The Role of *Amblyomma variegatum* in the Transmission of Heartwater with Special Reference to Guadeloupe

E. CAMUS AND N. BARRÉ

IEMVT-CIRAD
P.O. Box 1232
97 184 Pointe-à-Pitre Cedex
Guadeloupe, French West Indies

INTRODUCTION

In the early nineteenth century, heartwater was reported for the first time in South Africa.[1] It was probably also introduced from Senegal in Guadeloupe in the same period with African zebu cattle infested with *Amblyomma variegatum*.[2] However, if the disease has been actively investigated in South Africa since 1898, it was diagnosed only in 1980 in Guadeloupe.[3] A global study of heartwater transmission was lacking and different aspects were better documented for *A. hebraeum* than for *A. variegatum* in Africa. *A. variegatum* can be considered the most important vector in Africa because of its wide distribution, its close adaptation to domestic ruminants, and its efficacy as a vector.[4] It is the only vector of heartwater in the Caribbean.

In Guadeloupe, surveys and experiments have been conducted since 1982 to determine the following parameters of *A. variegatum*: the tick infestation rate on animals, the tick infection rate, the vector efficacy, the daily inoculation rate, additional pathogenicity induced by ticks, the delay of transmission after fixation, and the longevity of infected ticks. These parameters will be presented here and compared or supplemented with data from Africa on the same tick species and on *A. hebraeum.*

THE TICK INFESTATION RATE

In Guadeloupe, the most important hosts of *A. variegatum* are obviously the domestic ruminants. Nearly 100 per 100 of *A. variegatum* adults are observed on cattle and goats and also the majority of larvae and nymphs (TABLE 1).

The dogs probably play an important role in the local dissemination of *A. variegatum* in Guadeloupe because they are numerous (50,000) and most of them feral; they can be heavily infested and, experimentally, the *A. variegatum* female can engorge and lay viable eggs.[6]

Cattle egrets (*Bubulcus ibis*) move between islands and are perhaps involved in the recent dissemination of *A. variegatum* in the Lesser Antilles (J. Corn, N. Barré, and B. Thiebot, unpublished data). In Africa, *A. variegatum* is one of the major tick parasites of cattle over a wide area; it presents the widest distribution of the *Amblyomma* ticks, living in areas from 0 to 1590 meters high, where the rainfall ranges from 400 to 2800 mm.[7] Sheep and goats are infested to a lesser degree than cattle. Medium and large

TABLE 1. Hosts of *A. variegatum*[5]

	Cattle Goats (%)	Dogs Mongoose (%)	Chicken Caribbean Grackle Cattle Egret (%)
Adults	100	0	0
Nymphs	96.7	2.9	0.4
Larvae	94.9	4.6	0.6
Total	95.6	3.9	0.5

mammals as well as birds are frequent hosts of nymphs whereas larvae are recorded predominantly in birds and small mammals.[8]

The tick infestation rate of domestic ruminants is low in Guadeloupe, that is, in 50 per 100 of the farms where the animals are regularly sprayed with acaricides (20 treatments per year) (TABLE 2).

TABLE 2. Mean Tick Infestation in 22 Farms of Guadeloupe[9]

	Cattle	Goats
Male	1.23	0.11
Female	0.31	0.09
Nymphs	1.23	1.24
Larvae	9.55	11.3

In Senegal, without acaricide treatments, the tick burden is higher on cattle but lower on goats.[10] In Uganda,[11] Tanzania,[12] and Zambia,[13] the adult ticks mean is always higher than 10 for cattle during the rainy season.

Because of the persistent humidity, there is no absolute break in the activity of *A. variegatum* in Guadeloupe and the tick is established everywhere ruminants are present—that means almost all over the island.[5]

The low infestation rate in Guadeloupe compared to that in Africa is partly compensated by the high density of domestic ruminants and by the yearlong persistence of *A. variegatum.*

THE TICK INFECTION RATE

The tick infection rate (TIR) is a key parameter in the epidemiology of tick-borne diseases in general and of heartwater in particular. This parameter was developed in Australia to study babesiosis.[14]

Three different methods used in Guadeloupe to determine the TIR yield very close results.[9]

1. Inoculation of 50 individual tick homogenates collected from one farm into susceptible goats: 2 to 4% of females are considered infected.

2. Inoculation of 200 individual tick homogenates collected from 22 farms into mice and research of antibodies to *Cowdria* 5 wk later according to the Du Plessis method[15] yielded 2.7% infected females and no infected males.

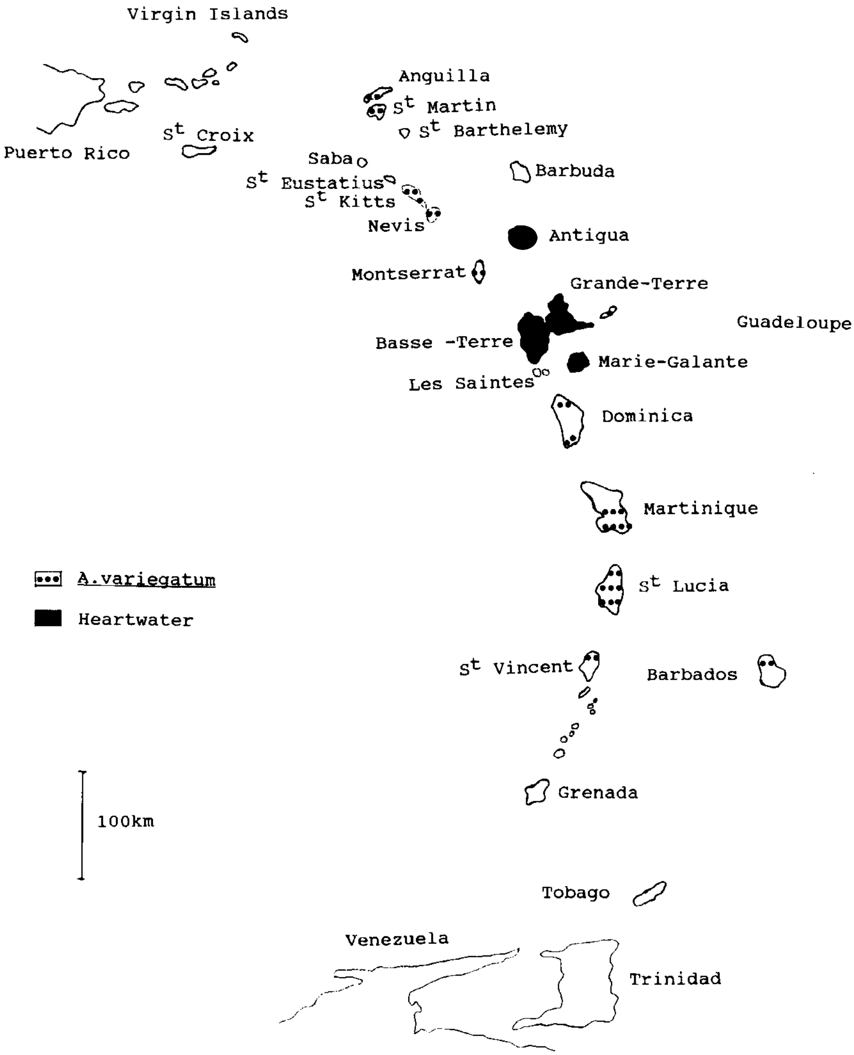

FIGURE 1. Distribution of *A. variegatum* and heartwater in the Caribbean.

3. Calculation of probability: Clinical cases of heartwater were diagnosed in every district in Guadeloupe but a survey with 19 pools of 105 ticks inoculated into susceptible goats indicated that only 12 of 19 pools were infected. Negative reactions (p) depend on the TIR (t) and on the size of the sample (n). Thus, $p = (1 - t)^n$. If we know (p) and (n), $t = 1\%$.

If the three islands of Guadeloupe (Grande-Terre, Basse-Terre, and Marie-Galante) are considered separately, and the data from Antigua examined (survey made in collaboration with the University of Gainesville, FL), the TIR is 1.2% for Basse-Terre, 0.6% for Grande-Terre, 2.5% for Marie-Galante, and 0.2% for Antigua (FIG. 1).

The TIR appears very low in Guadeloupe and Antigua which could be related to the very short rickettsemia in creole goats and creole cattle[9] and to the very low tick infestation rate.

In Madagascar, the TIR is also supposed to be very low considering the high percentage of adult cattle naturally exposed to *A. variegatum* and still susceptible to experimental heartwater.[16] In Senegal, a low number (1-14 nymphs and 1-11 adults) of *A. variegatum* is involved in the transmission of heartwater to sheep 2-4 wk before clinical cases;[17] in fact many others are present several months before the clinical disease and the TIR could be as low as 4% if only one tick is infected in each case.

The TIR looks higher in Zimbabwe with *A. hebraeum* collected on calves and on the ground and fed after moulting on susceptible sheep: 0-44.9% for males, 34.1-36.1% for females, and 0-13.4% for nymphs.[18]

Wide variations are observed in South Africa with *A. hebraeum* adults inoculated into mice[19]: 1.6-30%, the higher percentage being observed after outbreaks of heartwater. The importance of the TIR is fundamental in understanding the epidemiology of heartwater and in trying to control the disease. The low TIR in Guadeloupe is corroborated by the low cattle and goat infection rate (21% and 30%, respectively),[9] the unstable epidemiological situation, and the very slow dissemination of the disease (three islands infected for a century or more) compared to its vector (17 islands infested). If the TIR is also low in Senegal and Madagascar, which are infested with *A. variegatum,* the higher results observed in South Africa and mainly in Zimbabwe with *A. hebraeum* could be explained by a better vector efficacy of this last species and/or by a longer carrier state[20] and/or differences between stocks of *Cowdria.* It can be hypothesized that very pathogenic strains, such as those found in Senegal and Gardel (Guadeloupe), that induce rapid mortality on susceptible animals, shorten the rickettsemic period during which ticks can be infected. Also, such pathogenic strains are more likely to select resistant animals which could express also a shorter or a lower rickettsemia (to be confirmed).

THE VECTOR EFFICACY

The vector efficacy concerns the specific possibility for a tick to be infected and to transmit the infection. Thus far, the comparison deals only with the different stages of the same species and not with different species.

Comparison between Stages

Approximately 50% of *A. variegatum* nymphs and females and 9% of males are actually infective after a blood meal on a rickettsemic goat at the larval and nymphal stages.[9] However, the infectivity rate of nymphs (53%) appears higher (Chi 2, $p < 0.01$) than the infectivity rate of adults infected as nymphs ($p < 0.14$).

The American *A. cajennense* can experimentally transmit heartwater from larval to nymphal stage, but apparently not from nymphal or larval to adult stage;[21] the same phenomenon is observed with *A. sparsum,*[22] indicating that for some species nymphs are the most efficient or the only vector. However, *A. hebraeum* adults infected as nymphs are reported to be more efficient in demonstrating a carrier state on recovered animals,[20] suggesting differences between species. Adult *A. hebraeum* did not show the same ability in another experiment.[23]

Ticks Infected and Not Infective

A. variegatum larvae and nymphs fed during the rickettsemia can be infected without being infective, and the infection acquired in the larval stage can be transmitted only in the adult stage but not in the nymphal one. In these cases *C. ruminantium* may be present in the gut but not in the salivary glands. This could explain the difficulty in finding *Cowdria* in the salivary glands.[24] On the other hand, one single nymph, female or male, is sufficient to infect a susceptible animal.[9]

Transovarial and Transstadial Transmission

The transovarial transmission of *Cowdria* is described with *A. hebraeum* but seems to have a low incidence.[25] This transmission has failed in Guadeloupe with *A. variegatum.*[9] Males of *A. hebraeum* transferred from live or dead hosts to susceptible hosts are able to transmit *Cowdria* and have perhaps a higher epidemiological importance than previously suspected.[26] However, males of *A. variegatum* are unable to detach from a dead host during the days following death (N. Barré, unpublished data).

The *A. variegatum* strain of Martinique, occurring in that island that was apparently disease-free since 1948, has not lost its ability to experimentally transmit the Gardel strain of *Cowdria* (E. Camus, unpublished data).

As part of a survey on TIR, it is not sufficient to determine the infection of ticks; it is also necessary to evaluate what percentage of infected ticks can actually transmit heartwater.

THE DAILY INOCULATION RATE

The daily inoculation rate (DIR) was defined[14] to evaluate the epidemiological stability of babesiosis. The DIR represents the daily probability for an animal to be in contact with an infective tick.

The DIR is calculated in Guadeloupe, using the preceding data on tick infestation rate, tick infection rate, and vector efficacy.[9] The DIR is very low: approximately 0.20% for cattle and 0.14% for goats. The epidemiological situation of heartwater in Guadeloupe is unstable because the majority of susceptible goats (98%) and cross-bred cattle (96%) will not be infected and immunized during the period of natural resistance (3 wk for cattle and 15 days for goats). In Africa, the natural resistance can last 6 months for zebus[27] and 6 wk for goats.[28]

This unstable situation is confirmed by surveys of farms in Guadeloupe where nearly 10% of goats die from heartwater every year. The owners of cross-bred cattle take better care of their animals than do goat owners, often treating them against heartwater and babesiosis as soon as fever appears. Consequently, outbreaks are less frequent in cattle than in goats.

A stable situation could be achieved with a minimum DIR of 2% for cattle and 7% for goats. That means, compared to the Guadeloupean situation, inoculations 10 times higher for cattle and 50 times for goats. The infestation rates observed on cattle in East and South Africa are certainly high enough to maintain an epidemiological stability, even with a low tick infection rate. In contrast the stability is unlikely to occur for goats: a tick infection rate and a tick infestation rate both 7 times higher for the nymphs, the main vectors of heartwater to goats, would be needed. The calculation of DIR in Guadeloupe indicates that 63% of the infections to cattle and 91% of the infections to goats are transmitted by nymphs.

The unstable situation for goats in Guadeloupe is balanced by the existence of lineages of creole goats resistant to heartwater.[29]

OTHER CHARACTERISTICS OF THE TRANSMISSION

Two other features—the additional pathogenicity induced by ticks and the longevity of infected ticks—were never considered. Another feature (delay of the transmission) was examined both in Africa and Guadeloupe, and the last one (cycle of *Cowdria* in *Amblyomma*) derives from data from Africa.

Additional Pathogenicity Induced by Ticks

In Guadeloupe, heartwater seems to be more virulent when tick-transmitted than when needle-transmitted. In addition, when adult ticks are involved, fever is suppressed or very much shortened. This additional pathogenicity is induced by the tick itself and not by a particular stage of *C. ruminantium* in the tick, because tick homogenates are less virulent than tick transmission. The inoculation is not equivalent to natural infection, and, as a practical consequence, a standardized batch of ten infected nymphs is currently used to infect susceptible animals.

Tick saliva is perhaps associated with an immunodeficiency as it was hypothesized for acute dermatophilosis.[30] Such acute dermatophilosis is closely related to *A. variegatum,* and the additional pathogenicity could be a particularity of *A. variegatum.*

Delay of the Transmission after Tick Fixation

The transmission of heartwater occurs 2-3 days after the fixation of *A. variegatum* nymphs and 4 days after for adults.[9] Slightly shorter periods are observed with *A. hebraeum*: 27-38 h in the case of nymphs and 51-75 h in adults.[22] This period corresponds to the end of the fixation and the beginning of the bloodsucking phase.[5] To prevent the transmission of heartwater, an acaricide used every fourteenth day should remain effective for at least 12 days, which is the extreme limit of the best available acaricide.

Longevity of Infected Ticks

No difference can be observed between the longevity of infected and noninfected *A. variegatum,* between the weight of infected and noninfected *A. variegatum,* nor any correlation made between the weight of ticks and their longevity (E. Camus & N. Barré, unpublished data). The maximum longevity is 18 months for nymphs and 23 months for adults in laboratory incubators.[5]

The Cycle of Cowdria *in* Amblyomma

The light microscopic examination,[31] the EM,[32] the inoculation of organ homogenates, and the IFAT[33] demonstrate the presence of *C. ruminantium* in the following organs: gut (epithelial cells and lumen), malpighian tubules, salivary glands, hypodermis, rectal ampullae, saliva, and hemocytes. Different morphological aspects are observed: electron-dense and reticulated forms (gut); small, compact to larger colonies with individual organisms (salivary glands); and reticulation and dividing by binary fission (salivary glands).[24]

The previous hypothesis about the *C. ruminantium* transmission by regurgitation[31] is now replaced by a transmission via the saliva.[24] A delay of 15 days is necessary between the end of the infected blood meal and the presence of *Cowdria* in the tick gut.[32]. The most likely hypothesis for the cycle is then a multiplication of *Cowdria* in the gut epithelial cells and a transport to the salivary glands by the hemocytes. Some ticks cannot be infected, some are only infected but not infective, and some are infective. The infected ticks could be the ticks with *Cowdria* in the gut and the infective ones, ticks with *Cowdria* in the salivary glands.

CONCLUSION

The transmission of *Cowdria ruminantium* by *A. variegatum* in Guadeloupe presents some very different characteristics compared to *A. hebraeum* in Zimbabwe or in South Africa. In Guadeloupe the tick infection rate is much lower and nymphs play the major role. The differences between the *Amblyomma* species and the *C. ruminantium* stocks could explain different results. The different methods used to study the role of ticks in both situations may be also involved. More sophisticated methods, such as DNA probes, will be helpful in confirming the results.

SUMMARY

Heartwater has been diagnosed in Guadeloupe, Marie-Galante, and Antigua; it induces important losses among goats and European or cross-bred cattle when local zebu creole are highly resistant to infection.

Amblyomma variegatum is the vector of the disease in the Caribbean. The tick strain of Martinique, occurring in that island that has apparently been disease-free since 1948, has not lost its ability to experimentally transmit the disease. In Guadeloupe 97% of nymphs and nearly 100% of adults feed on cattle and goats. Some immature ticks (4.5%) feed on wildlife, including birds that may be involved in the spread of infected ticks. Only 1 to 4% of adult ticks are infected and only a proportion of infected ticks are really infective: 53%, 9%, and 50% of nymphs, males, and females, respectively. Nymphs play the major role in the transmission: they are more numerous than adults and engorge faster. Infected ticks have the same maximum longevity as noninfected ticks, that is, 18 months for nymphs and nearly 23 months for adults.

Cowdria is not transmitted immediately after tick fixation but after a delay of 2-3 days for nymphs and 4 days for adults. The disease is more often fatal when transmitted by tick biting rather than by needle transmission.

The daily infection rate that summarizes all the parameters is very low (0.14% and 0.20% for goats and cattle, respectively), resulting in an unstable epidemiological situation.

The transmission of *Cowdria* by *A. variegatum* in Guadeloupe shows significant differences compared with the transmission by *A. hebraeum* in Africa.

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