ENCEPHALITOZOON INFECTION IN A STILL-BORN FOAL

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ABSTRACT

A stud Clydesdale foal was still-born near full term. Macroscopic examination revealed a normal placenta, pulmonary atelectasis and faint white mottling of the kidneys. Microscopically there was severe lymphoplasmacytic interstitial nephritis. Numerous organisms resembling *Encephalitozoon cuniculi* were present in the affected kidneys. The organisms occurred in the areas of inflammation as well as in the renal glomeruli and intracellular cysts in the renal tubular epithelial cells and exhibited Gram positive staining. Ultrastructurally the organisms posessed a polar vacuole and a spiral filament typical of Microsporidia. The organisms were not detected in sections of the other organs examined.

Key words: *Encephalitozoon cuniculi*, microsporidia, abortion, still-birth, equine.

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INTRODUCTION

Encephalitozoonosis is a fairly common, ubiquitous latent infection of laboratory animals such as mice, rabbits, guinea-pigs, golden hamsters and rats[5]. It is also a fairly common infection in the Arctic fox where these are bred in Scandinavian countries[17]. Vávra et al. also reported yearly epidemics of encephalitozoonosis amongst suricates *(Suricata suricata)* in the Prague zoo in Czechoslovakia[17]. In South Africa this condition has been reported in laboratory rabbits, mice[6], wild dogs[15], dogs[1 14] and a kitten[16]. Although in some of these cases the parasite was identified as *Nosema*, subsequent clarification of the taxonomic position of the parasite by Cali, clearly indicates that these were in fact cases of encephalitozoonosis[4].

In South Africa canine encephalitozoonosis is generally sporadic but serological evidence indicates a prevalence of 18% in sera collected from Pretoria and Durban and 65-70% in certain breeding establishments[12]. The position in wild rodents in this country is unknown. Serum samples from laboratory rabbits showed a prevalence of 40%[13].

Although the canine disease was originally described by Plowright[10] as an "encephalitis-nephritis"-syndrome, in some instances the organisms disseminate more widely, affecting organs such as the liver, lungs and myocardium. The renal lesions in dogs usually comprise a severe, progressive widespread, sub-acute plasmalymphocytic interstitial nephritis accompanied by the presence of *Encephalitozoon* parasites in glomeruli and tubular epithelial cells in intracellular cystic forms, or freely in areas of inflammation, or in tubular lumens after rupture of such cysts[1 7 10].

Although Waller et al[18] found serological evidence of *Encephalitozoon* in horses no morphological description of lesions due to *E. cuniculi* infection in equines could be traced in a search of the literature. This report deals with an isolated case of a dead-born foal, showing renal lesions and organisms identical to those encountered in canine encephalitozoonosis.

A stud Clydesdale mare from the Clarens district in the Orange Free State delivered a still-born foal, near full term. The mare had aborted the previous season and this was the third foetal loss in the stud out of 9 pregnancies during that season. The mare showed no pyrexia or any other obvious clinical signs of systemic disease before, during, or after delivery of the foal.

The foal and placenta were rapidly cooled down in a deep freeze, but not frozen, and despatched to the Faculty of Veterinary Science, University of Pretoria, where a post mortem examination was conducted approximately 24 h after birth.

No placental abnormalities were identified. Foetal examination revealed mild hydrothorax, total atelectasis of both lungs, mild haemoperitoneum and congestion of the abdominal organs. The kidneys were congested and had an indistinct whitish mottled appearance of the cortex.

Specimens of the brain, spleen, liver, kidneys and intestines were fixed in 10% buffered formalin for histopathological examination. Sections were prepared according to standard procedures.

The only lesion of significance was encountered in the kidneys which consisted of a severe diffuse, interstitial lymphoplasmacytic nephritis (Fig. 1). Haematoxylin- and eosin-(HE) stained sections revealed numerous parasites morphologically resembling *E. cuniculi.* These parasites characteristically do not stain well with HE. However, application of Gram's stain using the Goodpasture as well as the Brown-Hopps methods, highlighted the distinctly Gram positive nature of the organisms which were morphologically indistinguishable from *E. cuniculi* (Fig. 2 & 4). Numerous intracellular cysts, most of which occurred in tubular epithelial cells (Fig. 3), were observed. Numbers of free-lying organisms were also seen in areas of cell infiltration, as well as in tubular lumens. Organisms were also encountered within endothelial cells of the glomerular tuft or lying freely in Bowman's space (Fig. 2). The organisms measured 2,4 x 1,3 μm. No parasites or signs of inflammation were

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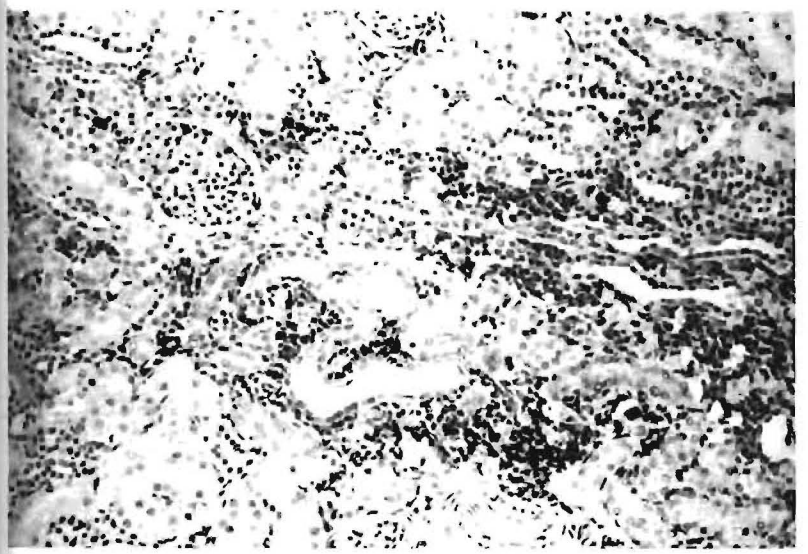

Fig. 1: **Diffuse lymphoplasmacytic interstitial nephritis in a stillborn foal. HE X 100**

seen in sections of the brain, liver, spleen or intestinal tract.

Formalin-fixed specimens of the kidney were transferred to 4% glutaraldehyde in Millonigs phosphate buffer, and postfixed in buffered one per cent osmium tetroxide. Thin sections were stained with uranyl acetate and lead citrate.

Microsporidial spores with the characteristic morphology of *E. cuniculi* were present in glomerular spaces as well as in renal tubular epithelial cells. The spores were generally electron-dense but in some a polar vacuole, and in others 5-7 coils of the polar filament, were visible.

Another mare of the stud was induced to foal at 370 d gestation by injecting 20 i.u. oxytocin (Oxytocin, Ciba-Geigy) intravenously. Foetal manipulation was necessary to correct an abnormal presentation. In spite of intensive supportive therapy, the foal died 3 d later. A post mortem and histopathological examination did not reveal any signs of *Encephalitozoon* infection.

Uterine biopsies were performed on the 2 mares which gave birth to the abovementioned foals. Histopathological examination of the samples did not reveal significant lesions.

Serum from the mare as well as from the rest of the stud was checked for antibodies against *Encephalitozoon* using fluorescein labelled antihorse IgG (FITC-GOAT X HORSE IgG, Zymed, California). These tests yielded negative results.

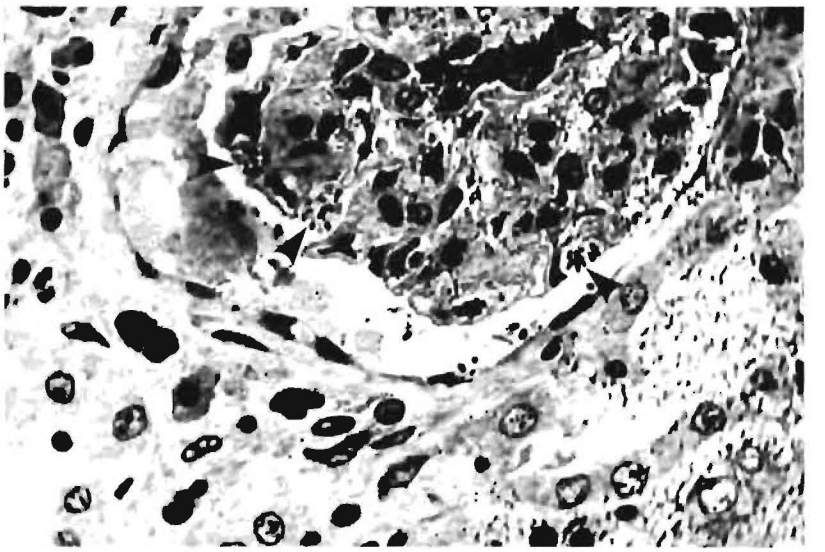

Fig. 2: ***Encephalitozoon cuniculi* parasites in the glomerular endothelium and some free-lying organisms in Bowman's space (arrow). Toluidine Blue X 1000**

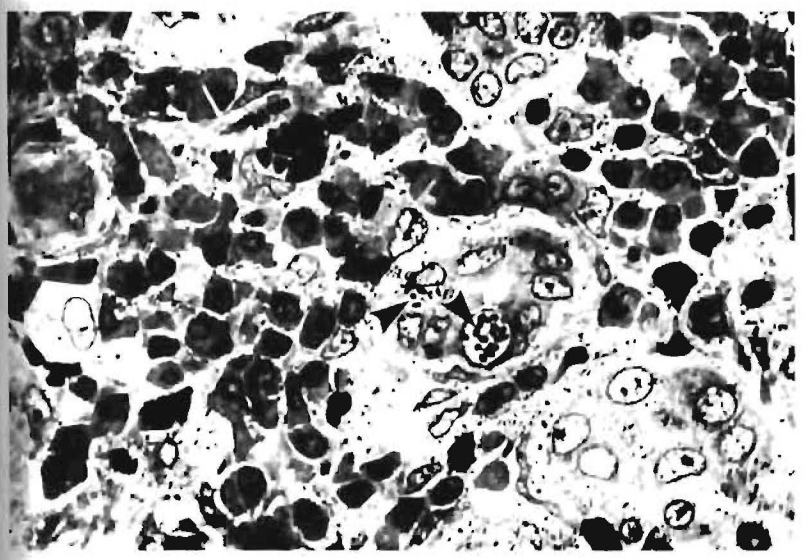

Fig. 3: **Intra-epithelial parasitovorous cysts in renal tubule surrounded by inflammatory cells. Gram's X 1000**

E. cuniculi is a parasite of warm-blooded vertebrates. It was originally confused with *Nosema* spp. which affects invertebrates such as bees as well as cold-blooded vertebrates[5]. It is commonly associated with laboratory animals, but several cases have been reported in dogs[1,3,7,14]. This case probably represents the first report of this parasite in equines. However, in 1988 a case of placentitis from a mare revealing numerous *Encephalitozoon* parasites (as well as Chlamydia) was diagnosed and taken up in the FIP collection in a conference organised by Parker[9]. Unfortunately such an unusual diagnosis was not suspected in the foal reported on in this paper, at the time of the necropsy, with the result that a urine sediment ex-

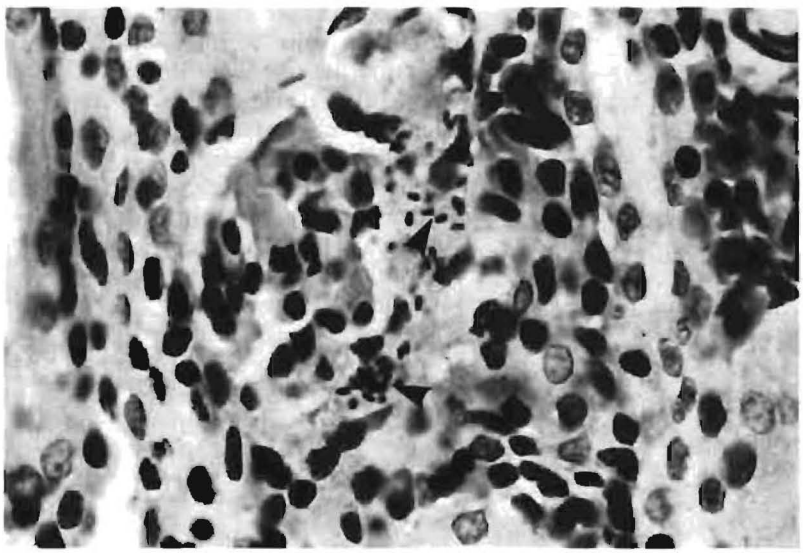

Fig. 4: **Free-lying *Encephalitozoon* organisms in an area of lymphoplasmacytic nephritis (arrow). Gram's X 1000**

amination was not carried out, nor were attempts made to culture or transfer the parasite to a laboratory animal.

The only evidence which substantiates the diagnosis of *Encephalitozoon* infection, is the histopathological and electron microscopical finding of numerous organisms in the kidneys of the subject which morphologically[2 3 5 14], as well as in its staining characteristics, resemble *E. cuniculi*. The ultrastructural demonstration of a single nucleus and the coils of the spiral polar filament are strong evidence of a Microsporidian. The presence of 5 to 7 coils, is indicative of *Encephalitozoon*[2 5 7 8]. The nature of the resultant inflammatory process is also consistent with *Encephalitozoon* infection, as it resembles very closely the pathology described in cases of canine encephalitozoonosis[1 3 7 10]. From a differential diagnostic point of view the authors are confident that the organisms are not *Toxoplasma, Besnoitia, Klosiella, Hepatozoon, Sarcocystis* or botryomycotic *Staphylococci*. The severity and diffuse nature of the ensuing nephritis caused by the infection, was beyond doubt the cause of death in this still-born foal.

The absence of antibodies against *E. cuniculi* in the mare is difficult to explain. One can only speculate that this may have been a different strain, or that the mare was only transiently infected or that the infection involved the foetus only. It is suspected that the parasite in man is not identical to *E. cuniculi*[11].

Transplacental infection is well documented[5] and this must explain the intra-uterine infection in this foal. The more common route in nature is the oral route of infection from infected litters or urine contamination of the feed and is probably the route by which the mare had picked up the organism. The intra-uterine death of this foal near term is also consistent with the manifestation of *E. cuniculi* infection in other species where neonatal mortality is a common finding[1 3 7 10 17].

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