A case of renal amyloidosis associated with udder cleft dermatitis in an adult dairy cow

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SUMMARY

Amyloidosis is a group of disorders characterised by the injurious deposition of abnormal proteins in tissues. Most common in cattle and other animals is secondary or reactive systemic amyloidosis associated with chronic inflammation, resulting in deposition of acute-phase serum amyloid A (SAA) in organs like the kidneys, liver, and spleen. While chronic diseases like mastitis, metritis and pododermatitis are usually the most common diseases identified as inflammatory sources, any persistent inflammation can trigger this disorder. Cattle affected by amyloidosis often exhibit symptoms like weight loss and kidney dysfunction. Here we present the case of a five-year-old Holstein Friesian referred to the University of Glasgow for weight loss and chronic diarrhoea. Clinical examination revealed low body condition score, watery diarrhoea, mild dehydration (5%), mild submandibular oedema, left renomegaly, and udder cleft dermatitis. Biochemistry and urine analysis indicated hypoalbuminemia with normal globulin levels and marked proteinuria. Given the poor prognosis, the animal was euthanised on welfare grounds. Gross post-mortem findings suggested a diagnosis of secondary amyloidosis, and histopathology confirmed SAA deposition in the glomeruli and renal medullary interstitium. In the absence of another grossly appreciable chronic inflammatory focus/ foci the udder cleft dermatitis was considered the likely contributing comorbidity.

KEY WORDS

Bovine; renal disease; amyloidosis; chronic inflammation; udder cleft dermatitis.

INTRODUCTION

Renal amyloidosis in cattle is uncommon, with abattoir reports ranging from 0.8 % to 5% (1,2). Amyloidosis is associated with chronic inflammatory diseases, such as reticuloperitonitis or any other long-term inflammation causing an elevated concentration of serum amyloid A (SAA), which can form aggregates that are systemically deposited, predominantly in the kidney, liver, and spleen (2,3). Even if the disease can affect different organs, the clinical signs draw attention when the kidneys are involved (4,5). There are no specific preventative measures for amyloidosis and the prognosis is usually poor (6).

CASE HISTORY

A 5-year-old Holstein Friesian cow was referred on 24th February 2021 to the University of Glasgow (UofG) School of Veterinary Medicine. The cow was 164 days in milk in her third lactation. According to the farmer's history, the cow had a mild milk drop (from 44 to 40 litres) in December 2020. On 9th February 2021, a significant milk drop (from 38 to 26 litres) associated with diarrhoea was noticed. The referring veterinary surgeon reported profuse diarrhoea, left renomegaly, and low body condition score. The cow was treated on farm with a three-day course of 15 mg/Kg bodyweight trimethoprim 40 mg - 200 mg sulfadiazine, IM, SID (Norodine®, Trimethoprim 4 mg/ml Sulfadiazine 20 mg/ml, Norbrook, UK); with no clinical improvement.

The cow was referred from a dairy farm milking 200 pedigree Holstein Friesian cows and averaging 12.500 kg per cow/year. Udder cleft dermatitis was reported sporadically on the farm (prevalence < 3 %). The farm was closed with biosecurity measures in place for visitors and contractors. A control plan was in place for paratuberculosis with all milking cows tested quarterly for antibodies in milk. All tests were negative in the previous 2 years. The herd had Bovine Viral Diarrhoea (BVD) negative status. Vaccinations against BVD, Leptospirosis, Infectious Bovine Rhinotracheitis and mastitis *(Escherichia coli-Staphylococcus aureus)* were performed yearly. Adult cattle were kept indoors all year round in cubicles, with some cows spending 2-3 weeks on pasture during the dry period. *Fasciola hepatica* was absent based on bulk tank mild antibody testing and abattoir reports.

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CASE PRESENTATION

On the day of the admission, the cow appeared bright, alert and responsive. Her Body Condition Score (BCS) was 2.00 (range 0-5; bodyweight 622 Kg (7)). A greenish and watery diarrhoea without visible blood was observed. Normal spontaneous micturition was observed, resulting in the voiding of pale yellow transparent urine. Mild oedema was observed in the submandibular area but not in the brisket. No jugular venous distension was observed. Mild dehydration was observed (5%). The mucous membranes were pink and moist and capillary refill time was less than 2 seconds. Examination of the oral cavity revealed no abnormalities. Palpable lymph nodes were normal in size and shape. The heart rate was 60 bpm (reference range: 40-80 (8)); there were no anomalies in frequency and rhythm. The respiratory rate was 30 bpm (reference range: 12-36 bpm (8)); no adventitious sounds were auscultated. Auscultation and percussion of both right and left abdomen sides revealed no abnormal sounds and rumination was regular (1 contraction every 40 seconds). No evidence of thoracic or abdominal pain was present on the "withers test". Rectal temperature was 38.0 °C (reference range: 38-39 °C (8)). On the trans-rectal examination, the left kidney was moderately enlarged but not painful on palpation. Palpation of other organs was unremarkable (rumen, intestines, sub-aortic lymph nodes, urinary bladder, uterus). A focal 10 cm diameter light red ulcerative lesion typical of a healing udder cleft dermatitis lesion on the skin of the ventral midline between the front udder quarters. Palpation of the rest of the udder was unremarkable and the milk was normal in colour and consistency. The California Mastitis Test (CMT) was negative for all quarters (9).

PROBLEM LIST

- Milk drop during mid-lactation
- Low BCS (2.00 out of 5)
- Chronic marked watery diarrhoea
- Enlarged and non-painful left kidney per rectum
- Mild submandibular oedema
- Mild dehydration (5%)
- Udder cleft dermatitis

DIFFERENTIAL DIAGNOSES

From most to least likely:

- Urinary tract diseases: Renal amyloidosis, Glomerulonephritis (GN), Pyelonephritis, Hydronephrosis, Cystitis.
- Protein-losing enteropathies: Paratuberculosis (*Mycobacterium avium* sub *paratuberculosis*-MAP), Gastrointestinal nematodes, Chronic salmonellosis.
- Hepatic failure: Fasciolosis.
- Heart failure: Congestive heart failure.

ANCILLARY TESTING

A blood sample for complete haematology and biochemistry was taken on 25th February 2021, and the results are reported in Table 1. The haematology was unremarkable. Biochemical features included hypoalbuminemia (19 g/L (31-38 g/L)) and mild hypoproteinaemia (53 g/L (63-89 g/L)). Globulins were within the normal range (34 g/L (30-48 g/L)).

A urine sample was collected by catheterisation of the urethra after careful disinfection of the vulva to evaluate renal functionality (Table 2). The urine sample was analysed with a dipstick (Multistix SG (Bayer)); urine pH was 8.5, and marked proteinuria (+4) was observed. Proteinuria was confirmed with sulfosalicylic acid precipitation; some white blood cells (WBCs) were observed on microscopy (Table 2).

Both kidneys were examined by ultrasonography: the left one by trans-rectal ultrasonography (7.5 MHz linear probe) and the right one trans-abdominally (2.5-5 MHz convex probe) in the dorsal part of the right paralumbar fossa (10). The ultrasonography of the left kidney showed renomegaly (15 x 35 cm). Similarly, the right kidney was evaluated trans-cutaneously, and renomegaly (15 x 35 cm) was observed; no other abnormalities, such as flocculent fluid or deformed sinuses, were observed (11,12). Ultrasonography of the bladder (7.5 MHz linear probe) was unremarkable. Ultrasonography of the heart (2.5-5 MHz

Table 1 - Haematological and biochemical parameters of a 5 year old cow referred for diarrhoea, wasting condition and renomegaly to the UofG.

Parameter	Result	Reference Interval
RBC	6.35	5-10 x 10^{12}
Hb	10.7	8.5-12.2g/dL
HCT	30.4	22-33 %
MCV	47.9	38-50 fl
MCHC	35.2	30-36/dL
WBC	9.72	4.6-12 x 10^9/L
Neutrophils	3.56	0.6-4.0 x 10^9/L
Lymphocytes	3.72	1.5-7.5 x 10^9/L
Monocytes	0.5	0.025-0.84 x 10^9/L
Eosinophils	0	0-9 x 10^9/L
Basophils	0.051	0-0 x 10^9/L
PLT	456	100-800 x 10^9
Toxic neutrophils	Absent	/
Phosphate	1.81	1.13-2.84 mmol/L
Calcium	2.24	2.2-3.3mmol/L
Magnesium	0.54	0.65-1.39 mmol/L
Sodium	137.3	135-157 mmol/L
Potassium	4.8	3.2-5.8 mmol/L
Total protein	53	63-89 g/L
Albumin	19	31-38 g/L
Globulin	34	30-48 g/L
Albumin/globulin ratio	0.56	0.88-1.31
GGT	22	0-27 U/L
GLDH	66.9	0-30 U/L
AST	62	0-140 U/L
ALK phosphatase	37	20-280 U/L
Creatinine	36	113-212 U/L
Urea	2.8	1.6-5.9 mmol/L

RBC: Red blood cells. Hb: Haemoglobin. HCT: Haematocrit, MCV: Mean corpuscular volume. MCHC: Mean corpuscular haemoglobin concentration. WBC: White blood cells. PLT: Platelet. GGT: Gamma-glutamyl transferase. GLDH: Glutamate dehydrogenase. AST: Aspartate aminotransferase. ALK: Alkaline.

Table 2 - Other ancillary tests of a 5 years old cow referred for diarrhoea, wasting condition and renomegaly to the UofG.

Urine dipstick	Proteinuria (+4): No other abnormalities were detected.		
Urine analysis	**Parameter**	**Results**	**Reference Interval**
	Quantitative Protein	351.7 mg/100ml	0- 25.0
	Qualitative Protein	+++	Considered physiological in ruminants
	Quantitative Creatinine	59.06 mg/100mL	0-142.3
	Protein creatinine ratio (UPC)	5.95	0.04.-0.25
	pH	8.5	/
	WBC (microscopy)	+	/
Urine culture and sensitivity	*Corynebacterium* Group G (formerly *Arcanobacterium haemolyticum*), *Aerococcus viridans* and *Bacillus* were isolated.		
Faecal culture and sensitivity	*Campylobacter jejuni* ssp *doylei* was isolated.		
Paratuberculosis	Serology (Ab ELISA): Negative (1%). Cut-off (P%): Negative </= 50%. PCR: negative.		
Parasitology	McMaster: no nematode eggs detected. Boray (faecal sedimentation): no liver egg flukes were detected.		

convex probe) was unremarkable. Other ancillary testing, such as faecal culture, *Mycobacterium avium* sub *paratuberculosis* - PCR, and parasitology, did not highlight any relevant findings (Table 2).

During the hospitalisation, which lasted three weeks, weekly haematology and blood biochemistry was carried out. Hypoalbuminaemia (in the range of 18-20 g/L (31-38 g/L)) was stable, and no further abnormalities were detected.

DIAGNOSIS

Based on the clinical examination and ancillary testing results, a non-infectious protein-losing kidney disease was diagnosed. The gold standard to differentiate between renal amyloidosis and glomerulonephritis in vivo would have been renal biopsy (5). Considering the poor prognosis for the mentioned diseases, it was decided to euthanise the animal using

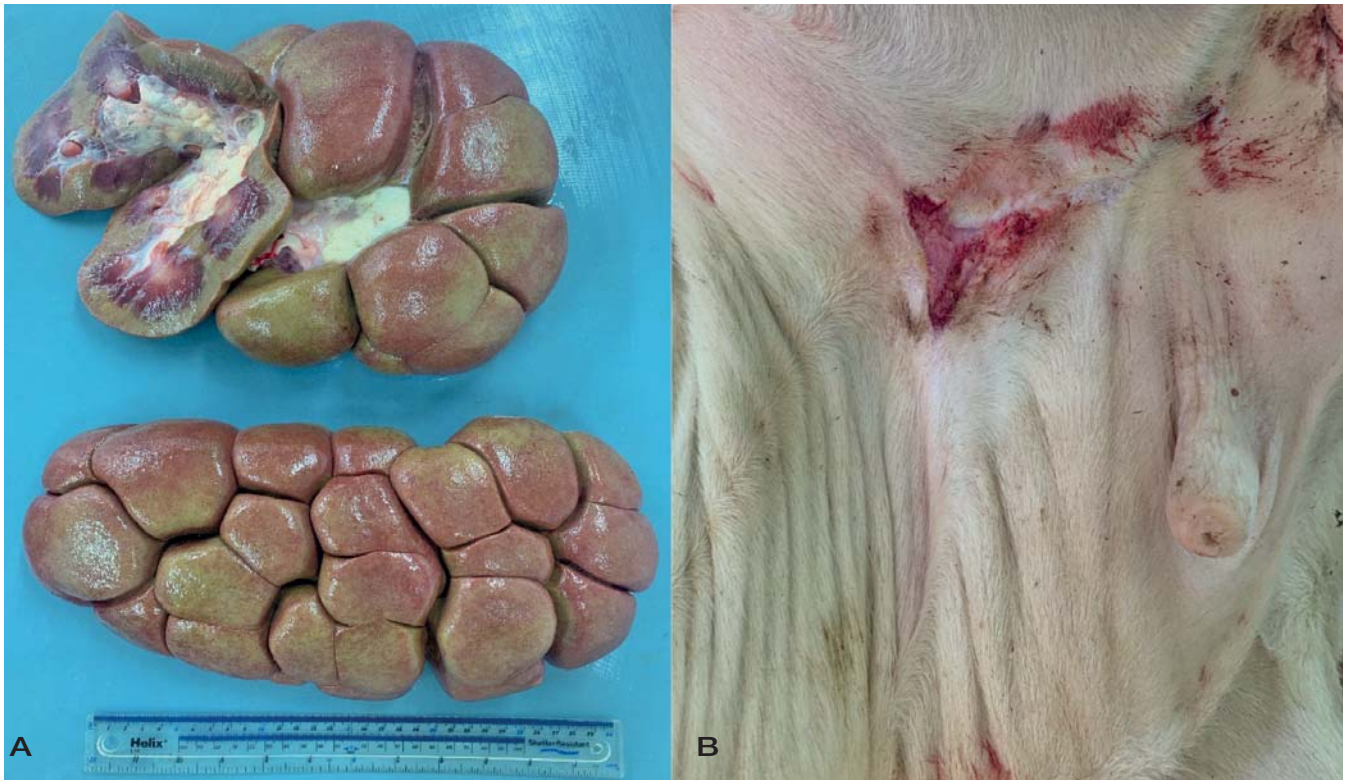

Figure 1 - Gross pathology images, kidneys bilaterally enlarged and udder cleft dermatitis.
A) The kidneys measured 15 x 20 x 35cm (normal size: 14 x 18 x 24), with pale yellow discolouration of the subcapsular surface and cortex.
B) The lesion on the udder was irregularly shaped, depressed, well-delineated, and dark red, indicating a chronic ulcerative dermatitis.

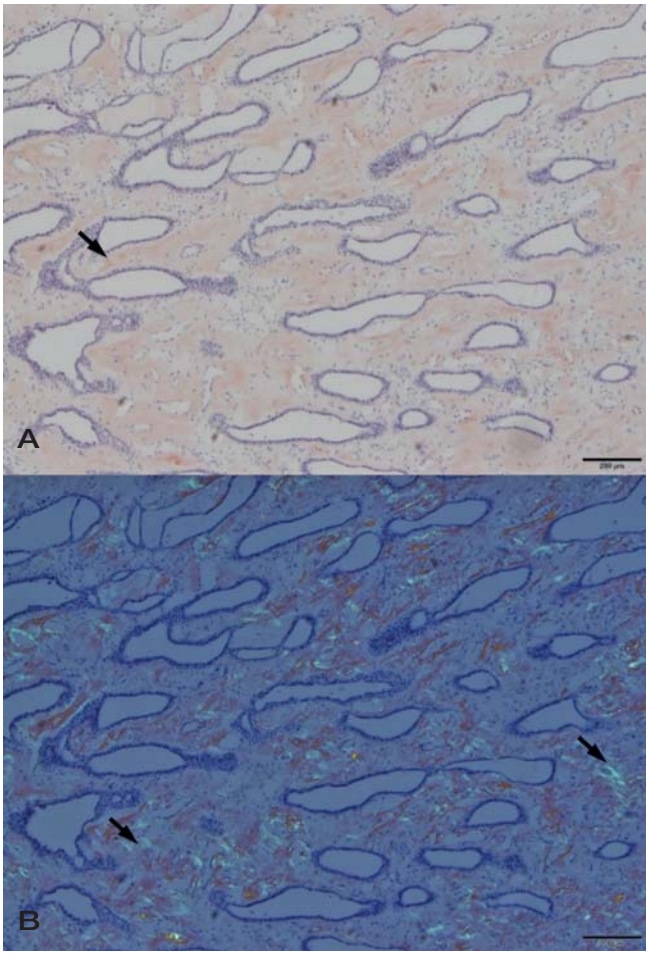

Figure 2 - Histopathology sections, medulla of the kidney.
A) Moderate expansion of the interstitium of the renal medulla by homogeneous amorphic congophilic material suspected to be amyloid (black arrow). Congo red, 6 um section, standard illumination, x 400, scale bar 200 um.
B) Apple green birefringence under polarised light (black arrows) confirms the congophilic material as amyloid. Congo red, 6 um section, polarised light, x 400, scale bar 200 um.

140 mg/kg, Pentobarbital Sodium, IV (Euthasol® Vet. 400 mg/ml, Dechra, UK). A post-mortem examination was performed.

On the gross pathology, both kidneys were enlarged (Figure 1A). The ventral subcutis was moderately expanded by clear to pale yellow gelatinous material (oedema). In the large intestines, the wall was markedly expanded by clear to pale yellow gelatinous material (oedema). On the skin of the udder, a lesion of approximately 10 cm diameter was observed between the two front quarters (Figure 1B).

On histopathology of the kidney, the glomeruli and interstitium of the corticomedullary and medullary regions were expanded by moderate to large amounts of pale eosinophilic homogenous material that on thick sections displays moderate to marked congophilia (Figure 2A) with apple-green birefringence in polarised light (Figure 2B) confirming that this was SAA substance.

The histopathology confirmed the SAA deposition in the glomeruli and renal interstitium of the medulla (4). A final diagnosis of renal amyloidosis was made.

DISCUSSION

Typical clinical signs of renal amyloidosis, such as chronic diarrhoea, weight loss, inappetence, peripheral oedema and reduced milk production, were potentially indicative of a wide range of disorders and, therefore, of low specificity. Renomegaly is more indicative of kidney disease, but it is a subjective evaluation, and when dilatation is subtle, it can be easily missed at trans-rectal palpation. Blood chemistry indicated hypoalbuminemia, suggesting a protein-losing disease rather than congestive heart failure as the cause of submandibular oedema. Heart diseases were then ruled out by ultrasonographic examination. Protein loss can be caused by kidney diseases, enteropathy or hepatic failure (5). Interestingly, the albumin level did not diminish below 10 g/L, which is considered the cut-off to create a change in the oncotic pressure to produce significant generalised oedema (5). Protein-losing enteropathies (such as paratuberculosis, gastrointestinal nematodes, and chronic salmonellosis) and fasciolosis can cause a clinical picture similar to the present case (5,13). These diseases were ruled out by ancillary testing. Faecal culture isolated *Campylobacter jejuni*, which was considered to be a secondary finding resulting from an opportunistic infection. In a study conducted by Wesley and collaborators, faecal samples were collected from 2,085 dairy cattle across 31 farm operations in the USA. *Campylobacter jejuni* was identified in healthy cows without any clinical signs (14). Having ruled out protein-losing enteropathy and liver failure, protein-losing nephropathy was considered the primary cause of the clinical signs.

Urinalysis was shown to be a useful tool in narrowing the diagnostic suspicion. The urinary dipstick showed marked proteinuria, which was indicative of kidney damage. It is worth remembering the importance of specifically testing proteinuria, like this case, with sulfosalicylic acid precipitation. In fact, alkaline urine pH can give a false positive result and lead to a misinterpretation (5). Among cattle diseases, pyelonephritis is the most common (5). Urinalysis revealed a lack of other evidence consistent with urinary tract inflammation and/ or infection. The small numbers of white blood cells observed in the present case was considered physiological based on the method of urine collection and the mixed culture of bacteria isolated was consistent with contamination. On urine culture, there was no evidence of *Corynebacterium renale* or *Escherichia coli*, which are commonly isolated in cases of pyelonephritis; the other isolated ones were considered non-pathogenic. These findings were supported by clinical examination, ancillary testing, and post-mortem findings. At the clinical examination, no signs of pain such as stranguria or renal pain at palpation were detected, and proteinuria in the absence of significant leukocyturia or renal pain made significant pyelonephritis unlikely, like a bladder infection. Ultrasonographycally, no signs of infection were detected in the kidney and bladder. Finally, post-mortem and histopathology did not reveal any evidence of pyelonephritis or cystitis, completely ruling out urinary tract infections. These findings were consistent with non-infectious protein-losing nephropathy, such as amyloidosis and glomerulonephritis. Clinically significant glomerulonephritis, such as renal amyloidosis, is rarely reported in cattle (5). Further pathological findings confirmed the presence of serum amyloid A in both glomeruli and renal interstitial

spaces, confirming the renal amyloidosis diagnosis (4). Considering no other sources of chronic inflammation were identified, udder cleft dermatitis was thought to be the inciting cause of the renal amyloidosis in this case (15).

CONCLUSION

For the first time, we report a case of renal amyloidosis in which udder cleft dermatitis was considered the causative factor in the absence of another identifiable inflammatory foci (15). There are no specific preventative measures for amyloidosis, and it was not considered relevant at the herd level. The present case highlights the importance of a comprehensive diagnostic approach, including history, clinical examination, ancillary testing, and post-mortem, to achieve the correct diagnosis of renal amyloidosis. Additionally, the causative inflammatory focus was identified by careful exclusion criteria.

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Author Contributions
Giovanni Capuzzello: Resources, Conceptualization; Investigation; Visualization; Data Curation; Writing - original draft. Isabella Nicola: Conceptualization; Writing - review & editing. Alexander Gray: Conceptualization; Investigation; Methodology; Data Curation; Writing - review & editing.

Conflict of Interest
The authors declare no conflict of interest.

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