Possible death of a buffalo calf (*Syncercus caffer*) due to suspected heartwater (*Ehrlichia ruminantium*)

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ABSTRACT

Rickettsial organisms resembling *Ehrlichia ruminantium* (the causative organism of heartwater) were demonstrated in brain smears and formalin-fixed brain sections derived from a buffalo calf that died on a private game reserve in northern KwaZulu-Natal. The possibility that the tick-free environment of a quarantine boma may have affected the calf's immunity, is discussed. These findings suggest that monitoring heartwater in wild ruminants and making brain smears as a routine during *post mortem* evaluations of wild ruminants, should be encouraged.

Key words: buffalo, *Cowdria ruminantium,* cowdriosis, *Ehrlichia ruminantium,* heartwater, South Africa.

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INTRODUCTION

Cowdria ruminantium,[4] the causative organism of heartwater, has recently been reclassified as *Ehrlichia ruminantium* based on genetic analysis as well as biological and protein characteristics.[6] *Ehrlichia ruminantium* is transmitted by 3 host ticks belonging to the genus *Amblyomma*[5]. *Amblyomma hebraeum,* the vector of *E. ruminantium* in South Africa, occurs mainly in the Limpopo, KwaZulu-Natal and in the Eastern Cape provinces of South Africa[23].

Heartwater is one of the most important tick-borne diseases of domestic ruminants in South Africa[18]. Mortality rates are high in susceptible cattle, sheep and goats introduced into endemic regions and peracute, acute, subacute and clinically inapparent forms of heartwater have been reported in these domestic ruminants[21]. In wild ruminants, such as the blue wildebeest (*Connochaetes taurinus*), springbok (*Antidorcas marsupialis*), bushbuck (*Tragelaphus scriptus*)[13], black wildebeest (*Connochaetes gnu*), blesbok (*Damaliscus albifrons*)[11], steenbok (*Raphicerus campestris*)[8], sitatunga (*Tragelaphus spekei*)[14] and eland (*Taurotragus oryx*)[25] deaths due to infection with *E. ruminantium* have been reported. To date, no deaths due to heartwater have been documented in the African buffalo. However, African buffalo[2,24] as well as eland, kudu (*Tragephalus strepsiceros strepsiceros*), blue wildebeest, giraffe (*Giraffa camelopardalis*)[15], sable (*Hippotragus equinus*) and tsessebe (*Damaliscus lunatus*)[16] are known to seroconvert after artificial infection and become carriers of the parasite. One African buffalo showed signs of heartwater after artificial infection with *E. ruminatium,* but it recovered spontaneously[24]. Different strains of *E. ruminantium* have the ability to grow in endothelial cell lines of sable antelope, buffalo and eland[20]. These findings suggest the possibility that wild ruminants play an important role in the epidemiology and spread of heartwater. This hypothesis is strengthened by the results of a study in the Kruger National Park of South Africa where it was shown that a wildlife cycle can maintain the transmission of *E. ruminantium* independent of domestic ruminants[17].

Numerous *E. ruminantium* isolates have been made, and named after the farm or country of origin. These strains differ in their pathogenicity for ruminants[3,7].

CASE REPORT

History

On 5 June 2000, a dead buffalo calf of approximately 5 months of age was discovered on a private game reserve in northern KwaZulu-Natal. It was the 2nd buffalo calf found dead within 2 months in this reserve. In addition, 1 blue wildebeest and one impala ram (*Aepyceros melampus*) had died of unknown causes. The dam of this buffalo calf came from the tuberculosis (TB) eradication programme carried out by KwaZulu-Natal Conservation Service (KZNCS) in quarantine bomas at Phinda Resource Reserve. The dam had initially originated from the Hluhluwe–Umfolozi Game Reserve and had been introduced onto the property about 1 year before this investigation. The calf was born in the private game reserve, which had been free of domestic ruminants for more than 10 years.

Pathology

Post mortem

The calf died during the morning of 5 June 2000. The carcass was kept cool until the *post mortem* could be carried out at 15:00 that afternoon.

Gross *post mortem* findings revealed the animal to be in good condition with a low tick burden. Although ticks were not specifically identified, some *Amblyomma* were present. *Rigor mortis* was still apparent. No signs of struggling or injuries were evident. The peripheral blood smear was negative for anthrax and blood parasites. The rumen contained some roughage; milk was found in the abomasum. Mild splenomegaly and hepatomegaly was present, as well as ascites. Whether hydrothorax was present, could not be determined, as the pericard was damaged when skinners opened the thorax. Lungs revealed a foamy, frothy material in the major bronchi and distal 3rd of the trachea. A few lobules in the cranial lobe appeared hepatized. Accentuation of lobulation due to interstitial oedema and emphysema in the lung was visible. Atrial petechiae were observed on the heart. In the brain blood vessels appeared engorged. A brain smear was performed and stained with Kyro-Quick stain (Kyron Laboratories)[19]. Numerous rickettsial-like organisms were observed in the endothelial cells of the capillary blood vessels, which was also seen in

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Giemsa-stained brain smears (Fig. 1). Unstained brain smears as well as samples of frozen brain were taken. Additional samples of brain, bone marrow, heart, kidney, liver, spleen, lymphonodes and lung were preserved in 10 % formalin for further histopathological investigation.

Microscopic examination

Brain. Severe congestion of meningeal and parenchymal vessels was evident. Prominent vasogenic oedema with widening of Virchow-Robin spaces, perineural spaces and dilatation of myelin sheaths in the central white matter neutrophils were present as well as diffuse gliosis with scant perivascular infiltrates of individual mononuclear cells. Distinct intracytoplasmic colonies of rickettsial-like organisms resembling *E. ruminantium* were identified within the endothelial cells (Fig. 2).

Spleen. Diffuse congestion of splenic sinuses was present while lymphoid follicles had a washed-out appearance with depletion of lymphocytes from the germinal centres (lympholysis).

Lung. Severe diffuse congestion was evident with engorgement of bronchial vasculature and alveolar wall capillaries. Effusion of fibrinous fluid had resulted in interstitial distension (interstitial oedema). There was scant interstitial infiltrates of mixed mononuclear cells and individual leukocytes. In individual lobules a severe inflammatory reaction was visible; bronchi were plugged with necrotic plugs consisting of fibrin, necrotic cell debris and infiltrating leukocytes (necrotic bronchitis). Peribronchial and intra-alveolar infiltrates contained innumerable neutrophils accompanied by proliferating alveolar macrophages (alveolar pneumonia). Large bacterial colonies consisting of bipolar, Gram-negative organisms resembling *Pasteurella* were observed in many of these necrotic areas.

Liver. The liver showed diffuse congestion with engorgement of hepatic sinusoides and portal vessels, and mixed mononuclear cell infiltrates in the portal areas (triaditis). Sinusoidal leukocytosis with individual neutrophils were present in hepatic sinusoides.

Kidney. The kidneys showed diffuse congestion with engorgement of glomerular and intertubular capillaries. Increased granularity of proximal convoluted tubular epithelial cells with increased eosinophilia and basally placed, hyperchromatic nuclei (tubular nephrosis) was evident.

Heart. Severe, diffuse congestion with engorgement of the myocardial capillaries were apparent. Multifocal haemorrhages were scattered throughout the myocard. Interstitial oedema was present with teasing apart of myofibres. Individual myofibres appeared amorphous and eosinophilic (myofibre hyalinization).

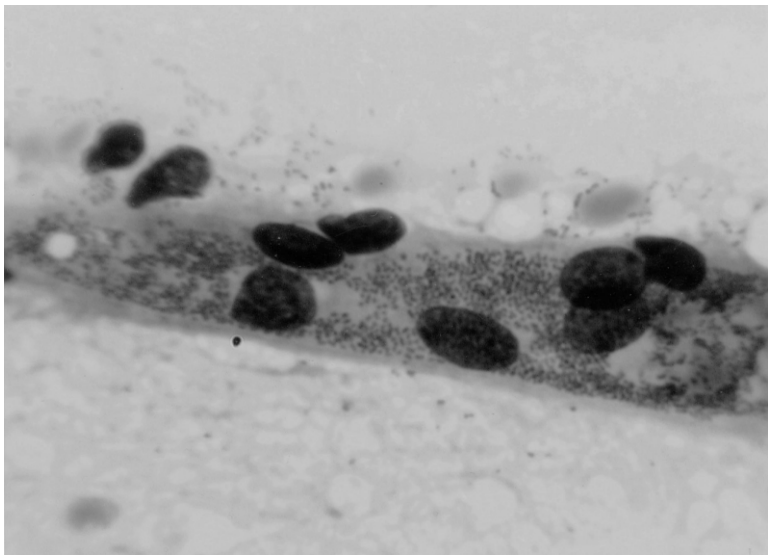

Fig. 1: **Many large colonies of small *Ehrlichia ruminantium*-like organisms, intracytoplasmically within endothelial cells of brain capillaries.** Giemsa; ×1000.

Immunohistochemistry

Immunohistochemical (immunoperoxidase) stains for heartwater were performed on formalin-fixed brain sections and revealed positive-staining granules in many endothelial cells of parenchymal capillaries. Confirmed cases of *Coxiella burnetti* and *Chlamydia psittaci* were used as negative controls and showed no positive staining.

Transmission electron microscopy

On transmission electron microscopic (TEM) examination, pleomorphic, coccoid, ovoid, filamentous, horseshoe-shaped and polygonal organisms, 0.49–2.7 μm in diameter and enclosed by a double-unit membrane were found in capillary endothelial cells, features that are characteristic of the *Ehrlichia* group.

DNA probes

Frozen brain tissue was also subjected to PCR and DNA probe examination in an attempt to confirm the identity of the organism. The pCS20 probe[22] is known to discriminate between *E. ruminantium* and other Ehrlichial organisms[1]. Unfortunately, the sample was in a too-advanced stage of decomposition and the results were inconclusive.

Diagnosis

The cause of death was suspected heartwater with complicating acute bacterial bronchopneumonia.

DISCUSSION

Demonstration of rickettsial organisms, resembling *E. ruminantium,* in brain

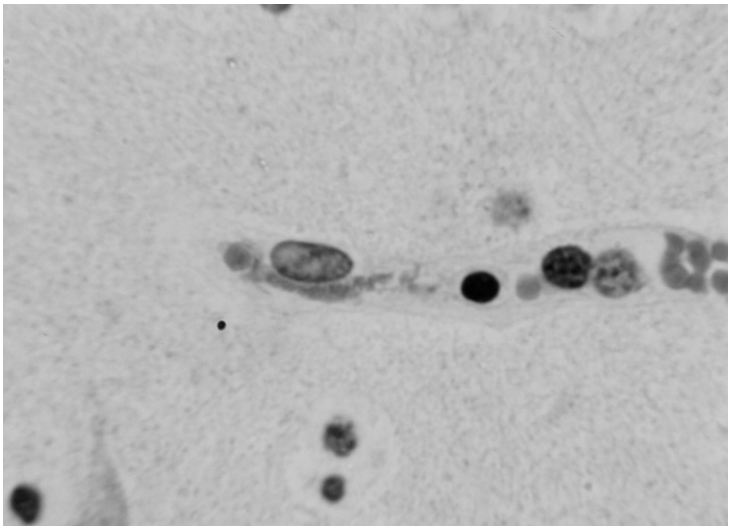

Fig. 2: **Formalin-fixed brain section showing a large colony of *Ehrlichia ruminantium*-like organisms within the cytoplasm of an endothelial cell.** H&E; ×1000.

smears, formalin-fixed brain sections stained with haematoxylin and eosin, positive immunoperoxidase staining of organisms with *E. ruminantium* antibody and identification of rickettsia in the brain sections on TEM, strongly support a diagnosis of heartwater. Whether this was the primary cause of death or whether death was the result of complicating bacterial pneumonia is debatable.

To the best of our knowledge, no mortalities as a result of heartwater have been reported in buffalo. This can be ascribed to several factors. If one considers buffalo to be at least as heartwater-resistant as the indigenous breeds of livestock, it is not surprising that relatively few fatalities due to heartwater have been reported from the field. Only small numbers of buffalo probably succumb to the disease and it is hardly possible to pick up sick or dying animals in the veld. The decomposed condition of most buffalo carcasses found in the wild often renders an investigation impossible. In addition, buffalo are not known to be susceptible to heartwater and therefore this disease might have been ignored as a possible differential diagnosis, especially in the absence of lesions typical of heartwater.

To date, only small numbers of buffalo have been artificially infected with heartwater to examine their resistance/susceptibility. Keffen[9] reported on the artificial infection of two 10-month-old buffalo calves. Both animals came from a heartwater-free area and were the 5th generation of animals bred in captivity. Neither of these buffalo showed any clinical signs. There was, however, no control group and the strain used for artificial infection is not mentioned. Andrew and Norval[2] described the infection of 4 susceptible weaner buffaloes with various isolates of heartwater. No clinical signs were detected in these animals but transmission of the disease to susceptible sheep *via Amblyomma* ticks was possible for 161 days. This demonstrates that despite the absence of clinical signs, *Ehrlichia* organisms can multiply in buffalo. This finding is supported by the results of Smith *et al.*[20], who cultured 19 different strains of *E. ruminantium* on endothelial cell lines of various wild African mammals, including buffalo. During a more recent investigation[24], 1 of 2 African buffaloes which were artificially infected with *E. ruminantium,* showed signs of heartwater[24]. Fourteen days after infection, the animals developed a temperature of 39.8 °C and higher. Typical signs of heartwater such as hyperesthesia, muscle tremors, opisthotonus and blinking of the eyes were present during the last 2 days of pyrexia. After 5 days, these signs disappeared spontaneously and the temperatures returned to normal. This confirms the susceptibility of buffalo to heartwater.

Animals in disease-free breeding programmes are kept in quarantine bomas, where ticks are absent. This can interfere with immunity to tick-borne diseases. Maternal immunity might be adversely affected in dams kept for long periods in tick-free bomas. Calves born in such bomas could be at risk as colostral antibodies are reported to be important in the protection calves from heartwater[12].

In addition, limited cross-immunity between the different strains of *E. ruminantium* has been reported[10]. Therefore, relocation of animals over large distances to other facilities could result in exposure to different strains of the organism and consequently susceptibility to the disease. This particular buffalo herd had been translocated 100 km from the Hluhluwe–Umfolozi Game Reserve, where the herd originated, before being kept in quarantine bomas.

The herd had spent about 9–12 months in the quarantine bomas, and protection of this weaner buffalo by maternal antibodies might have been low. The animal was also being weaned, which no doubt placed it under stress, and, in addition, it was suffering from pneumonia. All these stress factors together could have led to a higher susceptibility to heartwater and subsequently to death.

These findings show that the epidemiology of heartwater in buffalo is in urgent need of further investigation. It is recommended that buffalo herds translocated to heartwater-endemic areas should be monitored more closely. Game farmers should be encouraged to report all fatalities. In heartwater areas, brain smears should be taken as a routine from all mortalities in wild ruminants.

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