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Extraglomerular Lesions in Kidneys of Mink with Encephalitozoonosis

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Zhou Z.-y., K. Nordstoga and I. Bjerkås: Extraglomerular lesions in kidneys of mink with encephalitozoonosis. Acta vet. scand. 1992, 33, 33-41. – Extraglomerular renal lesions were studied by light and electron microscopy in 13 farmed mink which showed cataractous eyes associated with spontaneous encephalitozoonosis. The extraglomerular renal lesions consisted of multiple renal cysts, multifocal-to-coalescing interstitial nephritis and vasculitis. Tubular cysts of varying size were present in the corticomedullary junction and medulla. The inflammatory infiltrates were composed mostly of lymphocytes and plasma cells and usually accompanied an interstitial fibrosis. Vasculitis, perivasculitis and sclerotic arteries were frequently seen.

renal cysts; vasculitis; interstitial nephritis.

Introduction

Encephalitozoonosis is caused by the intracellular protozoon *Encephalitozoon cuniculi.* The parasite can infect many animal species including laboratory animals and several species of carnivores (*Bjerkås et al.* 1988, *Bjerkås* 1990, *Botha et al.* 1986, *Huldt & Waller* 1974, *Nordstoga* 1972, *Shadduck & Pakes* 1971, *Vávra et al.* 1971, *Wilson* 1979) and usually produces a subclinical disease. Clinical disease and death have been reported in rabbits and dogs. Encephalitozoonosis has caused heavy losses in blue foxes (*Nordstoga* 1972, *Nordstoga & Westbye* 1976), while mink *(Mustela vison)*, fed the same diet, have not been affected (*Nordstoga & Loftsgaard* 1986). However, more recently encephalitozoonosis has been diagnosed in mink in Norway. In the autumn of 1984, a large number of mink were reported to suffer from bilateral cataracts associated with encephalitozoonosis and a feedborn infection in the spring of 1984 was believed to be responsible for the problem in the mink (*Nordstoga & Loftsgaard* 1986).

Many studies on the pathology of encephalitozoonosis have revealed that the central nervous system and kidney are common target organs for this infection although many different cells, tissues and organs can be infected by the parasite. Renal involvement is observed in rabbits, dogs, cats and blue foxes suffering from the disease, and is manifested mainly as an acute or chronic interstital nephritis (*Botha et al.* 1986, *McCully et al,* 1978, *Nordstoga* 1972, *Plowright* 1952, *Sanford* 1987, *Testoni* 1974, *Van Rensburg & Du Plessis* 1971). This paper reports in detail the morphological findings of extraglomerular renal lesions in mink with spontaneous encephalitozoonosis. Glomerular lesions will be reported in a separate paper.

Materials and methods

Animals

The renal tissue was collected from 13 mink.

The mink were 1/2 or 1 1/2 years old. The etiological diagnosis was based on serological tests using antiserum against *Encephalitozoon cuniculi* and on demonstration of parasitic spores in cataractous lens (*Bjerkås et al.* 1988). All animals were free from plasmacytosis, as judged by the agar test (*Nordstoga & Loftsgaard* 1986).

Light microscopy

Pieces of the kidneys were fixed in 10% buffered formalin and embedded in paraffin. Sections were routinely stained with hematoxylin and eosin (HE) and elastin van Gieson (EVG).

Electron microscopy

Tissue blocks from 3 cases were chosen for ultrastructural observation. Small pieces from deparaffinized tissues were post-fixed in 2% osmium tetroxide solution, dehydrated in a graded ethanol series and embedded in Epon 812. Semithin sections were stained with toluidine blue for light microscopic survey. Ultrathin sections, for study of cystic epithelium and identification of inflammatory cells, were stained with uranyl acetate and lead citrate. The sections were examined and photographed using a Jeol 100S electron microscope.

Results

Macroscopic appearance

The kidneys of all affected animals had lesions consisting of variably sized, multiple cysts in the renal parenchyma, from barely visible, up to 0.5 cm in diameter. No other gross lesions were observed in the kidney.

Light microscopy

Tubular changes were frequently found. Tubules lying within the cortical or medullary lesions showed varying degrees of degeneration and necrosis. Many variably sized renal cysts were seen in medulla and corticomedullary junction (Fig 1). Most cysts were empty. Occasionally, a few cysts containing granular proteinaceous casts could be seen. Cystic epithelial cells were either low cuboidal or flattened. However, epithelial hyperplasia was also found (Fig 2). The parasite was not found within the epithelial cells or lumen of tubules. Pericystic cellular infiltration was slight but connective tissue proliferation was obvious.

A severe chronic interstitial nephritis was present in all animals. Inflammatory cells were distributed either diffusely or multifocally in the cortex and medulla. The cellular infiltrate consisted mostly of lymphocytes and plasma cells (Fig 3). Multifocal or coalescing areas of cellular infiltration were most extensive in the cortex and corticomedullary junction. Inflammatory cells were also scattered throughout the medulla. Occasional foci of infiltration were observed in the medulla. Lymphocytic and plasmacytic infiltration was frequently accompanied by a fibroblast proliferation.

Arteritis and periarteritis were pronounced. Numerous cellular infiltrates occurred within damaged vessel walls and perivascular areas (Fig. 4). Some arterioles showed fibrous changes and their lumens were often obstructed. Arterial sclerosis was often found. The arterial walls were very thickened and fibrocellular or fibromuscular intimal proliferation had narrowed the lumen (Fig 5). The internal elastic lamina was often interrupted in damaged arteries.

Massive cellular infiltrates, perivasculitis and sclerotic arteries were also found in the subpelvic connective tissues. In general, renal fibrosis was mild. Collagenization was

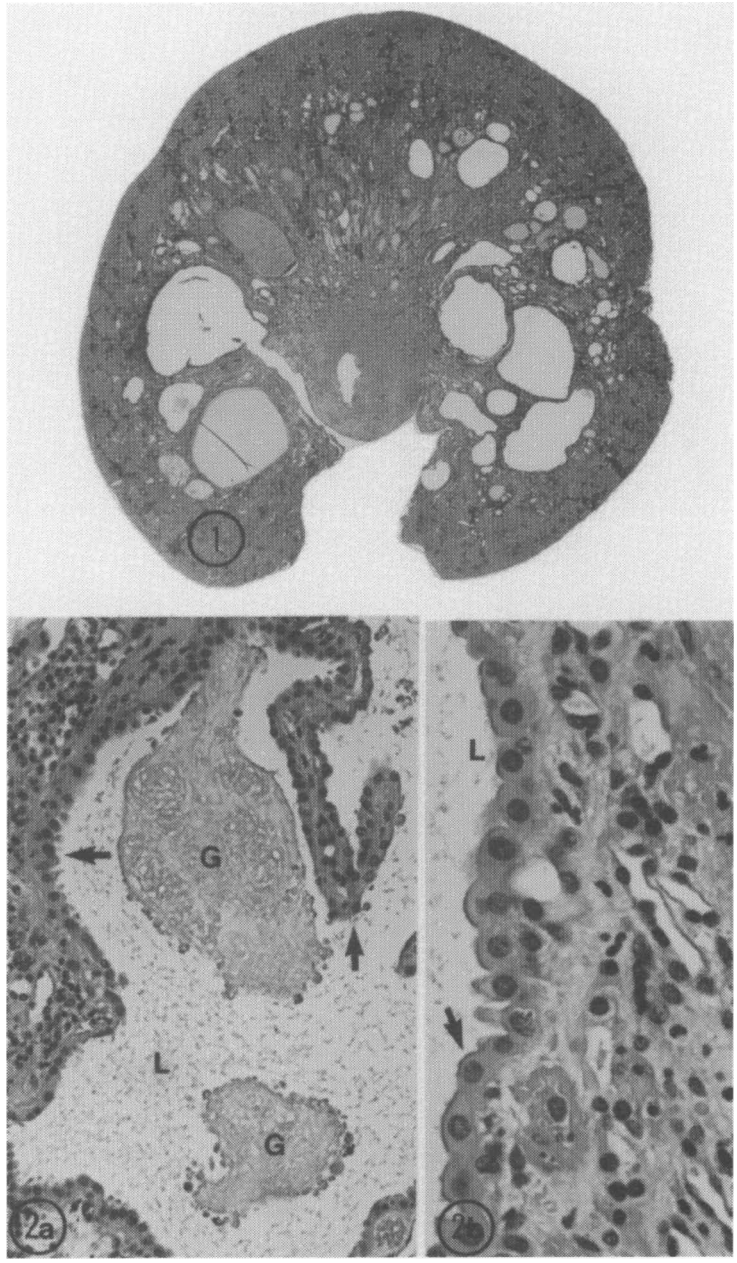

Figure 1. Variably sized, multible renal cysts in medulla and corticomedullary junction of affected mink. HE, x 5.

Figure 2. Tubular cyst walls. a.) Hyperplastic cyst walls (arrows). L = cyst lumen, G = granular proteinaceous casts. HE, x 100. b.) Non-hyperplastic cyst wall showing cuboidal epithelial cells (arrow). L = cyst lumen. HE, x 400.

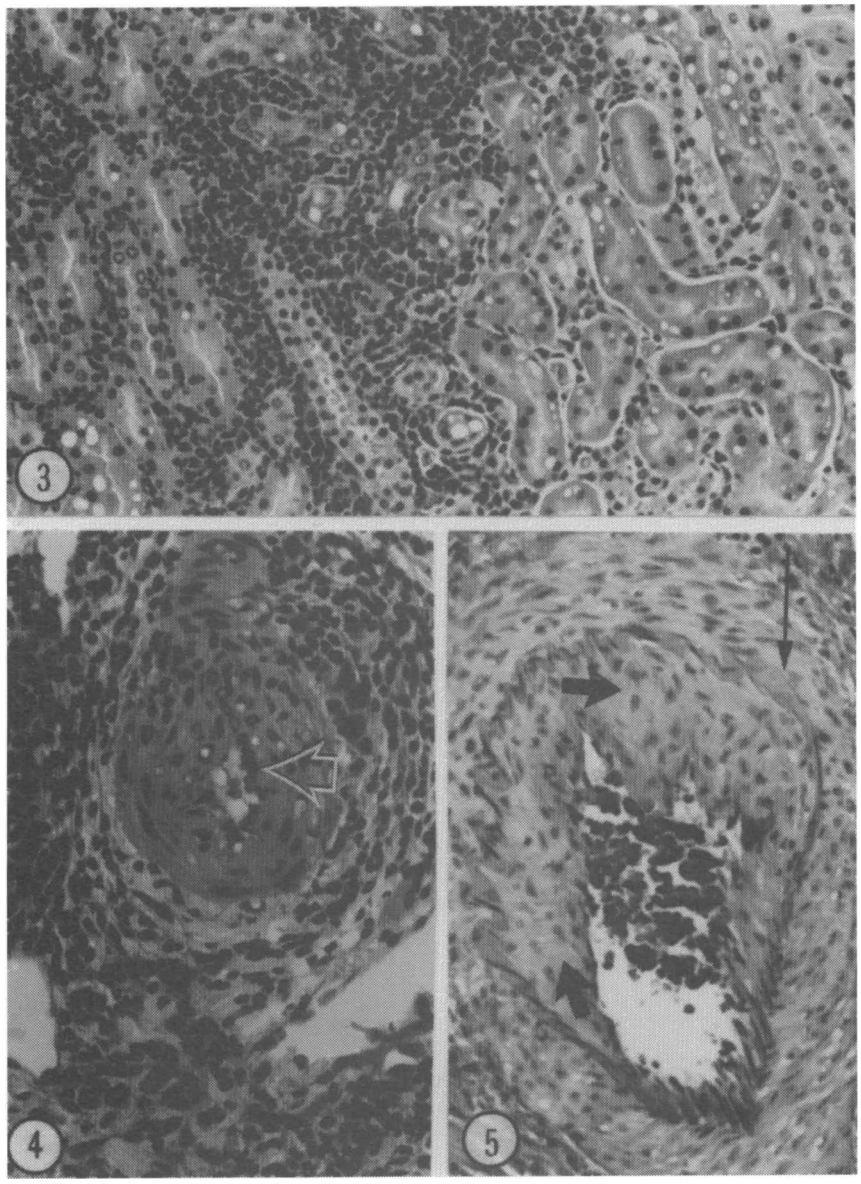

Figure 3. Cellular infiltrate in a cortical lesion. Note that the infiltrate consists mostly of lymphocytes and plasma cells. HE, x 160.

Figure 4. A small inflamed artery with an endothelial hyperplasia (open arrow); thickened media and a diffuse periarteritis. HE, x 190.

Figure 5. A sclerotic artery showing a thickened wall resulting from fibromuscular intimal proliferation (wide arrows) and a consequent reduction of the size of the lumen. The internal elastic lamina has fragmented in an area (arrow). EVG, x 200.

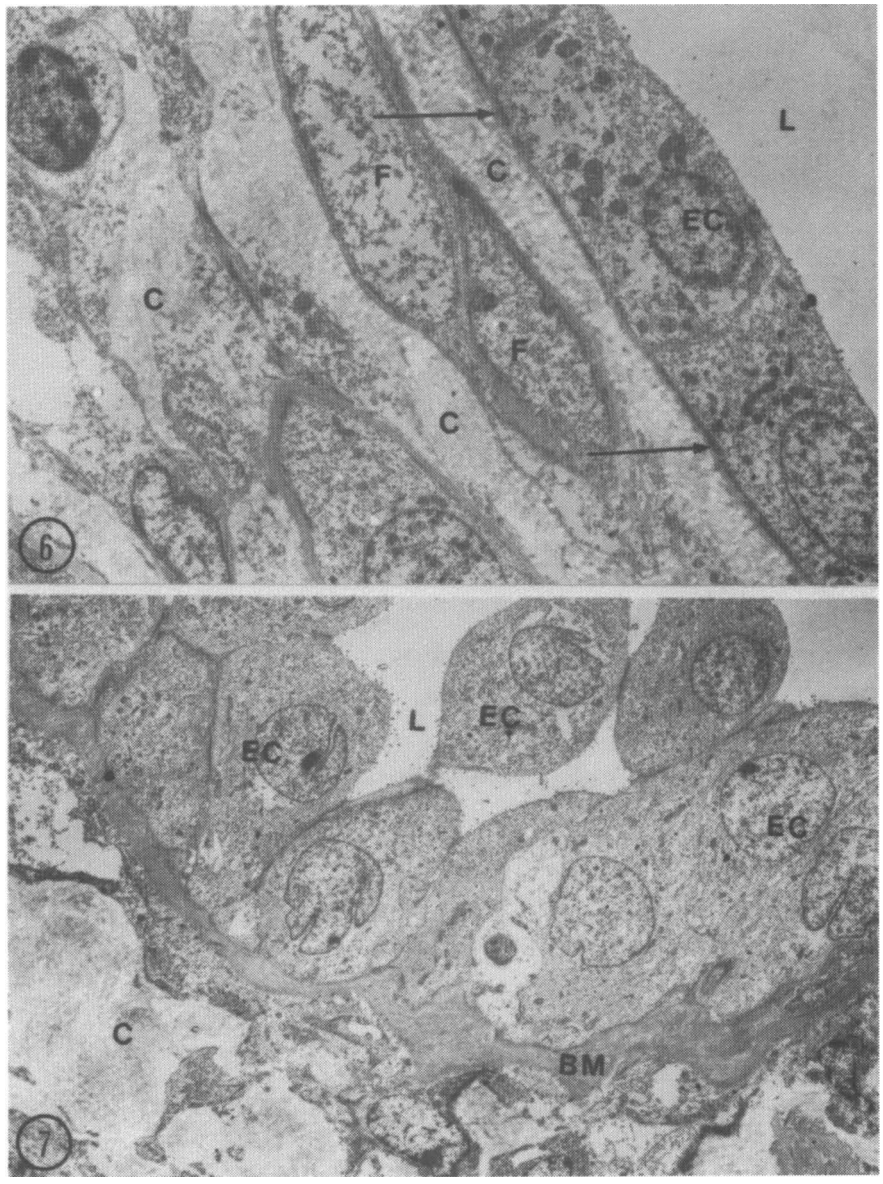

Figure 6. Electron micrograph showing flattened cystic epithelial cells (E C) and a thin basement membrane (Arrows). Note large amounts of collagen (C) around the cyst. L = cyst lumen, F = fibroblasts. x 4000.
Figure 7. Electron micrograph showing hyperplastic epithelial cells (E C), an irregular thickened basement membrane (B M) and peripheral collagen tissue (C). x 4000.

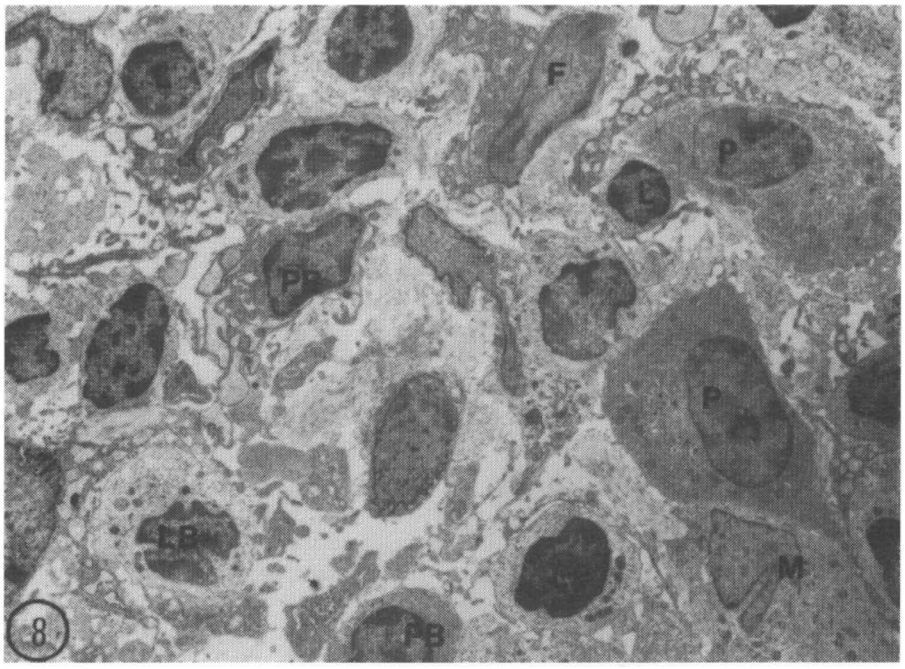

Figure 8. Electron micrograph of a cortical cellular infiltration showing plasma cells (P), plasmablasts (PB), lymphocytes (L) and lymphoblasts (LB). F = fibroblasts, M = macrophage. x 3000.

marked around the vessels. In addition, glomerulonephritis was present but the parasite was not demonstrated in the kidney tissues examined.

Electron microscopy

Ultrastructural examination of extraglomerular lesions revealed cystic epithelial changes and the nature of the cellular population. Two types of cyst epithelii were observed, either a cuboidal or a flattened epithelium. Most cysts showed flattened epithelial cells and a thin basement membrane (Fig. 6). The basement membrane was similar to that of noncystic tubules. The cytoplasm of the flattened epithelial cells contained many mitochondria and polyribosomes and the nucleus was spherical. The apical surface of the flattened epithelial cells had e few short microvilli. The hyperplastic cyst epithelial cells contained few organelles. Overall, the cyst epithelium had the same structure as a collecting duct epithelium. But hyperplastic cyst walls showed mostly an irregular thickened basement membrane (Fig. 7). Collagen tissue and a few lymphocytes and plasma cells were present around the cyst walls. No deposits were found in the epithelial basement membranes.

Mature lymphocytes with round dense nuclei surrounded by small amounts of cytoplasm were found scattered throughout the interstitium and were also observed within intact or degenerating tubular epithelium and the

lumen of tubules. Additionally, lymphoblasts with many free ribosomes were encountered. Plasma cells of varying maturity were found that ranged from plasmablasts with a central nucleus and moderate amounts of rough endoplasmic reticulum to mature plasma cells with an eccentrically placed nucleus and cytoplasm packed with parallel or distended endoplasmic reticulum (Fig. 8). Macrophages and fibroblasts were also seen scattered throughout the cellular infiltrates.

Vessel endothelial hypertrophy and hyperplasia were visible in some arterioles, and the lumen of the arteriole was usually obstructed by hypertrophic and hyperplastic endothelial cells. Occasionally, fibrin deposits within arterial walls could be found.

Discussion

This investigation has demonstrated that the kidney is a primary target organ of *E. cuniculi* infection in mink and that extraglomerular lesions consist of tubular cyst formation and chronic interstitial nephritis and vasculitis.

Renal cysts were numerous in the present material. Cystic disease may be caused by congenital and acquired events (*Maxie* 1985, *Confer & Panciera* 1988). Acquired cysts can be differentiated from congenital cytst on the basis of inflammatory scarring (*Crowell et al.* 1979, *Maxie* 1985). *Henriksen* (1988) reported polycystic kidneys in mink and suggested that the condition probably represented a hereditary disease. In this study, tubular cystic walls were lined by cuboidal, flattened or hyperplastic epithelium and were surrounded by some inflammatory cells and collagen tissue. There was chronic interstitial nephritis with variable scarring in the renal tissues. On the basis of above findings, it is concluded that the cysts were formed gradually and probably represent an acquired lesion associated with tubular damage caused by the parasite. The electron microscopic investigations demonstrated that the cysts originated from the collecting duct epithelium. Many authors have documented that *E. cuniculi* parasitizes the tubular epithelium of the renal medulla, usually the collecting duct tubules (*Flatt & Jackson* 1970, *Goodman & Garner* 1972, *Wilson* 1979). *E. cuniculi* is most common in lesions of the shortest duration and is absent from lesions of the longest duration (*Flatt & Jackson* 1970, *Wilson* 1979). In blue foxes, *E cuniculi* has been observed frequently in tubular cells of acute cases (*Nordstoga,* 1972). Although *E. cuniculi* was not demonstrated in the kidneys of the mink examined in this study, it was assumed that the parasite had dissappeared from tubular cells in these prolonged cases. However, it is not known for how long time the parasite persists in the tissues (*Goodman & Garner* 1972). In addition, there is no evidence of cystic changes being involved in renal lesions in blue foxes, dogs and rabbits with encephalitozoonosis (*Flatt & Jackson* 1970, *McCully et al.* 1978, *Nordstoga* 1972, *Nordstoga & Westbye* 1976). It must therefore be concluded that renal lesions in mink encephalitozoonosis are different from those occurring in other species, and that the mechanisms by which the cysts are formed are still obscure.

The appearance in this study of interstitial nephritis in mink encephalitozoonosis was basically similar to that reported in blue foxes, dogs, rabbits and other carnivores (*Botha et al.* 1986, *McCully et al.* 1978, *Nordstoga* 1972, *Sanford* 1987, *Vávra et al.* 1971). The renal vasculitis had features similar to those seen in blue foxes and dogs (*McCully et al.* 1978, *Nordstoga & Westbye* 1976). Arterial lesions in encephalitozoonosis were considered to be caused either by parasitism of

media smooth muscle cells (*Bjerkås* 1987, (*Bjerkås & Nesland* 1987) and endothelial cells (*Van Dellen et al.* 1978) or by a hypersensitivity reaction (*Nordstoga* 1972).

The pathogenesis of interstitial nephritis in many animals is unknown but is thought to be related to bacterial and viral septicemias or an immune response (*Thomson* 1988). Lesions consisting of multifocal interstitial nephritis and vasculitis and perivasculitis indicate a primary haematogenous dissemination of the infection (*Botha et al.* 1986). In the present study, although the parasite was not found in the renal tissues, the parasite has been demonstrated in the brains and the eyes of these mink (*Bjerkås et al.* 1988, *Bjerkås* 1990). This suggest that the parasite has been eliminated from most organs after a temporary parasiticemia in the early stage of the infection. A number of mechanisms may be involved in the development of the lesion. Immunological factors may play an important role in the pathogenesis of interstitial nephritis. In our peroxidase immunohistologic staining (*Zhou & Nordstoga,* unpubl. data), numerous plasma cells showing strong positive reaction for IgG were frequently seen in the interstitium. High proportions of plasma ceels suggested a local antibody response. Indeed, the presence of numerous lymphocytes and some macrophages would suggest that interstitial nephritis may also be associated with cell mediated immunity. However, until more detailed immunological studies are carried out, the role of humoral and cell mediated immunity in the pathogenesis of interstitial nephritis remains uncertain.

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Sammendrag

Ekstraglomerulære nyreforandringer hos mink ved encephalitozoonose.

De ekstraglomerulære forandringene i nyrene ble studert i lysmikroskopiske og elektronmikroskopiske preparater hos 13 mink. Alle dyra hadde dobbeltsidig katarakt og var spontant infiserte, sannsynligvis på fosterstadiet. Forandringene bestod av multiple cyster, multifokale betennelsesforandringer som delvis konfluerte til større lesjoner, og vaskulitt. De tubulære cystene var av noe forskjellig størrelse og forekom i medulla og på overgangen mellom medulla og cortex. Betennelsesinfiltratene bestod vesentlig av lymfocytter og plasmaceller og var ledsaget av interstitiell fibrose. Vaskulitt, perivaskulitt og sklerotisering i arteriene forekom ofte.

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