THE PATHOLOGY OF SUBCLINICAL INFECTION OF *ENCEPHALITOZOON CUNICULI* IN CANINE DAMS PRODUCING PUPS WITH OVERT ENCEPHALITOZOONOSIS

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ABSTRACT

The macroscopic, microscopic and clinical pathology and the serology of 2 clinically normal Staffordshire Bull Terrier bitches, both of whom produced pups with confirmed encephalitozoonosis, is described. Mild histopathological changes, similar to those seen in the infected pups, were observed. The spores of *Encephalitozoon cuniculi* were seen in the renal tubules of the kidney of one of the bitches. The serum urea concentrations of one of the bitches was elevated. A positive titre against *E. cuniculi* was obtained in both of the bitches. A 10-year-old girl who had had close contact with one of the infected litters of pups, seroconverted to *E. cuniculi.* Her two siblings were serologically negative.

Key words: Encephalitozoonosis, canine, dam, pathology, serology, *Encephalitozoon cuniculi,* human infection.

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INTRODUCTION

Encephalitozoonosis is a microsporidian infection that affects a wide variety of mammals[18]. In carnivores it is a sporadic, severe disease of the neonate usually culminating in the death of the animal. Although canine encephalitozoonosis in young animals has been well described, the pathology of the bitch bearing the infected litter, has not been investigated. Dams of pups infected with encephalitozoonosis usually appear to be clinically healthy[18].

Szabo & Shadduck[20] inoculated adult dogs intravenously with viable *Encephalitozoon cuniculi* spores. Histologic examination of the kidney, brain and liver revealed microfocal plasma cell and lymphocyte aggregates at the renal corticomedullary junction and in the medullary interstitium. No organisms were present in these areas or in other regions of the kidney.

Stewart et al[17] examined an adult bitch dosed with *E. cuniculi* and found plasma cell infiltrates in the meninges, lungs and spleen, together with multifocal plasmacytic interstitial nephritis and membranoproliferative glomerulonephritis. The animal did not develop clinical signs.

Experimentally infected adult vervet monkeys *(Cercopithecus pygerythrus)* were found to display macroscopic and microscopic lesions in the liver and kidney and rarely in the brain. The organisms were occasionally seen in the kidney and liver of the vervet monkeys. However, no clinical signs were recorded in these cases[22].

Several cases of clinical encephalitozoonosis have been reported in man[3 8 21], although identification of the aetiological agent of some of these cases has been disputed[6]. Antibodies to *E. cuniculi* have been found in persons living in or visiting the tropics and antibodies to *E. cuniculi* have also been noted in people suffering from malaria, tuberculosis, filariosis, schistosomosis and other diseases[1 7 14].

In the present study, 2 bitches from separate kennels, and their respective pups, which both developed typical encephalitozoonosis, were examined. Serum from 3 children, with close contact with one of the infected litters, was tested for antibodies to *E. cuniculi.*

MATERIALS AND METHODS

Pup A was one of a litter of 5 produced by an 18-month-old Staffordshire Bull Terrier bitch (Bitch A) at kennel A. Encephalitozoonosis had been diagnosed in a littermate a week previously. Two of the pups had already died and the remaining pup (still alive) was reported to have shown mild nervous signs (star gazing). Three children in the household at kennel A had had very close contact with the bitches and their litters.

A 5-year-old Staffordshire Bull Terrier bitch (Bitch B) and her pup were from a breeding kennel (B) of Staffordshire and Pit Bull Terriers where heavy losses had occurred in the litters over the previous 2 years.

Neither of the bitches showed any clinical signs of disease and they did not reveal any abnormalities upon physical examination. Serum was collected from each bitch and her respective pup, and also from all the other dogs on both properties (n=16). This was tested for antibodies to *E. cuniculi* as previously described[16].

The serum urea and creatinine concentrations of both bitches and their respective pups were determined, using the described methodologies[23].

Urine was collected from each animal. It was centrifuged at 2000G for 10 min and the sediment was stained with Gram's stain and examined.

Peripheral blood smears were made, using blood obtained from ear veins. These were stained with RapiDiff (Clinical Sciences Diagnostics, Division of C.S.M.L. Pty Ltd. P.O. Box 38939, Booysens, 2016) and examined under a light microscope.

Sterile thiopentone (Intraval Sodium, Maybaker (SA) Pty Ltd) was used to euthanase the bitches and a complete necropsy was conducted on both bitches and their pups.

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All the organs were examined grossly and specimens of the kidney, liver, lung, heart, stomach, pancreas, uterus and brain were placed in 10% neutral buffered formalin. Samples were routinely embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin, with Gram's stain and Masson's Trichome stain for histological examination. Numerous serial sections were cut from the kidneys of both bitches in order to locate the *E. cuniculi* organisms. One kidney from each animal was removed aseptically and primary kidney cultures were prepared as described[15].

Following confirmation of the disease in kennel A, serum was collected from the 3 children living in that household. After testing this serum, urine was collected from the sero-positive child, centrifuged at 900 G for 10 min and half the sediment was stained with Gram's stain. Phosphate-buffered saline was added to the remaining sediment which was then centrifuged at 900 G for 10 min and the sediment added to established Madin-Darby cell line of Canine Kidney cells (MDCK, Flow Laboratories). These cells were subpassaged twice weekly for 2 weeks. They were then stained with Giemsa stain and examined by light microscopy for the presence of *E. cuniculi*.

RESULTS

The clinical behaviour of both bitches was normal. Pup A was approximately 2 months old. It was slightly pot-bellied and enlarged kidneys were palpable. No other abnormalities were evident. Pup B showed a mucopurulent, bilateral ocular discharge and crusting around the nares. No further abnormalities were detected upon physical examination of the pup.

The results of the serology from all of the dogs on each property are shown in Table 2. Bitch A had a reciprocal titre of 640 and that of her pup was 160. Bitch B had a reciprocal titre of 40 and her pup had a titre of 640.

The results of urine examination and isolation of *E. cuniculi* in tissue culture are shown in Table 3. The urine sediment of Pup A revealed many Gram positive *E. cuniculi* spores. No *E. cuniculi* spores were present in the urine sediment of Pup B. No spores were seen in the urine from the child which had developed antibodies to *E. cuniculi* and spores were not detected in the MDCK tissue cultures.

Bitch A showed a mild diffuse pulmonary congestion. The renal capsule was difficult to strip and 2 focal pinpoint white spots were present on the cortical surface. The uterus contained a brownish necrotic substance.

Bitch B showed moderate congestion of the liver and spleen. The renal capsule was focally adherent.

The postmortem examination conducted upon Pup A, revealed a distended abdomen, increased consistency of the liver and mild proteinuria. The renal capsule was firmly adherent and the cortical surface mottled. The cortex was a pale, yellowish colour and petechiae were present on the cut surface. Small wedge-shaped whitish streaks extended from the cortex into the medulla.

Table 1: **Serum creatinine and urea concentrations of Bitch A and B and their respective pups**

Blood chemistry	Bitch A	Bitch B	Pup A	Pup B	Normal
S-urea mmol ℓ^{-1}	9,1	4,6	12,1	1,4	3,6-8,9
S-creatinine μmol ℓ^{-1}	111,0	91,0	72,0	47,0	< 133

Table 2: **Serological titres of dogs from Kennels A and B and the three children that had contact with the dogs from Kennel A**

Origin	Host	Total tested	Reciprocal titres							Total positive
			0	20	40	80	160	320	640	
Kennel B	Canine	20	15	1	1	1	1	-	1	5
Kennel A	Canine	5	1	-	-	1	1	-	2	4
Kennel A	Human	3	2	-	-	-	-	1	-	1

The only finding in Pup B was that the renal capsule was moderately difficult to strip.

The serum urea and creatinine concentrations are given in Table 1. No abnormalities or parasites were detected in the peripheral blood smears of any of the 4 dogs.

A moderate, multifocal, subacute, granulomatous interstitial nephritis was present in all sections of the kidney in Bitch A. The lesions consisted of foci or linear infiltrations comprised predominantly of plasma cells. A small centre of coagulative necrosis with haemorrhage, karyorrhexis, karyolysis and neutrophils was occasionally present. Rarely, the necrotic areas contained a few individual *E. cuniculi* spores. Only one colony of *E. cuniculi* spores was observed within a renal tubule, despite the numerous serial sections that were cut and examined. The mean size of the spores was 2,38 × 1,25 μm and they stained Gram positive.

The lesions in Bitch B were mild and consisted of subacute interstitial nephritis, characterised by tiny foci of lymphocytes, macrophages and plasma cells in the cortical and medullary interstitium. Despite numerous serial sections that were cut and examined, no

Table 3: **Demonstration of *E. cuniculi* by means of histopathology, urine examination and primary kidney tissue cultures**

	Urine examination	Tissue culture isolation	Histopathology
Bitch A	-	-	+
Pup A	+	+	+
Bitch B	-	-	-
Pup B	-	-	+

\+ = positive for *E. cuniculi*
\- = negative for *E. cuniculi*

parasites were seen.

The renal lesion in both bitches was accompanied by a mild, subacute, multifocal, granulomatous hepatitis, interstitial pneumonia, endometritis and encephalitis. The inflammatory infiltrate was predominantly lymphoplasmacytic in all areas. No *E. cuniculi* organisms were discerned in the sections stained by either HE or Gram methods.

Histopathology of Pup A revealed severe, subacute, multifocal, lymphoplasmacytic interstitial nephritis. Large interstitial foci of inflammatory cells occurred predominantly at the corticomedullary junction of the kidney. Several *E. cuniculi* organisms were seen in Pup A. These varied from densely-packed colonies to a few individual organisms present in the zones of inflammation. Often the colonies were not accompanied by any cellular response.

In addition to the renal lesions noted in Pup A, subacute, plasmalymphocytic intersticial myocarditis, multifocal granulomatous meningoencephalitis, multifocal hepatitis, mild gastritis and interstitial pneumonia were also noted. A small colony of Gram's positive *E. cuniculi* organisms was observed within an acinar cell of the pancreas.

In Pup B the renal lesions were extremely mild. Most glomeruli were unaffected, but occasionally, mild periglomerular fibrosis was encountered. Many serial sections of the kidney were recut and examined, but only 2 colonies of *E. cuniculi* were seen. Mild, subacute, perivascular, multifocal, lymphoplasmacytic meningoencephalitis, interstitial pneumonia and hepatitis were also noted in Pup B.

DISCUSSION

In contrast to the paucity of information available on the pathology in a bitch bearing a litter of pups infected with *E. cuniculi*, the pathology of blue fox vixens bearing infected pups has been extensively investigated[9,10]. Vixens are reported to be healthy and, with the exception of a thickening of the lamina propria of the uterine walls by predominantly mononuclear cells, no macroscopic or histopathological lesions are seen[10]. Parasites have not been detected in the organs of vixens giving birth to infected pups[10], although they must be present, since transplacental transmission has been conclusively demonstrated[11]. In this study, an inflammatory infiltrate into the submucosa of the uterine wall was noted, similar to that described in blue foxes[9].

In addition to blue foxes, transplacental transmission has been reported in rabbits[12], mice[13] and sporadically in the squirrel monkey (*Saimiri sciureus*)[2]. Circumstantial evidence for transplacental transmission has been reported in dogs[19], but has never been conclusively proved. The presence of lesions and a positive titre in both bitches and the presence of organisms in Bitch A, would suggest that the dams were the source of infection for the pups. Although transplacental transmission is the most likely means of transmission, it is possible that the organism could infect the pups during the birth process, or that the pups were infected from an environmental source.

The mild, lymphoplasmacytic, interstitial nephritis seen in these bitches appears to be similar to the lesions reported by Stewart[17] and Szabo & Shadduck[20]. Most of the extrarenal lesions seen in the 2 bitches could be described as a mild, focal, lymphoplasmacytic infiltration into various organs. The majority of the lesions appear to be a milder form of the lesions described in young pups suffering from encephalitozoonosis[4]. This type of lesion was seen in the liver, brain, lung and uterus of both bitches. It is significant however, that the renal lesions were fairly extensive in both bitches, although neither of the bitches displayed any untoward clinical or clinicopathological abnormalities. This leads one to speculate whether the renal lesions could have remained dormant and later manifested as a chronic interstitial nephritis entity in old age[18].

E. cuniculi organisms were observed in the kidney of Bitch A. This is a significant finding, previously unreported and it shows that the bitch can act as a source of infection for the pups. Stewart[17] and Shadduck & Szabo[20] observed a non-specific plasmalymphocytic interstitial nephritis in their experimentally-infected adult dogs, but did not find any parasites present in the organs examined. In addition, no parasites were noted in the blue fox vixens studied by Mohn[10], although parasites were seen in their placentae.

It will be noted from the results that although *E. cuniculi* organisms were observed in the kidney of Pup B, it showed rather mild lesions and did not display any clinical abnormalities. Encephalitozoonosis can manifest as a mild, moderate or severe, fulminating disease[20]. In cases of mild encephalitozoonosis where the animal is not overly sick, it may be possible that the renal lesions remain and eventually lead to chronic interstitial nephritis[18].

As may be noted from the histopathological results, *E. cuniculi* organisms were detected in the pancreas of Pup A which displayed an overt case of encephalitozoonosis. This particular lesion in the pancreas has been previously described only in vervet monkeys[22], where a lymphoplasmacytic infiltrate was noted. However the presence of the *E. cuniculi* organisms was not reported in this particular study. This finding serves to emphasise the widespread dissemination of the organism.

The serum urea concentrations of Bitch A and Pup A were elevated. This finding is in accordance with those of Botha[5] and is indicative of renal damage caused by the organisms.

The child that developed *E. cuniculi* antibodies, did not display any symptoms and has remained in good health to the present date. It is likely that this child became infected from Pup A or a litter mate. Pup A had large numbers of spores in the urine which would have allowed ample opportunity for infection. The fact that the other 2 siblings did not develop antibodies in spite of a similar exposure, would suggest that humans are not readily infected with *E. cuniculi*.

Both of the bitches and their respective offspring had positive serum titres to *E. cuniculi*, indicating that this is probably the most sensitive means of diagnosis. Organisms were seen in the histopathological specimens of 3 out of 4 dogs. However, in the case of Pup B, serial sections had to be cut and examined before organisms were seen. This illustrates the difficulty that may be experienced in confirming cases of encephalitozoonosis. Urine examination and preparation of kidney tissue cultures did not prove to be a very accurate means of diagnosis. These observations support the statement of Botha[5] that careful evaluation of clinical signs, clinical pathology, gross and histopathological findings, culturing and serological results is required to obtain a definitive diagnosis of encephalitozoonosis.

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