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DIAGNOSIS OF HEARTWATER AT POST-MORTEM IN RUMINANTS AND THE CONFIRMATION OF *COWDRIA RUMINANTIUM* IN MICE

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ABSTRACT

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Criteria that are required to confirm heartwater in ruminants and mice are discussed. In ruminants it entails the macroscopical and microscopical lesions as well as the identification of *Cowdria ruminantium* in brain smears or histological sections. Macroscopical lesions in the majority of animals that die of the disease include effusion of body cavities, hydropericardium, oedema of the lungs, brain, mediastinum, and its associated lymph nodes, and splenomegaly. The effect of specific chemotherapy on the morphology of heartwater organisms *in vivo* is outlined. A severe nephrosis in heartwater-infected Angora goats, treated after the first day of the febrile reaction, is described.

INTRODUCTION

Heartwater is sometimes misdiagnosed, even by experienced veterinarians, because gross lesions, considered as characteristic changes in fatal cases, may not be present in a low percentage of animals. This problem may be exacerbated by the fact that gross lesions may be inconspicuous or absent in animals specifically treated for heartwater.

The purpose of this paper is to describe the criteria that are required to confirm heartwater in ruminants and mice. The effect of specific chemotherapy on the morphology of *Cowdria ruminantium* in diseased animals is briefly discussed.

The diagnosis of heartwater in domestic ruminants

Anamnesis

It is important that a thorough anamnesis is obtained before a necropsy is performed on an animal that has died of suspected heartwater. Special attention should be placed on the clinical signs, vaccination and tick control programmes and the species, breed, age and origin of the animal (Van de Pypekamp & Prozesky, 1987).

Macroscopical pathology

The lesions in ruminants, game animals and mice infected with *C. ruminantium* were reviewed (Prozesky, 1987a). Most animals show effusion of body cavities, hydropericardium, oedema of the lungs, mediastinum and brain, splenomegaly and widespread petechiae.

The macroscopical lesions alone are not sufficient to make a diagnosis of heartwater. Alexander (1931) pointed out that splenomegaly and endocardial haemorrhages are the only changes in a small number of animals that die of the peracute form of the disease.

Microscopical pathology

The histopathological changes in animals that die of heartwater are of limited diagnostic value (Prozesky, 1987a). Confirmation requires the demonstration of *C. ruminantium* in either brain smears or histopathological sections. Heartwater colonies can be demonstrated in capillary endothelial cells of different tissues, especially the renal glomeruli (Fig. 1) and superficial grey matter of the cerebral cortex (Cowdry, 1926). Because of the low concentration of organisms in most fatal cases of the disease, it is often difficult to demonstrate heartwater colonies in tissue sections. Various staining methods may be applied to demonstrate heartwater organisms in tissue sections (Burdin, 1962). Toluidine blue or Giemsa stained sections are preferred by most workers for this purpose (Prozesky, 1987a).

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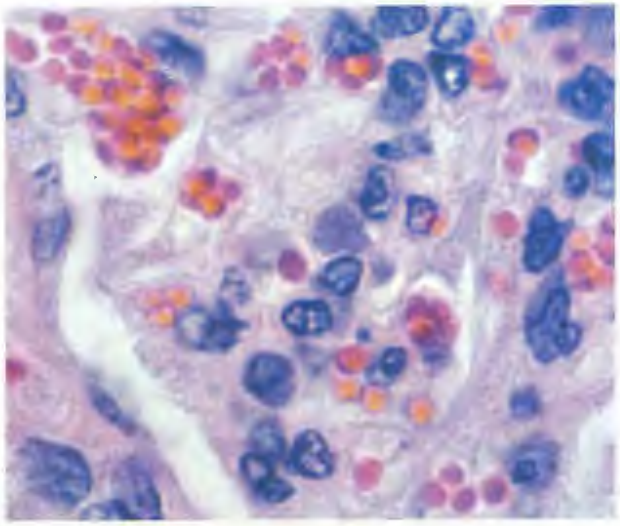

FIG. 1 A colony of heartwater organisms in an endothelial cell of a glomerulus in a sheep (arrow): HE × 3 000

Brain smears

The detection of *C. ruminantium* in capillary endothelial cells in brain smears was a major breakthrough in the diagnosis of heartwater (Purchase, 1945). Until then, the demonstration of *C. ruminantium* in smears from the intima of large blood vessels and in tissue sections were used to make a diagnosis (Jackson, 1931).

The preparation of brain smears is described in detail by Purchase (1945) and Uilenberg (1983). At present smears are prepared by crushing a small piece of hippocampus or cerebral grey matter *c.* 2 × 2 × 2 mm in diameter between 2 glass slides until the tissue has a soft pasty consistency. The material is then collected at one end of the slide which is held firmly in a horizontal position. The other slide, angled at about 45°, is used to make the smear by drawing the tissue along the horizontal slide. It is preferred to lift the angled slide slightly every 10 mm so that the completed smear has alternating thick and thin areas. This facilitates the microscopical detection of capillaries. Smears should be air-dried before staining. Different stains can be applied to demonstrate heartwater organisms in brain smears (Uilenberg, 1983). After fixation for about 1 min in absolute methanol or ethanol, smears are most often stained for 30 min in a 10 % Giemsa solution. Organisms are already differentiated after 5–10 min. CAM's Quick-stain* yields acceptable results and takes only 2–3 min to complete but is inferior if the results are compared with the

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Giemsa-stained smears (L. Prozesky, unpublished data, 1986). With both the Giemsa and CAM's Quick-staining methods, *C. ruminantium* stains a reddish purple to blue.

The number of colonies present in brain smears of animals that died of heartwater varies significantly from animal to animal. In the opinion of the author a diagnosis can be confirmed in most animals within 5 min of examining a smear. Fixed or unfixed smears are still suitable for diagnostic purposes for at least 1 month after they have been made (Uilenberg, 1983). Organisms can be demonstrated in brain smears prepared from animals in an advanced state of putrefaction.

The size of individual organisms ranges from *c.* 0,49 μm–2,7 μm in diameter (Prozesky, 1987c). Within a particular colony organisms are of the same size and internal structure with the exception of colonies containing giant organisms.

Diagnosis of heartwater in specifically treated ruminants

Pathology

The diagnosis of heartwater in animals is often complicated by specific treatment. Depending on the interval between infection and treatment, and between treatment and death, the macroscopical lesions vary significantly (L. Prozesky, unpublished data, 1986). A small number of treated animals do not fully recover, remain recumbent for days, and eventually have to be destroyed. In these animals macroscopical lesions are usually absent, and no organisms can be detected in brain smears. In most cases oedema of the brain is evident histologically (Prozesky, 1987b).

Severe kidney lesions have been reported in heartwater-infected Angora goats that were specifically treated after the first day of the febrile reaction (Prozesky & Du Plessis, 1985a). The kidneys were markedly swollen, light brown in colour and had a mottled appearance. On cut surface the pale cortex was clearly demarcated from the congested medulla (Fig. 2). Tubular epithelial cells were necrotic and many tubules contained hyaline, granular and cellular casts (Fig. 3). In animals with advanced lesions an interstitial fibrosis was present. Heartwater organisms could be demonstrated in brain smears of most of the animals.

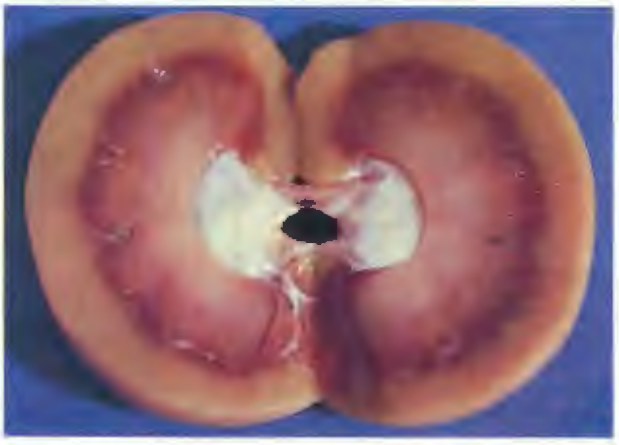

FIG. 2 Kidney of an Angora goat treated after the 3rd day of the febrile reaction

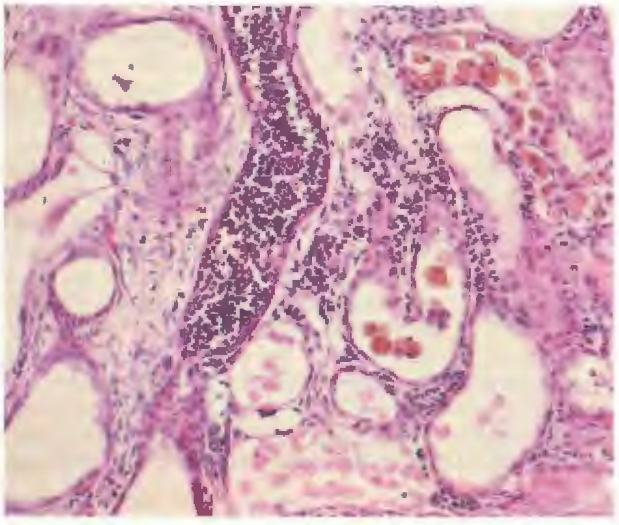

FIG. 3 Dilated convulated tubules contain cellular casts: HE × 400

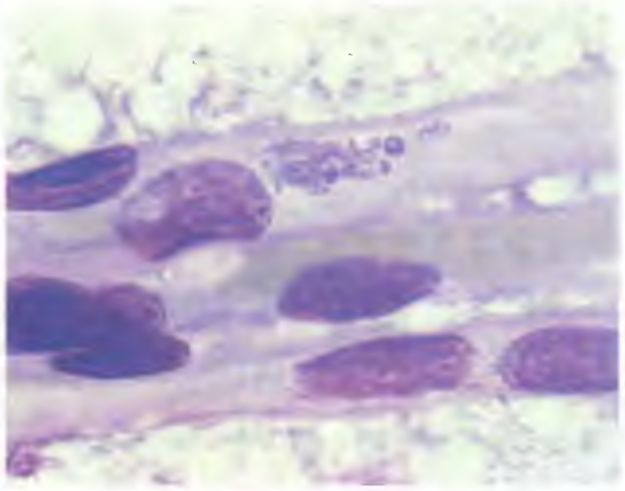

FIG. 4 Heartwater organisms in a Giemsa-stained brain smear prepared from an untreated animal (arrow): × 3 000

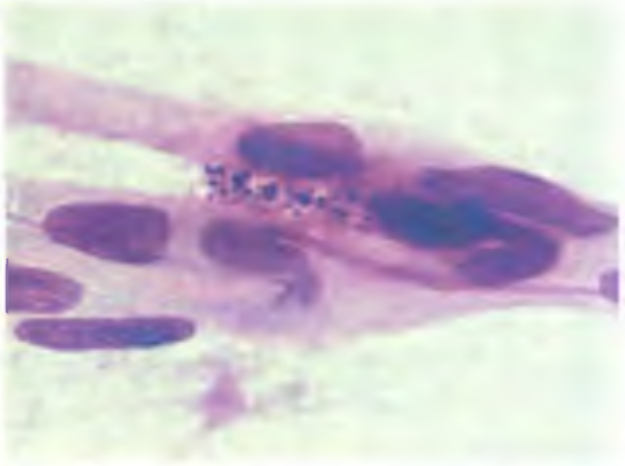

FIG. 5 Affected heartwater organisms in a brain smear prepared from a specifically treated animal (arrow): Giemsa × 3 000

Morphology of C. ruminantium

The identification of *C. ruminantium* in brain smears 48–60 h after an animal has been treated is often difficult. Apparently the small forms of the organisms are more severely affected by the chemotherapy than the larger forms. Affected organisms are poorly delineated and appear to fuse, making it difficult (Fig. 4 and 5) to distinguish them from phagosomes in endothelial cells, groups of blood platelets and the chromatin of capillary endothelial cell nuclei and mast cell granules. The absence of clearly identifiable organisms and characteristic lesions in treated animals makes the diagnosis of heartwater often very difficult if not impossible (L. Prozesky, unpublished data, 1987).

The confirmation of C. ruminantium *in mice*

Several heartwater isolates have been successfully established in mice (Du Plessis & Kümm, 1971; Prozesky & Du Plessis, 1985b; MacKenzie & Van Rooyen, 1981).

Information regarding the confirmation of heartwater in experimentally-infected laboratory animals is limited. Diagnostic criteria in mice differ to some extent from those in ruminants infected with *C. ruminantium*.

Pathological changes

Hydrothorax, hydropericardium, oedema of the lungs and splenomegaly occurred in the majority of animals inoculated with heartwater isolates infective for mice (Du Plessis, 1975; Prozesky & Du Plessis, 1985b). In mice infected with the Welgevonden isolate of heartwater, organisms were detected histologically in the endothelial cells of various organs (Prozesky & Du Plessis, 1985b), but the highest concentration of *C. ruminantium* was present in the lungs. Morphologically the Welgevonden isolate in mice was indistinguishable from the organisms described in the choroid plexus of sheep infected with the Ball 3 isolate (Pienaar, 1970).

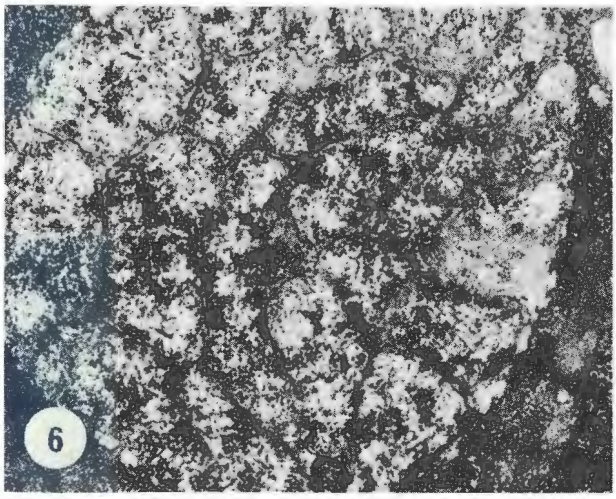

FIG. 6 Organisms in a peritoneal macrophage of a mouse infected with the Kümm strain of heartwater. × 9 800

In mice infected with the Kümm isolate developmental (electron-dense bodies) and morula stages were seen in various cells, including peritoneal macrophages and reticulo endothelial cells in the spleen and lymph nodes (Du Plessis, 1975). The detection of these stages (Fig. 6) in Giemsa-stained smears of peritoneal macrophages is routinely used for the demonstration of heartwater organisms in mice infected with the Kümm isolate (Du Plessis, 1982).

Organisms are detected in brain smears of mice, infected with either the Welgevonden or Kümm isolates, with much less frequency than in ruminants as a result of the relatively low concentrations of organisms in the brains of mice compared to other organs (J. L. du Plessis, personal communication, 1986). This phenomenon, however, needs to be further investigated as only a limited number of brain smears of mice infected with these isolates were examined.

To confirm the presence of *C. ruminantium* in mice unequivocally, infective material could be subinoculated into susceptible ruminants (Prozesky, 1987b).

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