The prevalence of *Cowdria ruminantium* in free-living adult *Amblyomma hebraeum* collected at a communal grazing area and in 2 wildlife reserves in South Africa

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ABSTRACT

In order to detect the prevalence of *Cowdria ruminantium* in the vector tick, *Amblyomma hebraeum,* free-living, unfed adult ticks were collected with the aid of pheromone/CO_2 traps. Ticks were collected at the Rietgat communal grazing area, as well as in the southwestern Kruger National Park and in the Songimvelo Game Reserve, all located in heartwater-endemic areas of South Africa. The presence of *C. ruminantium* in these ticks was determined by polymerase chain reaction (PCR) analysis. Ticks from the Rietgat communal grazing area were assayed in 2 batches and 4.7 % of the one and 11.3 % of the other were positive for infection, while 5.7 % of the ticks collected in the Kruger National Park and 25 % in the Songimvelo Game Reserve were positive. These results support the contention that a vector–wildlife cycle of transmission of *C. ruminantium,* the cause of heartwater in domestic ruminants, can be maintained in the absence of the latter animals.

Key words: *Amblyomma hebraeum,* communal grazing area, *Cowdria ruminantium* prevalence, PCR analysis, wildlife reserves.

Bryson N R, Horak I G, Venter E H, Mahan S M, Simbi B, Peter T F **The prevalence of *Cowdria ruminantium* in free-living adult *Amblyomma hebraeum* collected at a communal grazing area and in 2 wildlife reserves in South Africa**. *Journal of the South African Veterinary Association* (2002) 73(3): 131–132 (En.). Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

The advancement of our understanding of the epidemiology of heartwater in domestic and wild ruminants has undoubtedly been hampered by the poor diagnostic tests available to detect the presence of *Cowdria ruminantium,* the causative organism of this disease, in free-living, unfed vector ticks of the genus *Amblyomma.* The tests used in the past were indirect and many of these were cumbersome, expensive and involved the use of experimental animals, and their sensitivity and specificity were unknown. In the current project, free-living, unfed *Amblyomma hebraeum,* the principle vector of *C. ruminantium* in South Africa, were collected in heartwater-endemic regions and the presence of the organism determined by polymerase chain reaction (PCR) analysis.

Several studies to find a reliable method of detecting the presence of *C. ruminantium* in vector ticks have been conducted in southern Africa. In the first study, adult *A. hebraeum* were collected from cattle throughout the heartwater-endemic regions of South Africa, individually homogenized and the homogenates injected either intraperitoneally or intravenously into mice. Of the 240 ticks collected, 13 were positive for *C. ruminantium,* a figure based on the fact that 1 mouse died of heartwater and 12 others seroconverted[5]. In an extensive epidemiological study of 23 farms in heartwater-endemic regions of South Africa, 6 adult *A. hebraeum* were collected from each survey animal, and the homogenates of these ticks injected intravenously into mice[6]. Of these ticks 7 % were positive for *C. ruminantium.* More recently 292 free-living adult *A. hebraeum,* collected with pheromone/CO_2 traps near the Skukuza rest camp in the Kruger National Park, were subjected to PCR analysis and 1.7 % were found to be positive for infection with *C. ruminantium*[15].

In the southern lowveld of Zimbabwe, pools of free-living *A. hebraeum* nymphs (male or female) were fed on heartwater-susceptible sheep[9] and the prevalence of *C. ruminantium* infection in the various pools was calculated using a formula adapted from researchers in mosquito vector epidemiology[4,9]. The infection rate in the nymphs ranged from 0–13 %, in males from 0–45 %, and in females from 20–36 %. More recently adult *A. hebraeum* that had attached to sentinel cattle were collected in the southwestern lowveld of Zimbabwe in an area considered to be heartwater-endemic[12]. Of these ticks 1.7 % were positive on PCR analysis[12] while in the southern lowveld of Zimbabwe the infection rate in male *A. hebraeum* collected from cattle was 10.5 %, and 12.45 % in females and 3.16 % in nymphs[13].

In the current investigation pheromone/CO_2 traps were used to collect free-living, unfed, adult *A. hebraeum* in 3 heartwater-endemic regions of South Africa[2,3]. Ticks were collected at the Rietgat communal grazing area (25°24′S, 27°49′E) in North West Province, as well as in the vicinity of the Skukuza rest camp (24°58′S, 31°36′E) in the southwest of the Kruger National Park, Mpumalanga Province, and in the Songimvelo Game Reserve (28°57′–29°05′S, 30°52′–31°00′E), Mpumalanga Province. These ticks were sent to the University of Florida, United States Agency for International Development/Southern African Development Community Heartwater Research Project at the Central Veterinary Diagnostic and Research Laboratory in Harare, Zimbabwe. Here the DNA in the first batch of ticks from the Rietgat communal grazing area was extracted using phenol, chloroform, isoamyl-alcohol (PCIA)[8]. That in the second batch was initially extracted with PCIA, but problems with PCR inhibitors were experienced and these samples were re-extracted to purify the DNA by means of Q1A amp tissue DNA extraction columns in accordance with the manufacturer's instructions (Qiagen, CA, USA). The DNA of the ticks from the 2 wildlife reserves was extracted by means of Q1A amp tissue extraction columns. Thereafter the presence of infection was determined by the analysis of each tick with *C. ruminantium*-specific polymerase chain reaction (PCR) and DNA probe assays[15,16].

The prevalence of infection in the ticks

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Received: March 2002. Accepted July 2002.

Table 1: **PCR assay of *Cowdria ruminantium* infection in free-living, unfed adult *Amblyomma hebraeum* collected at 3 localities in South Africa.**

Locality	Number of adult ticks assayed	Prevalence of *C. ruminantium*
Rietgat communal grazing area	150[a]	4.7 %
Rietgat communal grazing area	284[b]	11.3 %
Kruger National Park	88[b]	5.7 %
Songimvelo Game Reserve	48[b]	25.0 %

[a]PCIA extraction.
[b]Q1A amp extraction.

collected from the various localities is summarised in Table 1. The 4.7 % infection rate in the batch of ticks collected at the Rietgat communal grazing area and extracted with PCIA corresponds to that in some of the previous surveys in South Africa[5,6,15], while the rate of 11.3 % in the batch extracted with Q1A amp DNA extraction columns is more in line with some of the higher rates recorded in Zimbabwe[13]. The prevalence of infection in the ticks collected in the Kruger National Park was 5.7 %, and that of those collected in the Songimvelo Game Reserve 25 %. It would, however, appear that not only do infection rates differ considerably from one heartwater-endemic region to another, but also with the various diagnostic techniques employed.

The high infection rates in free-living, unfed adult *A. hebraeum* at the Rietgat communal grazing area has important implications for endemic stability to heartwater in cattle in this region. The high prevalence of *C. ruminantium* in the ticks indicates that a fairly small population of parasitic ticks could ensure endemic stability and hence acaricide treatment applied solely to control tick damage should not be allowed as it could interfere with this stability.

The presence of *C. ruminantium* in 5.7 % of the unfed, free-living ticks collected in the Kruger National Park was higher than the 1.7 % recorded in a previous survey conducted here[15]. The prevalence of infection in ticks in the Songimvelo Game Reserve appears to be very high at 25 % and a larger sample than the 48 ticks analysed may have given another result. The occurrence of *C. ruminantium* in ticks in the 2 wildlife reserves is important epidemiologically because in neither reserve was there any contact between wild and domestic animals. A sylvatic life cycle for *C. ruminantium* has previously been suspected on the basis of infection in wild ruminants[10,14] and in free-living adult *A. hebraeum*[15]. The demonstration of infection in free-living, unfed adult *A. hebraeum* in the 2 wildlife reserves in the present study adds credibility to this belief.

Large animals such as African buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*) and giraffe (*Giraffa camelopardalis*) are the preferred hosts of adult *A. hebraeum*, while they, as well as a variety of smaller animals, are hosts to the immature stages of the tick[7]. Several of these animals are important not only because they sustain the tick population but also because they serve as reservoirs of *C. ruminantium*[1,11,14]. The presence of infection in the ticks in the 2 wildlife reserves is thus not unexpected as wild ruminants, *A. hebraeum*, and *C. ruminantium* are likely to have had a long association, with the former probably being the original hosts of the latter.

ACKNOWLEDGEMENTS

The community at Rietgat is thanked for granting us access to their grazing area, and Drs L E O Braack and R G Bengis and their staff for assistance in the Kruger National Park. The University of Florida/ USAID/SADC Heartwater Project, the Foundation for Research Development, the University of Pretoria and the Medical University of Southern Africa are thanked for financial support.

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