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Cowdria ruminantium identified in *Amblyomma gemma* using a DNA probe pCS20

WESONGA (F.D.), MUKOLWE (S.W.), FRED RURANGIRWA. Identification de *Cowdria ruminantium* dans la tique *Amblyomma gemma* par une sonde ADN, pCS 20. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46**, (1-2) : 179-181

Des tiques de l'espèce *Amblyomma gemma* ont été récoltées sur des animaux sauvages dans un ranch de 10 000 hectares, dans une région endémique pour la cowdriose au Kenya, proche de Nairobi. *A. variegatum* est le vecteur principal de la cowdriose au Kenya. E.A. LEWIS a incriminé *A. gemma* comme vecteur de la cowdriose, dans un rapport publié en 1947, sans donner de détails. Des *A.gemma* adultes ont été récoltés sur girafe (*Giraffa camelopardis*), bubale (*Alcephalus busephalus*), antilope canna (*Taurotragus oryx*) et autruche (*Struthio camelus*). Les tiques non gorgées prélevées sur girafe ont été nourries sur 3 moutons Dorper sensibles, qui ont été examinés quotidiennement pour des signes cliniques de la cowdriose. Toutes les tiques, y compris celles nourries sur les moutons, ont été disséquées et les intestins ont été testés sur la présence de *Cowdria ruminantium* à l'aide d'une sonde ADN, la pCS20. Aucun des moutons sur lesquels les tiques ont été nourries n'a montré de symptômes de la cowdriose pendant les 60 jours d'observation après la fixation des tiques. La sonde ADN a identifié *C.ruminantium* dans les tiques prélevées sur antilope et girafe.

Mots clés : Antilope - Girafe - Ovin - Cowdriose - *Cowdria ruminantium* - Isolement - Tique - *Amblyomma gemma* - Sonde ADN.

INTRODUCTION

Amblyomma gemma is the commonest species of *Amblyomma* on the Hopcraft ranch(4) located 20 km South East of Nairobi.This tick species has been reported to be capable of transmitting heartwater in the laboratory (15) and to be one of the vectors of heartwater in Kenya (6); however this was a single statement report and no details were given.

The main objective of the study was to establish if *A. gemma* is a vector of the disease on the ranch or if it simply acts as a reservoir of *Cowdria ruminantium* but playing little role in the actual transmission (of the disease). NEITZ (9) reported that infection in *Amblyomma* species can survive in a single tick generation for over 3 years.

Two methods were used to determine if the ticks were infected : feeding the ticks on susceptible sheep and probing the midguts of the adult ticks using a cloned DNA probe, the pCS20.

Because of its sensitivity and the large number of samples involved, a cloned DNA probe is suitable for detecting *Cowdria* in tick vectors (14). *C. ruminantium* has been detected in *A.variegatum* (14) using a cloned DNA probe, pCS20 derived from a strain of the parasite isolated from Crystal Springs (Zimbabwe).This probe is recommended for the detection of *C. ruminantium* as it has a high level of specificity and sensitivity (compared to another clone, the pCS9 from the Kiswani strain of Kenya).

MATERIALS AND METHODS

The study was conducted over a 3 month period, late October to January. This is the period of the short rains and there is a relative increase in the number of most tick species (including *A. gemma)* on the ranch (12).

Feeding of the ticks on sheep

Adult *A. gemma* ticks were collected from 4 species of wildlife eland, giraffe, ostrich and hartebeest, during the weekly cropping exercises.

The unengorged ticks were attached to sheep within 24 hours of collecting them from the wild animals. Three Dorper sheep were used in the experiment. A total of 104 unengorged ticks, all from the giraffe (very few ticks were obtained from the other 3 wild animal species) were attached on the sheep with the help of earbags. The earbags were opened after 9-12 days. By this time, some of the female ticks were fully engorged and dropping off. The earbags were left on the sheep up to 70 days to allow the males to engorge and drop off.

During the entire period of tick feeding, the sheep were monitored by daily rectal temperature records and clinical inspection.

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DNA separation from the mid-guts of *A. gemma* and probing for *C. ruminantium*

The separation of the DNA material from the tick mid-guts and the subsequent DNA probing for *C. ruminantium* was done as described by WAGHELA *et al.* (14) the only difference being that the mid guts were pooled in groups or batches of 10 according to the animal species from which they were collected.The exception to this group size was for the ostrich and hartebeest where only 5 and 2 ticks respectively were collected.

RESULTS

Feeding of the ticks on sheep

None of the sheep on which *(A. gemma)* ticks were fed showed heartwater symptoms for up to 70 days after tick attachment. One of the sheep died of pneumonia 48 days after tick attachment. A brain crush smear was made and on observation there were no *Cowdria* colonies.

Detection of *C. ruminantium* DNA in the ticks

The pCS20 probe detected *C. ruminantium* in 2 out of the 3 tick batches collected from the giraffe and in the batch (one) from the eland. All the ticks from the ostrich and hartebeest were negative.

DISCUSSION

Whereas it is known that A. *gemma* can become infected and transmit *C. ruminantium* under laboratory conditions, the results of this experiment do indicate that there is indeed a good chance of this tick species acquiring the infection in an endemic area and subsequently transmitting the infection to susceptible livestock under natural conditions.

However, the efficiency of this tick species as vector compared to *A. variegatum* is not known. Further experiments need to be done to ascertain this.

There is little information on the host range of the immature stages of *A. gemma*. Since transmission of infection is transstadial, it is important from the epidemiological point of view to know the hosts on which these immature stages feed and thus acquire infection. The next phase of this study will focus on this.This will be useful in providing information regarding the control strategies to be adapted in cases of outbreaks especially in the non-endemic areas. *A. gemma* appears to play a bigger role in the epidemiology of heartwater in Kenya than it is thought.

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***Amblyomma gemma* ticks were collected from wild animals on a 20,000 acre game ranch in a heartwater endemic area in Kenya, close to Nairobi. *A. variegatum* is the main vector of heartwater in Kenya. E.A. LEWIS, 1947, in a one sentence report has implicated *A.gemma* to be a vector of heartwater without giving any details. Adult *A. gemma* were collected from giraffe *Giraffa camelopardalis,* hartebeest *Alcephalus busephalus* , Eland *Taurotragus oryx* and ostrich *Struthio camelus* during cropping exercises. The unengorged ticks were fed on 3 susceptible Dorper sheep which were monitored daily for the clinical symptoms of heartwater. All the ticks, including those that were fed on sheep were dissected and the guts probed for the presence of *Cowdria ruminantium* using a cloned DNA probe, the pCS20. None of the sheep on which the ticks were fed showed heartwater symptoms up to 60 days from the attachment of the ticks. The DNA probe identified *Cowdria ruminantium* in the ticks collected from eland and giraffe.**

Key words : Eland - Giraffe - Sheep - Heartwater - *Cowdria ruminantium* - Isolation - Tick - *Amblyomma gemma* - DNA probe.

WESONGA (F.D.), MUKOLWE (S.W.), FRED RURANGIRWA.
Identificación de *Cowdria ruminantium* en *Amblyomma gemma* mediante el uso de ADN probador pCS20. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46**, (1-2) : 179-181

Se recolectaron garrapatas de *Amblyomma gemma* en reses bravías, en un rancho de 10 000 hectáreas, en una zona endémica para la cowdriosis en Kenia, cerca de Nairobi. *A. variegatum* es el principal vector de la cowdriosis en Kenia. En un reporte de 1947, E. A. LEWIS, menciona *A. gemma* como vector de la cowdriosis, pero no proporciona mayor detalle. Los adultos de *A. gemma* se colectaron de jirafas (*Giraffa camelopardalis*), búbalos (*Alcephalus busephalus*), antílope (*Taurotragus oryx*) y avestruces (*Struthio camelus*) durante diversas prácticas de cultivo. Las garrapatas, no ingurgitadas, fueron alimentadas sobre tres ovejas Dorper susceptibles, en las cuáles se controlaron diariamente los síntomas clínicos de la cowdriosis. Todas las garrapatas, incluyendo aquellas alimentadas en ovejas, fueron disecadas y se examinaron los intestinos para la presencia de *Cowdria ruminantium* , mediante la utilización de clones probadores de ADN, el pCS20. Sesenta días después del inicio de la alimentación de las garrapatas, ninguna de las ovejas mostró síntomas de cowdriosis. El probador de ADN identificó *Cowdria ruminantium* en las garrapatas colectadas de antílopes y jirafas.

Palabras calves : Antílope - Jirafa - Ovino - Cowdriosis - *Cowdria ruminantium* - Aislamiento - Garrapata - *Amblyomma gemma* - Sonda de ADN.