

RESEARCH COMMUNICATION

THE PRESENCE OF *COWDRIA RUMINANTIUM* ANTIGEN IN VARIOUS TISSUES OF *AMBLYOMMA HEBRAEUM* IMAGINES AS DETECTED BY AN ENZYME-LINKED IMMUNOSORBENT ASSAY

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ABSTRACT

VILJOEN, G. J., NEITZ, A. W. H., BEZUIDENHOUT, J. D., OBEREM, P. T. & VERMEULEN, N. M. J., 1988. The presence of *Cowdria ruminantium* antigen in various tissues of *Amblyomma hebraeum* imagines as detected by an enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, 55, 239–240 (1988)

Investigation into the presence of *C. ruminantium* antigen, using an enzyme-linked immunosorbent assay (ELISA) in various tick tissues and haemolymph of adult *Amblyomma hebraeum* ticks revealed that the organism invades a number of body parts and can be demonstrated in *A. hebraeum*. In females, the gut, salivary glands, hypodermis and synganglion and in males, the salivary glands and gut showed the highest concentration.

INTRODUCTION

Early studies by Cowdry (1925) on the development of *Cowdria ruminantium* in the tick *Amblyomma hebraeum* revealed that the organism was present in the epithelial cells of the intestine and in the lumen of the gut. He was, however, unable to find the organism in the salivary glands of the tick. The deduction was therefore made that the transmission might occur as the result of regurgitation during feeding. Bezuidenhout (1981) found that the infectivity of saliva collected from infected *Amblyomma hebraeum* females was very low. In 1987, Kocan, Bezuidenhout & Hart demonstrated *C. ruminantium* to be present in midgut epithelial cells and salivary glands of nymphal *A. hebraeum* ticks.

Further investigations regarding the localization of the organism in the tick would be of value in the study of the survival of the organism in the tick, its transstadial transmission in the vector and its transmission from vector to host. In the present work therefore, various organs of infected ticks were dissected and screened for *C. ruminantium* by an enzyme-linked immunosorbent assay (ELISA) with both heartwater positive and heartwater negative sera.

MATERIALS AND METHODS

Analytical quality reagents were used. All glassware and equipment were sterilized with 70% (v/v) ethanol and the buffers by filtration through 0,22 µm filters (Millipore).

Detection of *C. ruminantium* antigen in *Amblyomma hebraeum* imagines

(a) Collection of tick tissue and haemolymph

A. hebraeum larvae were allowed to attach on laboratory infected sheep (Bezuidenhout, 1981). In the nymph or adult stage the ticks were allowed to feed on either heartwater-infected or susceptible sheep. The adult ticks were immediately collected when they dropped off and dissected in ice-cold buffered lactose phosphate. Of the *A. hebraeum* Ball 3 strain-infected males and females, the salivary glands, brain, Malpighian tubules, gut, rectal sac, hypodermis and intestinal remnants were dissected and collected. Of the *A. hebraeum* Kümme strain-infected females (Kümme strain-infected males were not available), the salivary glands, brain, hypodermis, gut, malpighian tubules, rectal sac, ovaries and intestinal remnants were dissected and collected. Tissues obtained

from 25 ticks (for each group) were suspended in 0,5 ml BLP. Haemolymph was collected from the stumps of severed trochanters and the amount collected from 25 ticks (for each group) was made up to 0,5 ml with BLP. Four groups, containing tissues from 25 ticks per group, were stored in a liquid nitrogen freezer and later screened with ELISA in quadruplet.

(b) Preparation of tick tissue and haemolymph extracts

Samples were thawed and homogenized for 2 min with an Ultra Turrax at 4 °C. The homogenates were then sonified for 10 s with a sonifier using a micro-tip with output control at 3 and centrifuged for 30 min at 1 000 × g in a Rotor 40, using a Beckman L5–65 ultracentrifuge. The supernatants were then centrifuged for 30 min at 10 000 × g and the resultant supernatant centrifuged at 30 000 × g for 30 min. The final sediment was resuspended in 0,5 ml of 0,154 M NaCl. This represented the crude extract.

Preparation of antiserum

Four heartwater negative sheep, as determined by the ELISA, were used. Heartwater Ball 3-infected and heartwater non-infected *A. hebraeum* nymphae were used to infect the four sheep (Neitz, Viljoen, Bezuidenhout, Oberem, Putterill, Verschoor, Visser & Vermeulen, 1986). Serum from the 2 heartwater positive-infected and the 2 heartwater negative-infected sheep was prepared as described by Viljoen, Vermeulen, Oberem, Prozesky, Verschoor, Bezuidenhout, Putterill, Visser & Neitz (1985). The blood samples (10 ml) were collected 4 weeks after treatment of the infected sheep and left to clot. After 4 h the coagulated blood was centrifuged for 10 min at 300 × g in a Piccolo bench top centrifuge at room temperature. The sera were siphoned off, divided into 1 ml batches and stored at –75 °C.

Protein determinations

Protein was determined by the high temperature Biuret-Folin method as described by Dorsey, McDonald & Roels (1977), using bovine serum albumin as standard.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA method described by Neitz *et al.* (1986) was used. Serum solutions were made up as follows: 1 ml of serum was diluted 1:30 with a 0,05 M Tris and 0,154 M NaCl buffer (pH 7,4). As antigens, 10 µg protein/ml of the crude extracts were used. ELISA screening was performed at least in quadruplet on each of the four groups of tick tissues. P/N ratios were calculated by dividing the absorbance values obtained from positive-infected serum, by the absorbance value from negative-infected serum.

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RESULTS

Investigations into the presence of *C. ruminantium* antigen in the various tick tissues and haemolymph of adult *A. hebraeum* ticks revealed that the organism invades a number of body parts (Table 1). In females, the gut and synganglion (brain) and in males, the salivary glands and gut showed the highest signals.

TABLE 1 ELISA screening¹ of *Cowdria ruminantium* (Ball 3- or Kümm-strain) antigen in *Amblyomma hebraeum* males and females

Antigen screened	Material source			
	P/N ratio ²			
	Male (Ball 3 strain)	Female (Kümm strain)	Male (Ball 3 strain)	Female (Ball 3 strain)
Salivary glands	1,8 ± 0,2	1,6 ± 0,1	1,9 ± 0,1	2,2 ± 0,2
Synglanglion	1,2 ± 0,1	1,8 ± 0,1	1,3 ± 0,1	2,1 ± 0,2
Haemolymph	1,4 ± 0,2	1,3 ± 0,2	1,1 ± 0,1	1,4 ± 0,2
Malpighian tubules	1,1 ± 0,1	1,1 ± 0,1	1,1 ± 0,1	1,2 ± 0,2
Gut	1,7 ± 0,1	1,9 ± 0,2	2,3 ± 0,2	2,7 ± 0,3
Ovaries	—	1,1 ± 0,1	—	1,1 ± 0,1
Rectal sac	1,1 ± 0,1	1,1 ± 0,2	1,2 ± 0,2	1,4 ± 0,2
Hypodermis	1,4 ± 0,2	1,6 ± 0,1	1,3 ± 0,1	1,6 ± 0,1
Intestinal remnants	1,5 ± 0,1	1,4 ± 0,2	1,3 ± 0,2	1,4 ± 0,1

¹ Mean of four determinations

² Antigen was screened with heartwater positive-infected (P) and heartwater negative-infected (N) sheep sera

DISCUSSION

Several tissues of both female and male *A. hebraeum* ticks seem to harbour *C. ruminantium* organisms. *C. ruminantium* are present in the males, although the males are poor vectors of the disease. This may be related to their slower feeding habits (Ilemobade, 1976). The presence of antigen in the salivary glands and in the gut suggests that the pathogen can be transmitted as the result of salivation and/or regurgitation during feeding. The latter mode was proposed by Cowdry (1925). *C. ruminantium* absence in the ovaries is consistent with the notion that the organism is not generally transmitted transovarially from generation to generation (Neitz, 1968). *In vivo* investigations by Bezuidenhout (1988) showed *C. ruminantium* organisms to be present in the synganglion of Kümm strain-infected *A. hebraeum* females but absent in the Ball 3 strain-infected *A. hebraeum* females, while we demonstrated organisms to be present in both. In contrast to our findings, Bezuidenhout (1988) demonstrated organisms to be present in the

malpighian tubules of both strains. These differences may be due to differences in the stage and rate of infection or the feeding stage of the ticks at the time of organ dissection. It might also be possible, however, that due to the undetermined sensitivity of this ELISA, some of the organs found to be negative by us may contain a low concentration of *C. ruminantium*.

It should be borne in mind that transmission of a pathogen by its vector to a host could be by one or more of the following routes; salivation, regurgitation during feeding, faeces or coxal or dermal secretions entering the wound made by the mouthparts of the vector. However, the survival of the labile *C. ruminantium* organism is highly unlikely in an external environment (Camus & Barré, 1982; Uilenberg, 1983).

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