Journal of Veterinary Internal Medicine
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ACVIM American College of Veterinary Internal Medicine

Case Report
J Vet Intern Med 2017;31:598–603

Aminoaciduria Caused by Fanconi Syndrome in a Heifer

N. Cesbron, L. Dorso, A.-L. Royer, G. Dervilly-Pinel, and J. Hervé

A case study of renal tubular dysfunction consistent with idiopathic Fanconi syndrome is reported in an 18-month-old Holstein heifer. The clinical, biochemical, and histopathological features are described. The heifer had clinical signs of growth retardation, wasting, and persistent diarrhea. Biochemical blood analysis identified hypokalemia, hyponatremia, and hypochloremia. Urinalysis identified glycosuria, proteinuria, and acidic pH. Histological examination of the kidney disclosed mild tubular necrosis with proteinaceous casts in the lumina of renal tubules. We performed LC-HRMS on urine to confirm Fanconi syndrome. Using this technique, we identified severe generalized aminoaciduria suggestive of idiopathic renal Fanconi syndrome in this heifer.

Key words: Cattle; high-resolution mass spectrometry; liquid chromatography; renal tubular dysfunction.

An 18-month-old Holstein-Friesian heifer was referred for a 6-month history of poor body condition and persistent diarrhea. Before admission, the heifer had received only standard anthelmintic treatment (ivermectin, 0.5 mg/kg, topically[a]) in October 2014 and 1 injection of a nonsteroidal anti-inflammatory drug (meloxicam, 0.5 mg/kg, SC once[b]) in January 2015.

Upon arrival, the heifer had lethargy, weakness, enophthalmos without abnormal skin turgor (suggesting emaciation and loss of adipose tissue rather than dehydration), mild anorexia, and severe watery malodorous diarrhea. Severe emaciation was evident when comparing its weight and size to the breed standards (Fig 1): 250 kg vs 490 kg expected and 1.30 m height at the shoulder against 1.35 m expected. Urination appeared normal, but the urine was slightly cloudy. Urine dipstick evaluation[c] was performed upon arrival and disclosed a specific gravity of 1.020, aciduria (pH 6), hemoglobinuria (3+), hematuria (4+), glycosuria (3+), and proteinuria (3+). Glycosuria was not associated with clinical signs of hyperglycemia such as polydipsia or polyuria. Weakness, emaciation, and abnormal urinalysis findings suggested chronic renal disease associated with substantial protein loss, which was further investigated.

Abbreviations:

LC-HRMS	liquid chromatography–high-resolution mass spectrometry
PAS	periodic acid Schiff

A CBC[d] disclosed mild leukocytosis ($16.1 \times 10^3/\mu L$; reference range: $4.0–12.0 \times 10^3/\mu L$) with neutrophilia ($8.5 \times 10^3/\mu L$; reference range, $0.6–4.0 \times 10^3/\mu L$) and slight monocytosis ($1.1 \times 10^3/\mu L$; reference range, $0–0.8 \times 10^3/\mu L$), suggestive of mild inflammation. Because the hematocrit was normal (41%; reference range, 24–46%) and in the absence of any signs of dehydration (normal skin turgor, normal urine output), the heifer was considered adequately hydrated.

Serum biochemistry results included increased serum creatinine concentration (24 mg/L; reference range, 0–12 mg/L) as well as a mild increase in urea nitrogen (500 mg/L; reference range, 50–200 mg/L). Liver enzyme (alkaline phosphatase and γ-glutamyl transferase) activities were normal. Evaluation of serum electrolyte concentrations identified hypokalemia (<1.5 mmol/L; reference range, 3.8–5.2 mmol/L), hypochloremia (76 mmol/L; reference range, 95–110 mmol/L), and hyponatremia (118 mmol/L; reference range, 145–160 mmol/L). Serum phosphorus and calcium concentrations were within the reference range.

Serum protein concentration was increased (85 g/L; reference range, 60–80 g/L), whereas serum albumin concentration (41 g/L; reference range, 30–43 g/L) was normal. Serum protein electrophoresis identified a slight increase in alpha-globulin concentration with an albumin:globulin ratio of 1.07. Because proteinuria was not associated with hypoproteinemia, nephrotic syndrome was excluded.

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This work was performed in Oniris, Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering.

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Submitted May 2, 2016; Revised August 30, 2016; Accepted September 26, 2016.

DOI: 10.1111/jvim.14605

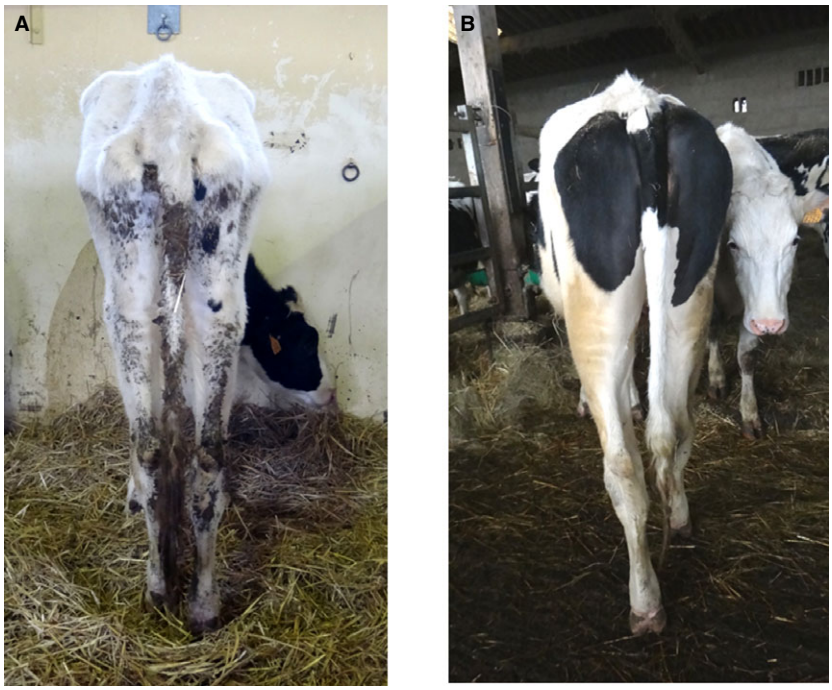

Fig 1. Affected heifer at 18 months of age (**A**) as compared to an age-matched Holstein heifer (**B**). The 2 animals are of similar size except for marked thinness in muscle mass of the hindquarters and fat over bony prominences in the affected heifer.

On day 2, complete urinalysis[e] confirmed severe proteinuria (3 g/L) with the presence of nucleated cells (30 WBC/μL and 0/μL RBC; reference range, up to 5 red cells or leukocytes per μL), aciduria (pH 6.3; reference range, 7–9), and isosthenuria (urine specific gravity 1.018; reference range, 1.020–1.050).

The abnormal laboratory results (hypokalemia, hyponatremia, glycosuria, proteinuria, and aciduria) were consistent with renal tubular dysfunction suggesting possible Fanconi syndrome.[1] The heifer died suddenly on day 2, and serum glucose and bicarbonate concentrations were not measured.

Necropsy identified marked and diffuse serous atrophy of fat (compatible with emaciation and weight loss) and abundant malodorous diarrhea without any gross intestinal findings.

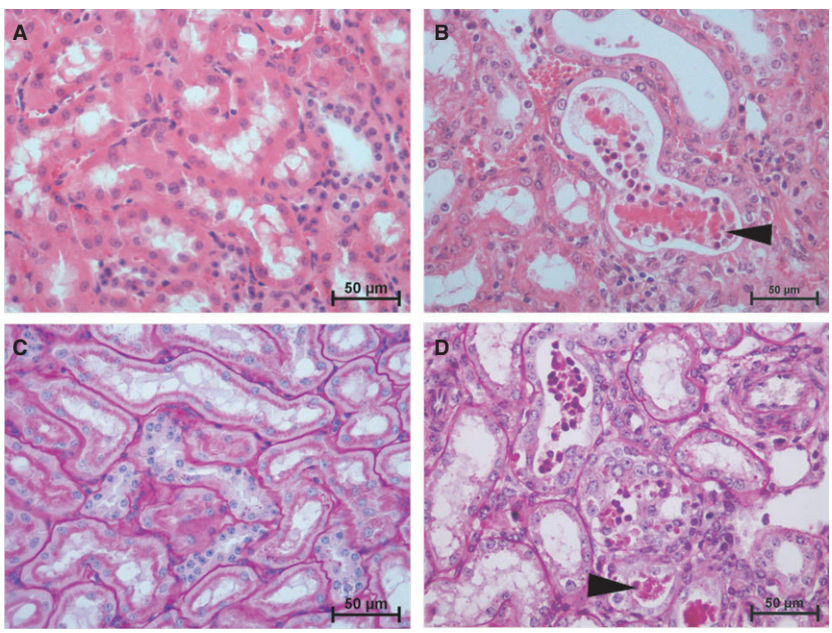

Fig 2. Histological examination of age-matched control kidney (**A** and **C**) and affected kidney (**B** and **D**), hematoxylin and eosin staining (**A** and **B**), and periodic acid Schiff staining (**C** and **D**), ×400. Cytonuclear debris from tubular cells are observed in the lumina of distal convoluted tubules (**B**, arrowhead) associated with acidophilic PAS-positive proteinaceous casts (**D**, arrowhead).

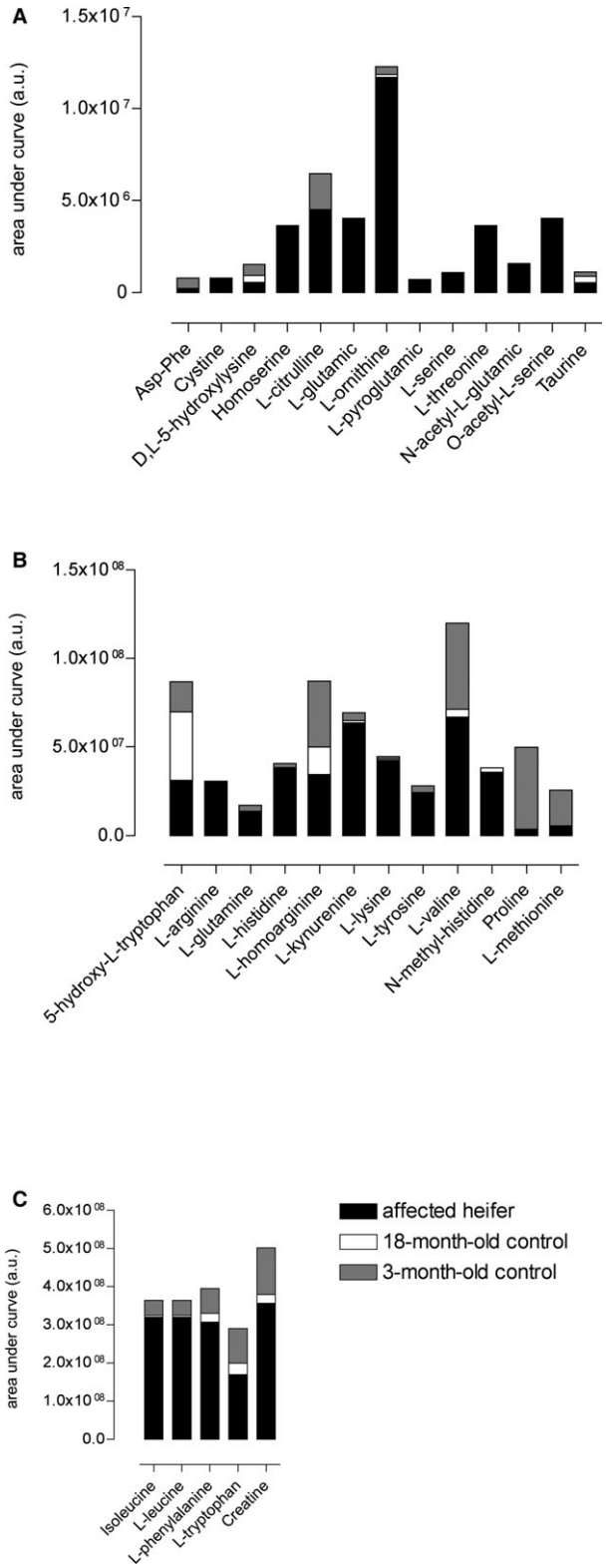

Fig 3. Severe generalized aminoaciduria in affected heifer. Data were obtained using a LC-HRMS full scan performed on urine samples of the affected heifer as compared to controls. Control 1 is an 18-month-old Holstein-Friesian heifer without renal failure, and control 2 is a healthy 3-month-old male Holstein-Friesian calf. Quantifications of peak areas (expressed in arbitrary units, au) for amino acids and their catabolites are reported as stacked bars. Because relative quantities vary considerably among amino acids, data were subdivided to produce Figure A (range, 0–1.5 $\times$ 10^7), B (range, 0–1.5 $\times$ 10^8), and C (range, 0–6 $\times$ 10^8).

Both kidneys were grossly normal. Histological examination of the kidney (Fig 2) identified slight and focal thickening of Bowman's capsule by periodic acid Schiff (PAS) staining. Other regions of the glomeruli were normal. No histological abnormalities were detected in the proximal tubules. Intranuclear inclusion bodies suggestive of lead poisoning were not observed. We observed accumulation of rare acidophilic proteinaceous casts that were strongly positive for PAS in the lumen of the distal convoluted tubules associated with rare cytonuclear debris (Fig 2B and D), compared with an age-matched control kidney (Fig 2A and C). These findings were consistent with mild and focal tubular necrosis and mild proteinuria. The renal cortical interstitium had mild nonspecific multifocal lymphoplasmacytic infiltration.

In humans, renal lesions in Fanconi syndrome are mild and include cuboidal glomerular epithelium, epithelial vacuolization, variation in size of the proximal tubules, interstitial fibrosis associated with lymphoplasmacytic infiltration and degenerative changes.[2] In dogs with Fanconi syndrome, histological renal lesions also are mild and nonspecific with interstitial fibrosis and tubular atrophy. Affected dogs often exhibit karyomegaly in scattered tubular cells.[3]

In our case, renal histological lesions were limited. Neither epithelial vacuolization nor karyomegaly was observed. We reported mild focal injury to renal distal convoluted tubules, characterized by the presence of necrotic tubular cells in the lumina of tubules. This focal injury was unlikely to have resulted from any toxic event (no antibiotic exposure or heavy metal poisoning). Altogether, these histological findings were not specific for Fanconi syndrome.

We performed liquid chromatography–high-resolution mass spectrometry (LC-HRMS)[f] on the affected heifer's urine for amino acid profiling purposes. Control samples included 2 urine samples from an 18-month-old Holstein-Friesian heifer (referred to as control 1) and from a 3-month-old male Holstein (referred to as control 2). External calibration for positive ionization mode was performed using a standard mixture of caffeine, L-methionyl-arginyl-phenylalanyl-alanine acetate, and Ultramark 1621.[g] Cattle urine samples (250 μL) were filtered through a 10 kDa polyethersulfone membrane[h] under centrifugation at 10,000 g for 20 minutes at 5°C. Ten μL of a solution containing isotope-labeled standards at concentrations of 5 ng/μL each in ethanol/water (75 : 25) was evaporated to dryness under a stream of nitrogen at 30°C, and 30 μL of filtered and diluted urine sample was added. Then, reverse phase chromatographic separation was performed.[i] The mobile phase consisted of water containing 0.1% acetic acid (solvent A) and acetonitrile containing 0.1% acetic acid (solvent B).[j] The gradient elution (25 minutes) started with 5% solvent B which was held for 2.4 minutes, after which solvent B was gradually increased to

Table 1. Urinary amino acid detection by LC-HRMS full scan.

Amino acid	Retention Time	Peak Area (Arbitrary Units)		
		Affected Heifer	18-month-old Control	3-month-old Control
Cystine	0.49	7.9×10^5	ND	ND
Isoleucine	0.84	3.2×10^8	4.7×10^6	3.9×10^7
L-arginine	0.47	3.0×10^7	8.1×10^4	4.7×10^5
L-citrulline	0.50	4.5×10^6	ND	2.0×10^6
L-glutamic acid	0.50	4.0×10^6	ND	ND
L-glutamine	0.50	1.4×10^7	ND	3.1×10^6
L-histidine	0.47	3.8×10^7	1.8×10^5	2.3×10^6
L-leucine	0.84	3.2×10^8	4.7×10^6	3.9×10^7
L-lysine	0.46	4.2×10^5	6.9×10^5	1.3×10^6
L-methionine	0.74	5.6×10^6	ND	2.0×10^7
L-ornithine	0.46	1.2×10^7	1.6×10^5	4.1×10^5
L-phenylalanine	1.30	3.0×10^8	2.2×10^7	6.6×10^7
L-serine	0.50	1.0×10^6	ND	ND
L-threonine	0.51	3.6×10^6	ND	ND
L-tryptophan	2.77	1.7×10^8	3.0×10^7	9.1×10^7
L-tyrosine	0.74	2.4×10^7	ND	3.9×10^6
L-valine	0.63	6.7×10^7	4.5×10^6	4.9×10^7
Proline	0.46	3.7×10^6	ND	4.6×10^7
Taurine	0.43	5.3×10^5	3.4×10^5	2.6×10^5

Retention time and peak area for each amino acid identified in urine samples are presented. ND, non detected.

successively achieve 25% at 4.5 minutes, 70% at 11 minutes, and finally 100% at 14 minutes, which was held for 2 minutes. The system then was returned to initial conditions (5% B) during the remaining 9 minutes. The flow rate was 400 μL/min, and the column oven and sample tray were held at 35°C and 4°C, respectively. The injected volume was 5 μL. For HRMS acquisition[i], nitrogen was used as sheath gas and auxiliary gas at flow rates of 55 and 10 arbitrary units (au), respectively. The ion transfer tube temperature was set at 350°C. The injected volume was 5 μL. A positive polarity mode was used. Finally, LC-HRMS profiles were obtained operating at full scan mode (65–1,000 m/z).

Relative quantifications of urine amino acids are presented in Figure 3. Retention time and peak area for each amino acid are listed in Table 1. As shown in Figure 3, the urine sample from the affected heifer exhibited generalized severe aminoaciduria. Indeed, except for proline and L-methionine, which are overrepresented in the control 2 urine sample (Fig 3B), all amino acid and catabolite concentrations were higher in the patient urine sample as compared to controls. Data obtained for proline and L-methionine in the control 2 urine sample are notable because the sample originally came from a 3-month-old male Holstein. At this age, kidney development is incomplete and tubular transport systems are still maturing, which might explain these findings.

By contrast, the severe generalized aminoaciduria in the urine sample of the affected heifer that had no histological signs of intoxication (cadmium, lead, copper, antibiotics) is most consistent with a diagnosis of Fanconi syndrome.

Fanconi syndrome was first described in 1931 by the Swiss pediatrician Guido Fanconi and is well described in humans and dogs (especially the Basenji). In humans, Fanconi syndrome is a proximal convoluted renal tubular disorder characterized by impaired proximal reabsorption of glucose, amino acids, phosphate, and bicarbonates leading to increased urinary excretion of these substances. Clinical signs at presentation generally include polyuria, polydipsia, moderate dehydration, and body weight loss despite normal appetite. Blood analysis identifies hypocalcemia, hypophosphatemia, hyponatremia, hypokalemia, and hypochloremia. Urinalysis findings include glycosuria with normal blood glucose concentration, proteinuria and, less frequently, aminoaciduria, phosphaturia, and increased urine sodium concentration.

Idiopathic Fanconi syndrome is described in several breeds of dogs.[4–8] To our knowledge, as far as cattle are concerned, a single case of paradoxical glycosuria has been reported.[9] In that case, an emaciated 9-month-old Simmental bull had serum biochemical abnormalities including hypophosphatemia, hypokalemia, hypochloremia, increased serum total bilirubin concentration, and increased activities of aspartate aminotransferase, γ-glutamyl transferase, and glutamate dehydrogenase. Urinalysis identified glycosuria and ketonuria, but neither proteinuria nor bicarbonaturia was identified.

Although the classical form of Fanconi syndrome in humans is characterized by severe generalized aminoaciduria, it is also associated to several pathologies (e.g, cystinosis, Lowe's syndrome, tyrosinemia) with different patterns of aminoaciduria. In patients with idiopathic Fanconi syndrome, marked urinary excretion of threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, leucine, isoleucine, arginine, and phenylalanine has been described.[1] In our case, we identified increased excretion of leucine, isoleucine, phenylalanine, tryptophan, and valine associated with moderately increased excretion of arginine, glutamine, histidine, and lysine.

Aminoaciduria could result from a defect in energy generation or an impaired coupling of energy generation to the process of amino acid reabsorption in the kidney. Another possible explanation for the generalized defect in amino acid handling is a primary disorder of the lipid bilayer of the membrane allowing increased efflux of the transported amino acids or sodium.[1]

Fanconi syndrome can be either inherited or acquired from a wide variety of toxic tubular injuries. A disease involving multiple defects in renal tubular reabsorption and resembling Fanconi syndrome in humans is described in several breeds of dog (Basenji, Norwegian elkhound, Shetland sheepdog, Schnauzer) and is suspected to be inherited.[4,6,10] In particular, a deletion in the last exon of the FAN1 gene was strongly associated with Fanconi syndrome in a cohort of 78 Basenjis.[11] Numerous cases of acquired and transient Fanconi syndrome in dogs were reported associated with copper storage hepatopathy or intoxication by numerous substances such as heavy metals (cadmium, lead), antibiotics (tetracycline, aminoglycosides) or jerky treats.[3,5,7,8,12–15] Recently, 4 cases of chlorambucil-induced Fanconi syndromes in cats were reported.[16] Transient and acquired Fanconi syndromes also were reported in quarter horses.[17]

In our case, no nephrotoxic treatment or environmental exposure (antibiotics or other) was reported. Liver enzyme activities were normal allowing us to exclude Fanconi syndrome caused by copper storage hepatopathy. Finally, lead poisoning was eliminated because no neurological signs were reported in this heifer and because renal histological examination did not identify either enlargement or vesiculation of nuclei of tubular cells, or irregular acid-fast intranuclear inclusion bodies in renal tubules.

Considering both the young age of the patient and its history of chronic disease, an inherited form of Fanconi syndrome was favored. Inherited Fanconi syndrome could result in persistence of immaturity of the renal proximal tubules. In our case, although there was no histological evidence of renal dysplasia, we cannot be certain the affected heifer ever had normal proximal tubule function.

To our knowledge, ours is the first report of Fanconi syndrome in cattle confirmed by LC-HRMS scan of urine.

Footnotes

[a] Ivomec®Pour-on bovin, Merial SAS, Villeurbanne, France
[b] Metacam®, Boehringer Ingelheim Santé Animale, Reims, France
[c] Urivet-100 reagent strips (KitviaND)
[d] Complete blood cell counts were determined from EDTA blood samples using the MS9-5s analyzer calibrated for cattle (Melet Schloesing, Osny, France)
[e] Cytological and biochemical examination of urine (LDH Vet, Oniris)
[f] LC-HRMS instrument: 1200 Infinity Series high performance liquid chromatography system (Agilent Technologies, Santa Clara, California, USA), coupled to a single-stage Orbitrap (Exactive HCD; Thermo Fisher Scientific, Les Ulis, France) equipped with a heated electrospray ionization source (H-ESI II)
[g] Calmix-positive® (Thermo Fisher Scientific, Les Ulis, France)
[h] VWR International, Fontenay-sous-Bois, France
[i] Hypersil Gold C_{18} column, 100 mm × 2.1 mm, 1.9 μm particle size (Thermo Fisher Scientific, Les Ulis, France)
[j] Acetonitrile, water and acetic acid LC-MS Chromasolv® were purchased from Flucka (Steinheim, Germany)

Acknowledgments

The authors are grateful to JM Nicol for clinical referral of the heifer and to D Boucher for standard laboratory investigations.

Grant support: No external funding was used for this project.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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