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RESEARCH COMMUNICATION

DEMONSTRATION OF *COWDRIA RUMNANTIUM* IN *AMBLYOMMA HEBRAEUM* BY FLUORESCENT ANTIBODY TECHNIQUES, LIGHT AND ELECTRON MICROSCOPY

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ABSTRACT

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Positive identification of *Cowdria ruminantium* in *Amblyomma hebraeum* was obtained by direct and indirect fluorescent antibody techniques, and by light and electron microscopy. Colonies of organisms could only be demonstrated with certainty in the epithelial cells and lumen of the alimentary tract of ticks.

INTRODUCTION

The morphology and distribution of *Cowdria ruminantium* in *Amblyomma hebraeum* was first studied by means of conventional histological sections and Giemsa-stained smears (Cowdry, 1925). Cowdry found groups or colonies of organisms in epithelial cells, and sometimes in the lumen, of the alimentary tracts of ticks which had fed on animals infected with heartwater. *Cowdria* organisms were never detected with certainty in any other tissue of the vector and it was speculated that infection took place either by regurgitation or by excretion of infective organisms from the alimentary canal.

Ticks were found to be a very effective amplifying system for C. *ruminantium* and a suspension of *A. hebraeum* nymphae, infected during their larval stage, can in fact be used to vaccinate cattle and sheep against heartwater (Bezuidenhout, 1981).

Due to difficulties encountered in the *in vitro* cultivation of *Cowdria,* isolation and purification of organisms derived from the tick vector became essential. The possibility of obtaining a purified source of organisms for the development of serological tests, and for other purposes, prompted a study of *C. ruminantium* in the tick.

Some observations on the morphology and distribution of *C. ruminantium* in *A. hebraeum* as found under fluorescent, light and electron microscopy are reported herein.

MATERIALS AND METHODS

Experimental ticks

Adult ticks belonging to 2 strains of *A. hebraeum* (Spes Bona X and Spes Bona Q) were used in these studies. The ticks were infected with *C. ruminantium* by feeding the larval stage on reacting sheep, as previously described (Bezuidenhout, 1981). Control ticks from both strains were fed throughout their parasitic life cycle on heartwater-susceptible sheep.

Light and electron microscopy

Unfed and partially-fed adult ticks were used. Ticks were restrained in paraffin wax, covered with phosphate buffered saline (PBS) and cut in half from the anterior to the posterior end along the mid-dorsal line. One half of each tick was fixed, embedded and sectioned for light and electron microscopy using techniques described by Kocan, Ewing, Holbert & Hair (1982). The remaining halves of the ticks have been processed for animal inoculation studies to be conducted at a later stage.

Fluorescent antibody (FA) studies

Adult ticks, unfed and prefed, from the infected and control groups were used. Whole ticks were frozen and sectioned (2–4 μm) at –18 °C using a cryostat microtome. Sections were fixed and stained for direct (DFA) and indirect (IFA) fluorescent antibody studies according to standard methods. For the DFA, serum from a goat with a titre of 1:2560 against the mouse adapted heartwater-Kumm strain (Du Plessis & Kumm, 1974) was conjugated with fluorescein isothianate according to previously described methods (Peacock, Burgdorfer & Ormsbee, 1971) with certain modifications (Oberem, Bezuidenhout, Viljoen, Neitz & Vermeulen 1984).

After the globulins had been precipitated and labelled, the conjugate was adsorbed to liver and tick powder to reduce non-specific fluorescence.

The IFA tests were done using mouse (anti-Kumm) as well as goat and bovine (anti-Ball 3) sera. Frozen sections were also stained with Giemsa (Purchase, 1945), methyl green and pyronin Y and acridine orange (Burdin, 1962).

RESULTS

Light microscopy

Thick epoxy-embedded sections (1 μm) differentiated with Mallory's stain revealed colonies of *C. Ruminantium* similar to those described by Cowdry (1925) in *A. hebraeum* and by other workers in the mamalian host (Pienaar, 1970; Stewart & Howell, 1981). In *A. hebraeum* these colonies were found most frequently near the basement membrane of the gut epithelium (Fig. 1 & 2). They varied greatly in size (3–45 μm) and shape as well as in the number and size of organisms within them. With the exception of a single finding of highly suspicious colonies in the malpighian tubules, organisms have not as yet been seen in any of the other tissues. Colonies were not observed in sections of tissues from the control ticks. Frozen sections stained with Giemsa, methyl green and pyronin Y and acridine orange gave unsatisfactory results.

Electron microscopy

Up to now, it has not been possible to study all the different types of colonies seen under the light microscope with the electron microscope. However those that were found closely resemble previously described forms occurring in the mammalian host (Pienaar, 1970). Organisms 0,25–1,2 μm in diameter are contained in a membrane-bound vacuole in the cytoplasm of gut epithelial cells. Furthermore, every organism is surrounded by a double unit membrane (Fig. 3 & 4).

Fluorescent antibody studies

In both DFA and IFA studies colonies showing specific fluorescence were found in the gut epithelium and corresponded exactly with those viewed under normal white light (Fig. 5 & 6). Their morphology and distribution in the gut also corresponded with those of the colonies found in thick electron microscope sections differentiated with Mallory's stain. There was minimal non-specific fluorescence of the gut and other organs but the

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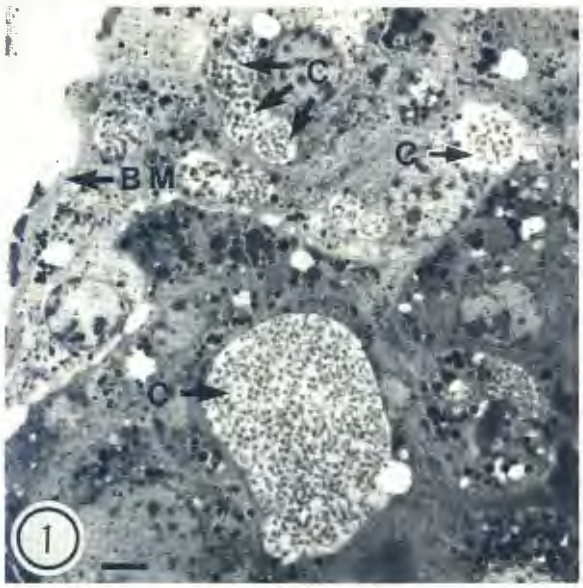

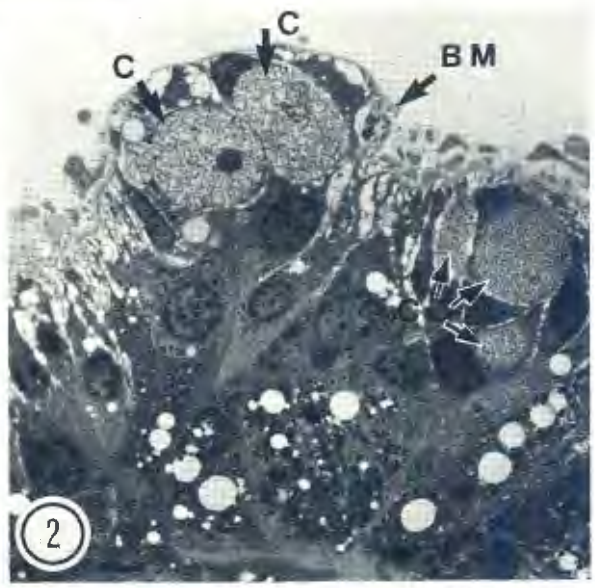

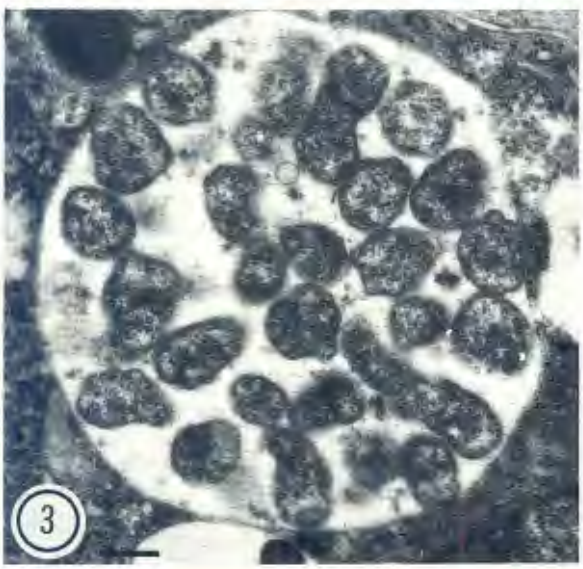

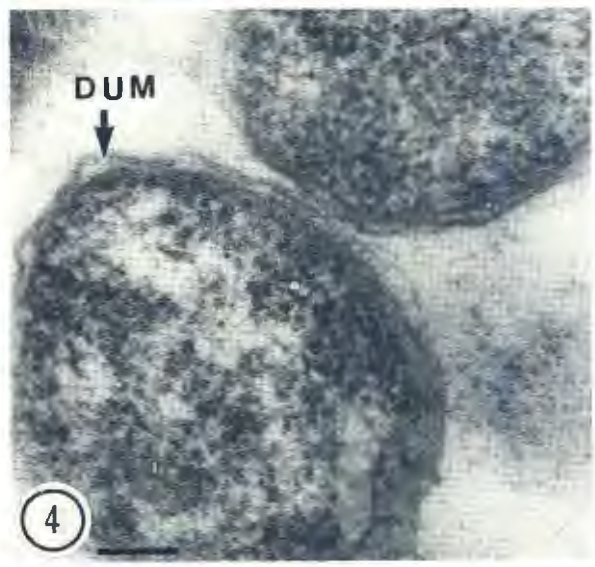

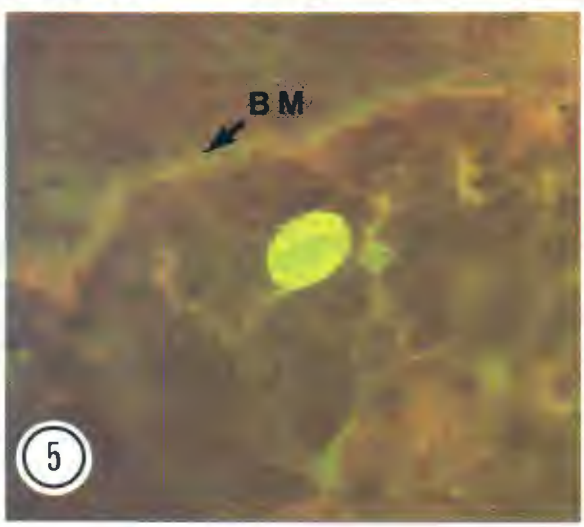

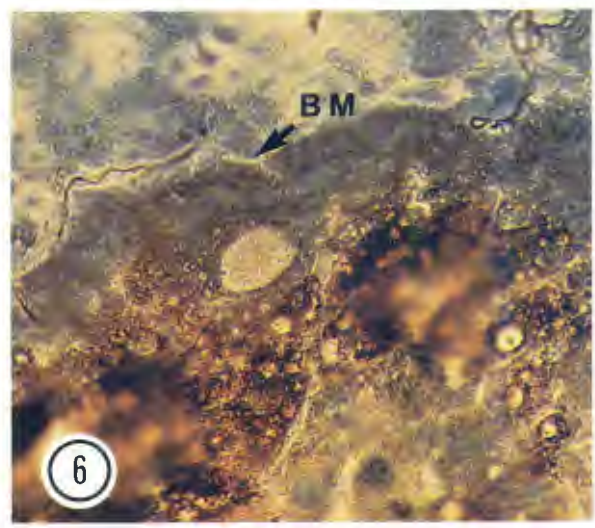

FIG. 1 One large and numerous small colonies of *Cowdria ruminantium* (C-arrows) in gut epithelial cells near the basal membrane (BM-arrow) in prefed *Amblyomma hebraeum* male. Mallory's stain; × 1 200. Bar scale = 5 μm

FIG. 2 Colonies of *Cowdria ruminantium* (C-arrows) in gut epithelial cells near the basal membrane (BM-arrow) in prefed *Amblyomma hebraeum* female. Mallory's stain; × 1 100. Bar scale = 5 μm

FIG. 3 Electron micrograph of a *Cowdria ruminantium* colony in a gut epithelial cell from a prefed *Amblyomma hebraeum* male, × 6 500. Bar scale = 0,5 μm

FIG. 4 Electron micrograph of 2 *Cowdria ruminanium* organisms from the colony illustrated in Fig. 3, showing the typical double unit membrane (DUN-arrow) that surrounds these organisms, × 43 000. Bar scale = 0,1 μm

FIG. 5 A colony of *Cowdria ruminantium* in gut epithelial cell of prefed *Amblyomma hebraeum* female, showing specific fluorescence after direct fluorescent antibody staining; 3 μm frozen section × 1 000; BM = basal membrane

FIG. 6 The same colony as that shown in Fig. 5, viewed under normal light. The section was counter-stained with Evan's Blue

cuticle, muscle and guanine crystals in the malphigian tubules stained brightly. From these preliminary studies it appears that the IFA, regardless of the origin of the positive sera used, gave slightly better results than the DFA. The pleomorphism of colonies was clearly demonstrated in the FA studies and the number of colonies per frozen section varied from one in some ticks to more than 150 in others. Small fluorescing intracytoplasmic organisms of a suspicious nature were seen once only in salivary glands, where they were present in a few cells only.

No specific fluorescence was observed in control ticks.

Discussion

The light microscopic morphology and distribution of *C. ruminantium* in *A. hebraeum,* as described by Cowdry (1925), were confirmed using other methods.

Electron micrographs of some types of colonies seen in thick sections with the light microscope were obtained. Further studies to demonstrate and describe the others are under way.

Positive fluorescence of *Cowdria* in the vector tick, using DFA and IFA tests, has been observed for the first time. the apparent absence of *C. ruminantium* in tissues other than the gut is puzzling because it has been found that homogenates prepared fom salivary glands, and also to a lesser extent from malpighian tubules, hypodermis, rectal ampules and haemolymph, produced heartwater after inoculation into susceptible sheep (Bezuidenhout, unpublished data, 1984). This aspect must be investigated further.

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