstration of *C. ruminantium* in *A. variegatum* under natural conditions

Samples of partially engorged adult *A. variegatum* were collected from apparently healthy cattle and also from animals which died of heartwater (table 3).

TABLE 3 Experimental initiation of heartwater in sheep and cattle with the suspension of adult *Amblyomma variegatum* collected from the field

Origin of ticks	Animal inoculated	Result
Collected on heartwater positive cow	Sheep 516	Reacted thermally and recovered <sup>a</sup>
Collected on trade cattle	Sheep 561	Died of heartwater
Collected on breeding cattle	Sheep 567	N° reaction <sup>b</sup>
	Sheep 512	Died of immediate shock
Collected on heartwater-positive cow	Ox 522	Died of immediate shock
	Ox 525	Died of immediate shock

<sup>a</sup> was positive for heartwater on blood subinoculation

<sup>b</sup> animal proved resistant on challenge inoculation.

Approximately 70 p. 100 of the ticks in each batch were males and the rest were females. They were kept in an incubator maintained at 28 °C and relative humidity (RH) of 80-95 p. 100 for 4 days. Three females and 7 males from

each batch were randomly selected and were ground up in a mortar with 10 ml cold (4 °C) phosphate buffered saline (PBS) pH 7.3. Sterile sand was added to aid thorough grinding and the final suspension was obtained by filtering the mixture through Whatman grade 1 filter paper. Of each suspension, 2 ml were injected intravenously into individual experimental animals. Due to high mortality resulting from intravenous injection of tick suspension, the experiment was limited to only 4 trials (table 3).

## 3) Transmission of heartwater by *A. variegatum* under controlled conditions

Four batches of engorged female *A. variegatum* collected from apparently healthy cattle were kept separately in an incubator maintained at 28 °C and RH of 80-95 p. 100. All subsequent stages were reared under the same conditions. Two to 3 weeks after hatching, a random sample of about 300 larvae from each batch was ground up as already described for adults and 5 ml of the suspension injected intravenously into an experimental goat ; while a second batch of 300 was allowed to feed on another goat. Using this procedure, it was concluded that neither batch of larvae was infected with *C. ruminantium*. Uninfected nymphs and adults were obtained from a proportion of these larvae by feeding on goats raised free of ticks.

Initiation of infection in ticks with *C. ruminantium* was carried out with larvae and nymphs (table 4). In both cases, the procedure consisted of placing ticks on animals using the ear-bag technique. The animals were infected by intravenous inoculation of D225 stabilate the same or the day previous to placement of ticks. This method was also adopted in the transmission experiments (table 4) except that adults were fed on scrotum of goats using the scrotal-bag technique. It was found that preattachment of males, as has been shown for *Amblyomma hebraeum* by LOUNSBURY (9), was a condition for female attachment in *A. variegatum*. Thus, in general, males were first allowed to attach before females were placed, the period of male attachment varying usually between 2 and 6 days.

Engorged larvae and nymphs were collected daily after they dropped into the bags and were allowed to moult into the next stage. Similarly, engorged females were collected and allowed to lay eggs which hatched into larvae.

TABLE 4 Experimental transmission of heartwater by *Amblyomma variegatum* to bovine and caprine species.

Expt.	Tick Batch	L → X → A → L → N → A					
		L	X	A	L	N	A
1	S1	I 16 (40)	X 13 (16)				
2	S2	I 1331 (1800)	X 15 (20)	X 1F (1F)**	O 721 (550x2)	O 60 (72)	O 3F, 8M (5F, 8M)
3	S3	I 76 (110)	X 20 (26)	X 4F, 5M (4F, 6M)	O 1120 (1400)	O 26 (32)	O 8F, 10M (12F, 12M)
4	S4	O 1400 (1700)	I 140 (198)	X 3F, 9M (5F, 9M)			

\* Additional 9 uninfected males were also attached.

L = larvae; N = nymphs; A = adults.  
 F = female; M = male.  
 I = stage at which infection was initiated.  
 X = stage at which infection was transmitted.  
 O = stage failing to transmit infection.

Figures are the number of ticks which successfully attached and fed to engorgement, except males.

Figures in parenthesis represent the number of ticks placed.

Experimental animals were infested with the different stages of *A. variegatum* about 2-3 weeks after emergence and the criteria for successful transmission were the presence of clinical signs of heartwater and demonstration of *C. ruminantium* in brain squash smears of infested animals. All animals which did not contract the disease were subsequently challenged with D225 stabilate to establish their susceptibility.

The hypothesis that (a) one infected *A. variegatum* could transmit heartwater (18) and (b) that male ticks could effect heartwater transmission (2) was tested in the following experiments. One of the female ticks (table 4) which had transmitted infection as nymphs was used. Nine uninfected males raised in the laboratory were first allowed to attach on a goat before the female was placed. In the second experiment, 60 males were randomly selected from a batch infected as larvae. Twenty each were placed on 3 susceptible goats.

#### 4) Epidemiology of heartwater under static but improved animal husbandry as obtained on Shika Agricultural Research station, near Zaria

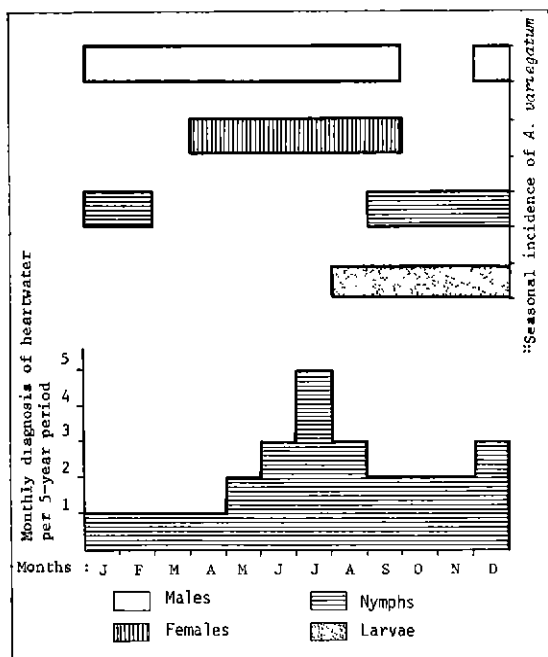
A case study of an outbreak of heartwater on this research station was undertaken as an

example of a situation that can occur under static but improved husbandry system. The farm lies about 22 km north-west of Zaria. Presently, its main function is to provide facilities for research in forage and animal production. At the end of 1973, there were 345 sheep of local breeds (Uda and Yankassa) and 488 cattle of which 21 were purebred Friesians, 250 were Friesian-Zebu cross-breeds and the rest were indigenous Zebu breeds. All animals were maintained under a close system of management. The animals were routinely sprayed against ticks fortnightly or at long intervals during the dry season (November to April) and weekly during the wet season (May to October).

## RESULTS

### 1) Incidence of heartwater in relation to seasonal activity of *A. variegatum*

The seasonal incidence of *A. variegatum* (11) and the incidence of heartwater based on brain smear examination (table 1) have been combined and graphically illustrated in figure 1. Table 2 illustrates the relationship of the animals which died of heartwater and the proportion infested with adult *A. variegatum*.



\*Based on data of Mohammed (11).

Fig. 1. — Comparative incidence of heartwater over a period of five years and seasonal incidence of *Amblyomma variegatum*.

## 2) Demonstration of *C. ruminantium* in *A. variegatum* under natural conditions

Inoculation of suspension of adult *A. variegatum* into experimental animals resulted in immediate shock and fatality in 3 out of 6 animals used at different times. The details of the results are presented in table 3. Two of the 3 animals which survived inoculation, contracted heartwater, demonstrating that *C. ruminantium* is present in *A. variegatum* under natural conditions.

## 3) Transmission of *C. ruminantium* by *A. variegatum* under controlled conditions

The details of transmission trials are shown in table 4. Initiation of infection with *C. ruminantium* was achieved with both larvae and nymphs of *A. variegatum*. Transstadial transmission was consistently successful, but transovarial transmission could not be demonstrated as checked in the filial generation.

Heartwater was successfully transmitted by one infected tick (female) as shown in table 4; only one out of the three goats infested with male ticks, however, contracted the disease. The two goats which did not react were fully susceptible on challenge inoculation.

## 4) Case study of heartwater on Shika Agricultural Research station, 1974

For 5 years prior to 1974, only 2 confirmed cases of heartwater were reported from the station, one in a sheep (1972) and the other in a calf (1973). However, towards the end of May, 1974, there was an outbreak of the disease, first in the milking herd of about 35 cows and then sporadically in other herds. In addition, clinical cases of anaplasmosis and babesiosis were diagnosed. The milking herd suffered serious losses not only due to death but also to marked drop or even cessation of milk yield. This herd had been restricted to the barn and was fed mainly on hay and silage for one year until April, 1974 when the cows were turned out to graze on the pasture due to shortage of food. This coincided with the beginning of the annual rains. The records showed that after February, 1974 the animals had not been sprayed for protection against ticks. The first death due to heartwater occurred on May 31, 1974. Subsequently, six confirmed cases occurred in quick succession. Mass treatment with oxytetracycline and Berenil (Farbwerke Hoechst A.G.) was immediately instituted and all animals were sprayed against ticks. Daily rectal temperature was taken and any animal showing fever of unknown origin was again treated with oxytetracycline. No further cases occurred after these measures were instituted.

## DISCUSSION

It is clear from the body of evidence obtained in the present study that the incrimination of *A. variegatum* as a vector of heartwater in Nigeria is conclusive.

While caution is dictated in interpreting figure 1 as relates to annual incidence of heartwater because of the limited and restricted data collection, certain trends are, nevertheless, evident and call for comments. The major peak incidence of heartwater which occurs during the female activity (fig. 1) suggests that, in nature, females are more efficient than other stages in transmitting *C. ruminantium*. This is probably due to favourable climatic conditions during the seasonal activity of the female, since in the experiments (table 4) no difference was observed in the relative ability of both nymphs and females to transmit infection.

Since transovarial transmission did not occur and infection was easily initiated with larvae and nymphs (table 4), it is suggested that the overlap in the activity of infected adults and uninfected larvae and nymphs (fig. 1) provided opportunity for these latter two stages to become infected. This can only occur when a non-tick reservoir of infection exists during this period of overlap. This point will later be further amplified.

The persistence of heartwater immediately after the period when females are found must be related to nymphal activity; while the minor peak would seem to be a result of male activity (fig. 1). Field observations showed that feeding activity of males is restricted to the period immediately following attachment and tails off or ceases later because of limitation imposed by their integument. Consequently, their ability to transmit infection must be limited by this peculiar feeding characteristic. This observation is supported by transmission trials with male *A. variegatum* and the low incidence of heartwater immediately following the minor peak in spite of the presence of both nymphs and males. However, the role of the males becomes important again when their attachment is required as a pre-requisite for female attachment.

The transmission of *C. ruminantium* under controlled experiments confirms and extends the observations of DAUBNEY (4), NEITZ (17) and UILENBERG (20) that the pathogen is transstadially transmitted. It also shows that, like *Amblyomma hebraeum* (9), *A. variegatum* does not transmit *C. ruminantium* transovarially.

The 1974 outbreak of heartwater in Shika farm coincided with the breakdown of tick control measures after 5 successive years in which only 2 cases of the disease were diagnosed and during which time tick control was uniformly good. Since transovarial transmission does not occur, there must have been a reservoir of infection other than ticks on the farm to precipitate this outbreak. NEITZ (12, 13, 14, 16) showed that antelopes were susceptible to heartwater and in most cases did not manifest clinical signs. He suggested that they might

act as reservoir hosts. NEITZ (15) also demonstrated that recovered animals could serve as a carrier of the pathogen for up to 60 days after clinical recovery. Furthermore, NEITZ *et al.* (19) and ILEMOBADE (6) found that immune animals could, after reinfection, maintain *C. ruminantium* for a length of time sufficient to infect ticks. HAIG (5) demonstrated that the organism persisted in mice for up to 90 days.

There are no game animals in Shika; rodents, however, are present. The tick control programme was rigid as was evidenced by the fact that when this control broke down, not only was clinical heartwater diagnosed, but also clinical anaplasmosis and babesiosis occurred. It is clear, therefore, that under such a rigid tick control programme, *A. variegatum* could not depend on ruminants on the farm for feeding, for maintaining their life-cycle, nor for maintaining a persistent heartwater infection. It follows, therefore, that *A. variegatum* population used animals other than the farm animals to complete their life-cycle.

The upsurge and build-up of tick population immediately following the breakdown of tick control indicate that during the rigid spraying period, *A. variegatum* had maintained themselves on bait animals other than the ruminants. Since infestation of the cattle led to an outbreak of heartwater, these bait animals must, in all probability, also have served as reservoir for *C. ruminantium*. As *Amblyomma* sp. maintain themselves on a wide variety of hosts, the identification of bait animals under the situation in Shika will remain an open question. It can be speculated, however, that rodents may fulfill such a role.

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## SUMMARY

### Epidemiology of heartwater in Nigeria

Evidence from epidemiological and laboratory studies demonstrated that *Amblyomma variegatum* is a vector of heartwater in Nigeria. Initiation of infection in ticks was carried out using larvae and nymphs

of *A. variegatum*. Transstadial transmission was consistently successful, but transovarial transmission could not be demonstrated. Although no difference was observed in the relative ability of both nymphs and females to transmit *C. ruminantium* under laboratory conditions, field studies suggested that, in nature, females play a more important role in the transmission of the pathogen than do other stages.

Evidence was obtained that a single female *A. variegatum* is capable of transmitting infection and that males could also transmit infection although not regularly.

The role of animals other than ticks in the epidemiology of heartwater relative to the outbreak at a research farm has been discussed and it is speculated that rodents could serve as a reservoir of infection.

## RESUMEN

### Epidemiología de la heartwater en Nigeria

Estudios epidemiológicos y ensayos en laboratorio mostraron que *Amblyomma variegatum* es un vector de *Cowdria ruminantium*, organismo causal de la cowdriosis en Nigeria.

Se inocularon larvas y ninfas de *A. variegatum* para realizar la infección inicial. La transmisión trans-estadial del parásito siempre tuvo éxito; en cambio no se pudo demostrar una transmisión transovarial.

No se observó en laboratorio ninguna diferencia entre ninfas machos y hembras en cuanto a su capacidad relativa de transmitir *C. ruminantium*. Sin embargo, según los estudios sobre terreno, se sugiere que, en la natura, las hembras desempeñan un papel más importante en la transmisión del agente patógeno que los otros estadios.

Se ha demostrado que una sola hembra de *A. variegatum* puede transmitir la infección; las garrapatas machos igualmente pueden transmitir dicho parásito, pero lo hacen menos regularmente que las hembras.

A propósito de una epidemia ocurrida en un centro de investigaciones, se discute el papel que ciertos animales, otros que las garrapatas, podrían haber desempeñado en la epidemiología de la cowdriosis.

En este caso, ciertos roedores podrían haber servido de reservorio de infección.

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