Oxalate nephrosis in Zwartble sheep

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Oxalate nephrosis is an important differential diagnosis for dullness, inappetance and sudden death in the Zwartble breed

ABSTRACT
The Zwartble breed is originally from Friesland, in the northern Netherlands, but has been established in the UK and Ireland for over 100 years. Used for both meat and fibre production, Zwartbles are prolific, milky and show strong mothering ability. During the period December 2013 to March 2014, a three-year-old Zwartble ewe and a three-week-old Zwartble lamb from different flocks were presented with a history of sudden death following a period of dullness and inappetence. At necropsy, the kidneys were pale and shrunkken with poor demarcation between cortex and medulla. Histological examination showed interstitial fibrosis, urolithiasis and nephrocalcinosis with the presence of intra-luminal, and some intracellular, yellow, translucent calcium oxalate crystals in the renal tubules. These cases are notable for the rarity of oxalate nephrosis in sheep, a possible breed predisposition to the condition in Zwartbles (Zwartble nephrosis) and a likely similarity to other inherited primary hyperoxalurias (especially PH2 or L-glyceric aciduria) described in human patients and in some breeds of cats, dogs and cattle.

OVERVIEW
In man, the primary hyperoxalurias (PHs) are rare, inherited disorders of glyoxylate metabolism resulting from deficiencies of activity of specific enzymes in the liver and other tissues. There are two characterised PHs in man: PH1 is caused by deficiency of the enzyme alanine-glyoxylate aminotransferase-1 (AGT-1) in the liver, while PH2 results from reduced glyoxylate reductase/hydroxypruvate reductase (GR/HPR) activity in the liver and other tissues. A small number of human cases of PH (described as non-PH1 or PH2) fit the established diagnostic criteria for hyperoxaluria without demonstrable deficiency in either AGT-1 or GR/HPR activity and the cause of these cases in unknown. PHs result in over-production of oxalate by the liver, which is counter-balanced by increased renal excretion with resultant super-saturation of the urine with calcium oxalate, leading to urolithiasis, nephrocalcinosis and, in severe cases (usually PH1), systemic oxalosis. Secondary hyperoxaluria may occur due to dietary or other exposure to large amounts of oxalate or oxalate precursors. Ingestion of some plants including beets, kale and rape may cause oxaluria in sheep although this is uncommon with a low incidence of oxalate nephrosis in the population as a whole. Ruminating animals are tolerant of oxalate due to degradation to harmless carbonates or bicarbonates by rumen microflora. Pre-ruminant lambs are more susceptible and hyperoxaluria has been reported in six-week-old Suffolks fed a concentrate contaminated with oxalate-forming mould.

Severe oxalate nephropathy, possibly corresponding to PH2 (L-glyceric aciduria) has been described in Zwartble sheep and is sometimes termed Zwartble nephrosis, although no characterisation of the gene/enzyme involved has been reported to date. L-glyceric aciduria has been reported in domestic shorthaired cats and the polymorphisms of the feline glycolate reductase gene (GRHPR) resulting in reduced enzyme activity have been identified. Genetic testing is now possible to characterise important GRHPR mutations in domestic shorthairs to identify homozygous affected animals and heterozygous carrier cats. However, not all oxalate nephropathy in cats is due to L-glyceric aciduria. Ragdoll cats show a nephrosis in which oxalate crystals are present in the renal tubules. However, both PH1 and PH2 can be excluded on the basis of urine oxalate and liver enzyme analysis, and the aetiology and probable inheritance of familial renal disease in this breed has yet to be elucidated and the term oxalate-like nephrosis is best used to describe this condition.

Primary hyperoxaluria has been described in Tibetan spaniels and koala bears, although there are as yet no reports of the actual enzymes and associated polymorphisms causing the condition in these species. Similarly, reports of severe primary hyperoxaluria and renal oxalosis in Beefmaster cattle in the US are not fully characterised, although autosomal recessive inheritance can be reasonably assumed.

CASE STUDIES
Two cases of oxalate nephropathy in Zwartble sheep originating from different flocks were examined by the Northern Ireland Agri-Food and Biosciences Institute (AFBI) Disease Surveillance and Investigation Branch between December 2013 and March 2014. A three-year-old Zwartble ewe was presented in December 2013 with a history of sudden death following a period of dullness and inappetence. The ewe was at grass and being fed supplementary concentrate during the pre-lambing period and had previously been routinely treated for the prevention of fasciolosis and vaccinated against clostridial disease and pasteurellosis.
A three-week-old lamb was presented in February 2014 with a history of sudden death following a period of non-specific malaise. The lamb was indoors on the ewe and had access to creep feed, and had been treated with oxytetracycline.

In both cases, the gross post-mortem findings were unremarkable save for observable renal pathology. In the ewe, the kidneys were pale and shrunken and showed poor demarcation between cortex and medulla. In the lamb, the kidneys were observed to be swollen with pale cortices. No significant organisms were recovered from cultures of lung, liver, spleen or small intestine in either case. Washings taken from the abomasum and small intestine showed only a small number of coccidial oocysts of no clinical significance in the ewe and the presence of significant numbers of cryptosporidial oocysts in the lamb.

Histological examination of haematoxylin and eosin stained sections of the kidneys from the ewe showed chronic interstitial nephritis with focal to coalescing infiltration of lymphocytes and plasma cells with marked fibrosis of the interstitium. The renal tubules showed either narrowing of the lumen due to the interstitial fibrosis or marked expansion (ectasia) and were lined by attenuated, degenerative or necrotic epithelium. Pale yellow-coloured, translucent, variable shaped crystals, often with wheat-sheaf morphology, were detected in the tubular lumen (urolithiasis) and incorporated into the tubular cells or interstitium (nephrocalcinosis) (Figure 1).

The crystals were considered to be morphologically consistent with calcium oxalate and this was confirmed by the use of alizarin red S stain. Calcium oxalate crystals characteristically stain red with alizarin red S at pH 7.0 but not at all at pH 4.2. Calcium phosphate and calcium carbonate, however, stain red at both pH 7.0 and pH 4.2. This difference allows presumptive identification of calcium oxalate deposits which can be confirmed by pre-treatment of sections with acetic acid which will dissolve calcium phosphate or carbonate but not oxalate (Figure 2).

Histological examination of haematoxylin and eosin stained sections of the kidney from the lamb similarly showed interstitial fibrosis, urolithiasis and nephrocalcinosis, but no nephritis. The renal tubules again showed either narrowing of the lumen or ectasia with protein casting and were lined by attenuated, degenerative or necrotic epithelium. Pale yellow-coloured, translucent, variable shaped crystals, often with wheat-sheaf morphology, were detected in the tubular lumen and incorporated into the tubular cells.

Sections treated with acetic acid and stained with alizarin red S at pH 7.0 were positive for the presence of red staining calcium oxalate crystals (Figure 3). The calcium oxalate crystals demonstrated by the various staining techniques also showed characteristic bi-refringence under polarised light (Figure 4).

A diagnosis of primary hyperoxaluria (PH) and oxalate nephrosis was made in both the ewe and the lamb on the basis of: a history which precluded access to oxalate-containing plants or other sources of oxalate; the breed and age of the sheep; confirmation of the presence of calcium oxalate crystals by use of the alizarin red S specific staining method; and the gross and histological findings of urolithiasis and nephrocalcinosis due to calcium oxalate crystals.

**SUMMARY**

Oxalate nephrosis and primary or secondary hyperoxaluria is uncommon in sheep in the UK and Ireland, and the incidence of oxalate nephrosis is much higher than average in Zwartbles. Oxalate nephrosis in Zwartbles is probably due to PH2 (L-glycerate aciduria) similar to that in other species, but the gene(s) and/or enzyme(s) involved and mode of inheritance have not been confirmed and reported to date.

Zwartbles are well established in the UK and Ireland, and have many useful breed characteristics; flocks of these sheep are seen regularly by some practitioners. Oxalate nephrosis is an important differential diagnosis for dullness, inappetance and sudden death in this breed. The cases described highlighted the age range over which the condition can occur with even very young lambs being affected.
PH2 can be confirmed by testing for elevated levels of urinary oxalate and L-glycerate where these tests are available or by post-mortem examination with demonstration of kidney lesions and the presence of calcium oxalate crystals by special stains in sheep not exposed to exogenous oxalate or its precursors.

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REFERENCES


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Reader Questions and Answers

1: ZWARTBLE NEPHROSIS IS DUE TO:
A: Deposition of struvite crystals in the urethra
B: Deposition of urates in the renal tubules
C: Deposition of calcium oxalate crystals in the renal tubules

2: PRIMARY HYPEROXALURIA TYPE 2 (PH2) IS CAUSED BY REDUCED ACTIVITY OF WHICH OF THE FOLLOWING ENZYMES?
A: Glyoxylate reductase/hydroxypyruvate reductase
B: Glutathione peroxidise
C: Glyoxalate aminotransferase

3: IN WHICH OF THE FOLLOWING SPECIES HAVE PRIMARY HYPEROXALURIAS NOT BEEN DESCRIBED TO DATE?
A: Cats
B: Dogs
C: Kangaroos

4: SECONDARY HYPEROXALURIA IS NOT CAUSED BY:
A: Ingestion of beets, rape or kale
B: Ingestion of oxalate forming moulds
C: Specific enzyme deficiencies in the liver and other tissues.

5: WHEN ALIZARIN RED S STAIN IS USED, CALCIUM OXALATE CRYSTALS STAIN RED/PINK:
A: At pH 7.0 but not at all at pH 4.2
B: At both pH 7.0 and pH 4.2
C: At neither pH 7.0 nor pH 4.2


Figure 3: Alizarin red S stained sections showing red calcium oxalate crystals in renal tubules. Sections of lamb kidney pre-treated with acetic acid and stained at pH 7.8.

Figure 4: Bi-refringence of calcium oxalate crystals in a section of ewe kidney, viewed under polarised light.