J. Comp. Path. 2014, Vol. 151, 162–165

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INFECTIOUS DISEASE

Membranoproliferative Glomerulonephritis in a Calf with Nephrotic Syndrome

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Summary

A 2-month-old Japanese black calf was presented with a history of weight loss, exophthalmos and subcutaneous oedema of the brisket. Urinalysis and serum biochemistry showed proteinuria and hypoproteinaemia suggestive of nephrotic syndrome. Microscopically, lesions in the kidney were characterized by proliferation of mesangial cells and diffuse thickening of the glomerular basement membranes with the appearance of double contours. Immune complex deposits were confirmed by electron microscopy and immunofluorescence using reagents specific for bovine immunoglobulin G, complement factor C3 and bovine viral diarrhoea virus (BVDV). Consequently, the glomerular lesion in this case was diagnosed as membranoproliferative glomerulonephritis. BVDV type 1 was detected in serum by nested reverse transcriptase polymerase chain reaction. Viral antigen was also identified in the glomeruli by immunofluorescence. These results suggest that BVDV may have been the cause of immune complex glomerulonephritis in this calf.

Keywords: bovine viral diarrhoea virus; calf; glomerulonephritis; kidney

Nephrotic syndrome is characterized by proteinuria, hypoalbuminaemia, oedema and hypercholesterolaemia (Maxie and Newman, 2007). In cattle, this syndrome is usually caused by renal amyloidosis. There are few reports (Wiseman *et al.*, 1980; White *et al.*, 1986; Doré *et al.*, 1987; Murray and Sharpe, 2009) of nephrotic syndrome due to glomerulonephritis in this species. In animals, glomerular lesions can be classified as proliferative, membranous or membranoproliferative glomerulonephritis (MPGN). The most commonly identified cause of glomerulonephritis in domestic animals is immune complex deposition (Maxie and Newman, 2007). Although bovine viral diarrhoea virus (BVDV) has been recognized as an important factor in immune complex glomerulonephritis in cattle (Cutlip *et al.*, 1980), to our knowledge there is only one report (White *et al.*, 1986) of nephrotic syndrome in a BVDV-infected cow. This report describes the morphological, ultrastructural and immunolabelling features of MPGN, which developed into nephrotic syndrome in a calf infected persistently with BVDV.

A 2-month-old Japanese black calf with poor growth rate and exophthalmos was referred to the Miyazaki University Animal Hospital. There was a recent history of respiratory difficulty and diarrhoea. Several cows on the farm had aborted. Blood was collected for serum biochemical examination. This analysis revealed significant hypoproteinaemia (31 g/l, reference range 62–75 g/l) due to hypoalbuminaemia (14 g/l, reference range 30–36 g/l). There was an increase in the concentration of creatinine (804.4 μmol/l, reference range 44.2–159.1 μmol/l) and blood urea nitrogen (49.1 mmol/l, reference range 3.6–8.9 mmol/l). Urinalysis revealed severe proteinuria. Nested reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect the presence of BVDV genetic material in serum, using primers described by Gilbert *et al.* (1999). A serum neutralization test using BVDV type I Nose strain was performed in microtitre plates as described by Schefers *et al.* (2008). BVDV type 1 was detected in

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.05.001

the serum by RT-PCR and although neutralizing antibody was not detected, the calf showed high levels of BVDV antibody detected by a commercial enzyme linked immunosorbent assay (ELISA) kit (Bio-X Diagnostics, Jemelle, Belgium).

The calf was humanely destroyed because of its rapid clinical deterioration and poor prognosis. On necropsy examination, subcutaneous oedema was observed in the brisket and there was marked ascites. The kidneys were pale and enlarged with diffuse red foci, up to 1 mm in diameter, on the capsular and cortical regions of the cut surfaces. Lesions were not seen in other organs.

The kidneys were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (2 μm) were stained with haematoxylin and eosin (HE), periodic acid methenamine silver (PAM) and Congo red stain. Microscopical examination of the kidneys revealed hypercellular glomeruli with proliferation of mesangial cells and matrix. Lobulated glomeruli showed irregular thickening of the basement membrane (Fig. 1). Double contours in the glomerular basement membrane (GBM) were identified with PAM stain (Fig. 2). There were marked hyaline droplets in the cytoplasm of proximal tubular cells. The interstitial spaces were infiltrated with lymphocytes and macrophages. The kidneys did not show amyloid deposition with Congo red stain.

Samples of formalin-fixed kidney were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in Epon 812. Semithin sections were stained with toluidine blue for light microscopy and ultrathin sections were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope. Ultrastructural examination revealed an increase in the mesangial cell population and thickening of the GBM with electron-dense deposits (Fig. 3).

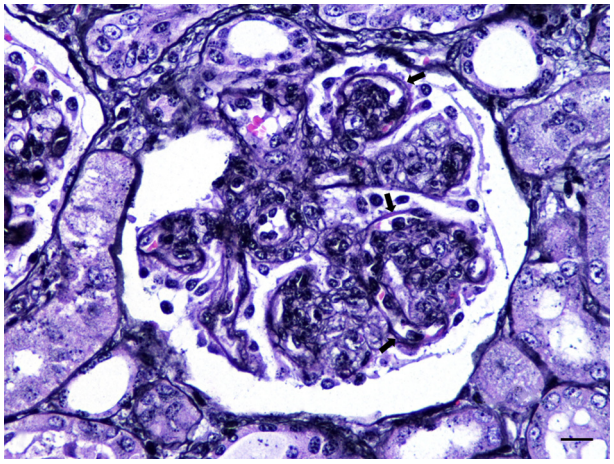

Fig. 2. Glomerular basement membrane with formation of clear double contours (arrow). PAM. Bar, 10 μm.

Histopathological changes in the glomeruli and the presence of electron-dense deposits were suggestive of immune complex deposition. Such complexes generally consist of immunoglobulin (Ig) G and complement factor C3 together with an antigenic component. Candidate antigens in ruminants may derive from organisms involved in purulent metritis, from BVDV infection or infections with *Trypanosoma congolense* or *Fasciola hepatica* (Cutlip *et al*., 1980; White *et al*., 1986; Marques *et al*., 2004). However, this case was only 2 months of age, was male, and

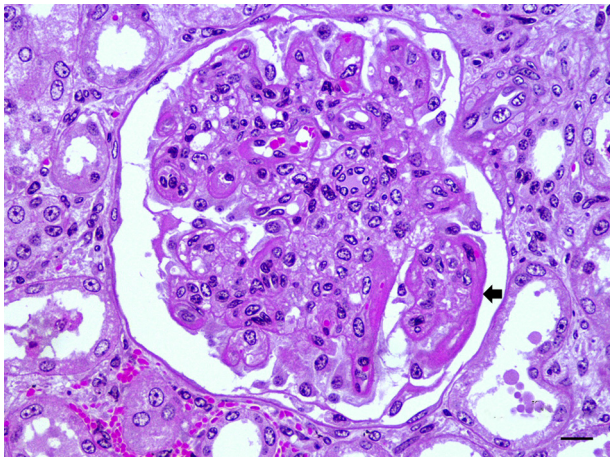

Fig. 1. Lobulated glomerulus with proliferation of mesangial cells and irregular thickening of glomerular basement membrane (arrow). HE. Bar, 10 μm.

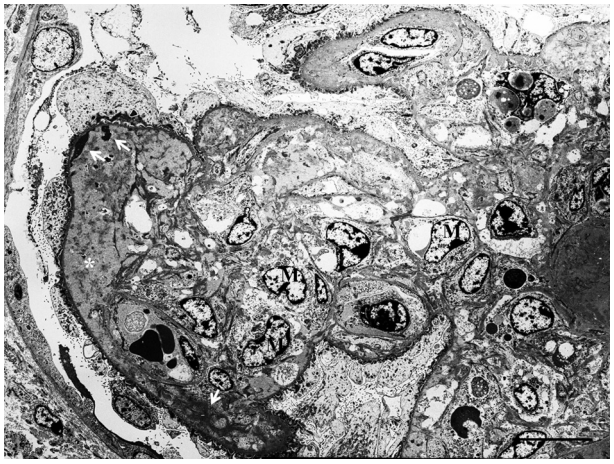

Fig. 3. Glomerular basement membrane is irregularly thickened (*) and contains electron-dense deposits (arrow). Increased mesangial cell population (M) near the capillary loop. Transmission electron microscopy. Bar, 10 μm.