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INFECTIOUS DISEASE

Urocystitis and Ureteritis in Holstein Calves with Septicaemia Caused by *Salmonella enterica* Serotype Dublin

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Summary

Salmonellosis is an enteric or multisystemic disease of global distribution that affects numerous animal species. Although *Salmonella enterica* has been associated with urinary tract lesions in man, information on urocystitis/ureteritis in cattle caused by salmonellae is lacking. This communication describes lesions of the inferior urinary tract in four Holstein calves with septicaemia caused by *S. enterica* subsp. *enterica* serotype Dublin. Examination of the urinary bladder revealed either diffuse irregular thickening (three cases) or petechiation (one case) of the mucosa. On histopathological examination, urocystitis with submucosal histiocytic, lymphocytic and plasmacytic infiltration and neutrophil transmigration through the urothelium was noted in all cases. In one case, a fibrinosuppurative ureteritis was detected. *Salmonella* Dublin was identified by culture, 16S rDNA sequencing and serotyping and *Salmonella* antigen was detected intralesionally by immunohistochemistry. Other lesions, indicative of septicaemia included hepatitis, enteritis, pericarditis, splenitis, lymphadenitis and pneumonia. We conclude that *S.* Dublin can be uropathogenic in cattle with septicaemia.

Keywords: cattle; pathology; salmonellosis; urinary tract infection

Salmonellosis is a zoonotic disease of worldwide distribution. The genus *Salmonella* includes >2,500 serotypes within two species: *S. enterica* and *S. bongori*. *Salmonella enterica* subsp. *enterica* is a major pathogen and can infect numerous animal species and man. Salmonellosis causes significant economic losses to the food animal industry and is of great public health concern. Numerous cases of food-borne illness, hospitalization and death are reported every year (www.cdc.gov/salmonella/index.html).

Salmonellosis in cattle usually results from infection by *S. enterica* subsp. *enterica* serotypes Dublin or Typhimurium after oral transmission through contaminated food, water or milk/colostrum. Infections are characterized clinically either by septicaemic and/or enteric disease, or by asymptomatic infection (Carrique-Mas *et al.*, 2010; Costa *et al.*, 2012; Uzal *et al.*, 2016). Even though both serotypes can cause enteric disease, there is a tendency for a differential clinical manifestation between them; while *S.* Typhimurium is generally associated with enteritis and diarrhoea in calves, *S.* Dublin is more often associated with systemic infections that can result in death after a peracute clinical course or acute disease with fever, respiratory signs, arthritis or abortion in pregnant cows. This septicaemic manifestation can result in chronic infection with asymptomatic shedding of the agent (Carrique-Mas *et al.*, 2010; Costa *et al.*, 2012; Uzal *et al.*, 2016).

Typical lesions include fibrinous enterocolitis in the enteric form and hepatitis, cholecystitis, embolic

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pneumonia, splenitis, lymphadenitis, arthritis and gangrene of the distal extremities, tail and pinnae in the septicaemic form (Uzal *et al*., 2016; Pecoraro *et al*., 2017). Urocystitis and urinary tract infection (UTI) caused by *S. enterica* subsp. *enterica* has been described in man (Tena *et al*., 2007; Na *et al*., 2013; Polat *et al*., 2014); however, descriptions in cattle are lacking. This work describes lower urinary tract lesions in four Holstein calves with septicaemia caused by *S.* Dublin.

Four Holstein calves aged between 2 and 4 months from two independent outbreaks of septicaemic salmonellosis were subjected to necropsy examination on two commercial dairy farms in Uruguay. In outbreak 1, in November 2015, three animals (cases 1–3) were examined, while case 4 was examined in outbreak 2, in May 2016. Tissue samples, including liver, spleen, kidneys, ureters, urinary bladder, adrenal glands, mesenteric lymph nodes, lungs, skeletal muscles, heart, forestomachs, abomasum, small and large intestines, tongue, skin and brain, were collected and fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4–5 μm) were stained with haematoxylin and eosin (HE).

Several tissues (i.e. urinary bladder, ureter, intestine, lung, kidney and/or liver) from cases 1, 2 and 3 were processed for identification of *Salmonella* antigen by immunohistochemistry (IHC). Antigen retrieval was performed by treating the slides with proteinase K (Dako, Carpinteria, California, USA; catalogue number S3004) for 5 min at room temperature (RT). The primary antibody was a mouse monoclonal IgG2b specific for the common lipopolysaccharide (LPS) core of *Salmonella* O-serogroups A, B, C1, C2, D, E1, E3, E4, F, G1 and G2 (ViroStat Inc., Westbrook, Maine, USA; clone 513, catalogue number 6371), applied at a 1 in 50 dilution for 45 min at RT. The secondary antibody was a goat anti-mouse Ig polymer dextran conjugate labelled with horseradish peroxidase (Dako EnVision + System, HRP, Dako; catalogue number K4001) applied for 30 min at RT and 3-amino-9-ethylcarbazole (Dako AEC substrate chromogen, Dako; catalogue number K3469) applied for 15 min at RT was used as the chromogen. Slides were then counterstained with Mayer's haematoxylin (Sigma–Aldrich, St. Louis, Missouri, USA; catalogue number MHS32) for 5 min at RT and coverslipped. Tissues from cases 1 and 2 were also subjected to IHC for identification of bovine respiratory syncytial virus (BRSV), bovine herpesvirus 1 (infectious bovine rhinotracheitis virus, IBRV) and bovine viral diarrhoea virus (BVDV). Tissues from case 2 were also subjected to IHC for the detection of bovine coronavirus (BCoV). All IHC was performed following validated standard operating procedures at the University of Minnesota Veterinary Diagnostic Laboratory, using appropriate positive and negative controls for each technique. Specifically for the *Salmonella* IHC, the positive control consisted of colon from a horse infected with *S.* Typhimurium.

Aerobic bacterial cultures were performed from fresh tissues and/or body fluids in all cases on MacConkey and blood agars (Oxoid, Basingstoke, UK) and on Salmonella–Shigella agar (Mast Group, Bootle, UK) and incubated at 37°C for 24–48 h. Bacterial isolates were identified by biochemical testing (Octavia and Lan, 2014) and sequencing of the 16S rDNA using a commercial sequencing service (Macrogen, Seoul, South Korea); the sequences were assembled and edited using BioEdit (Ibis Biosciences, Carlsbad, California, USA) and compared with sequences deposited in the Ribosomal Database Project. Serotyping was performed at the Instituto de Higiene, Universidad de la República, Montevideo, Uruguay, following the Kauffmann–White classification scheme (Grimont and Weill, 2007).

Outbreak 1 occurred over a 3-week period in a group of 45 3- to 4-month-old Holstein calves housed in collective pens. Ten calves became ill (morbidity 22.2%), eight died naturally (mortality 17.7%, lethality 80%) and one was humanely destroyed in the terminal stages of disease. Clinically, the affected calves showed depression, mucopurulent occulonasal secretion, dehydration, tachypnoea, abdominal breathing, cough and fever. Three calves were subjected to necropsy examination; all had moderate diffuse thickening of the mucosa of the urinary bladder, which had irregular surfaces and poorly-defined areas of pale red discolouration. In the lungs, there were numerous, 2–3 mm diameter, dark red foci disseminated throughout the caudal lobes over the pleural and parenchymal surfaces (i.e. embolic pneumonia) and the cranioventral lobes had a pattern of fibrinosuppurative bronchopneumonia. The liver was mildly enlarged with diffuse ochre–orange discolouration of the parenchyma.

Outbreak 2 occurred in 2- to 3-month-old Holstein calves housed in collective grazing pens. Clinically, affected calves had diarrhoea, weakness, tenesmus and polyarthritis. Five of 20 calves died (mortality 25%). Post-mortem examination of one calf revealed dehydration and mild fibrinous peritonitis. The mucosal surface of the urinary bladder had moderate petechiation (Fig. 1). The mesenteric lymph nodes were moderately enlarged and the small intestinal contents were sparse, yellow in colour and pasty.

On histological examination of the urinary bladder, in all four cases there was severe (cases 1

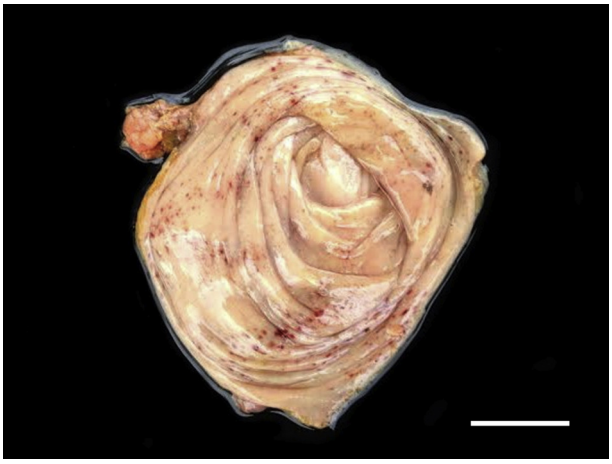

Fig. 1. Urinary bladder, Holstein calf (case 4). Multifocal widespread mucosal petechiation. Bar, 3 cm.

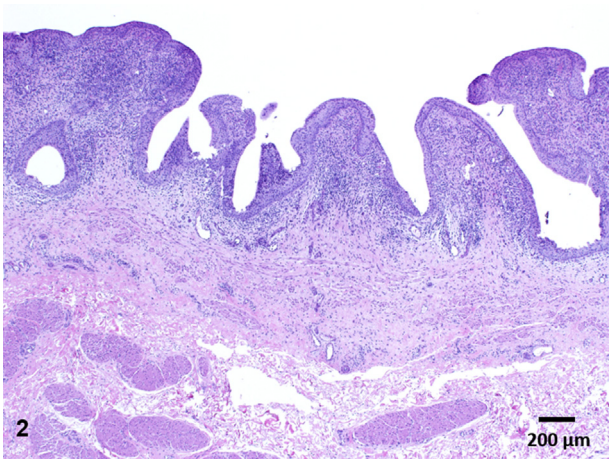

Fig. 2. Urocystitis, Holstein calf (case 1). The submucosa of the urinary bladder is markedly and diffusely hypercellular due to inflammatory cellular infiltrate and there is moderate hyperplasia of the mucosa. HE.

and 2), moderate (case 3) or mild (case 4) urocystitis characterized by submucosal histiocytic, lymphocytic and plasmacytic infiltration and neutrophil transmigration through the urothelium (Figs. 2 and 3). In case 1, severe fibrinosuppurative ureteritis was detected. Collectively, other microscopical lesions included multifocal necrotizing neutrophilic and histiocytic hepatitis, moderate multifocal enteritis with neutrophilic and necrotizing cryptitis, fibrinous epicarditis with epicardial petechiation, multifocal neutrophilic and fibrinous splenitis and lymphadenitis and multifocal neutrophilic and histiocytic tubulointerstitial nephritis. These lesions were consistent with septicaemia in all cases. In addition, two of the animals in outbreak 1 had bilateral cranioventral pneumonia, with fibrinonecrotizing, neutrophilic and histiocytic alveolitis and bronchiolitis.

IHC for *Salmonella* spp. revealed infrequent immunoreactivity in the urinary bladder mucosa of case 1 (Fig. 4) and in the urinary bladder, kidney and intestine of case 2; however, no *Salmonella* antigen was demonstrated by this technique in sections of ureter, intestine and lung of case 1, lung of cases 1 and 2 and urinary bladder, kidney, liver and intestine of case 3. In the IHC-positive tissues, the size and shape of the immunoprecipitates were similar to those found in the positive control (Supplementary Fig. 1). The IHC for BRSV, IBRV and BVDV were all negative in the lung of cases 1 and 2. Case 2 was also negative for BVDV and BCoV in intestine.

Bacterial colonies morphologically and biochemically consistent with *S. enterica* were isolated from the urine, liver, lung and mesenteric lymph node from cases 1–3 and from the urine, kidney, liver, lung, spleen, mesenteric lymph node and faeces from case 4. In most plates, in all cases, mixed cultures

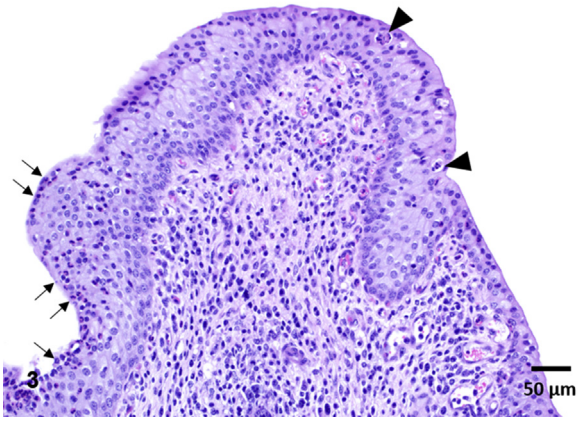

Fig. 3. Urocystitis, Holstein calf (case 1). Higher magnification of the urinary bladder shown in Fig. 2 depicting neutrophil transmigration through the urothelium (arrows) with formation of mucosal micropustules (arrowheads), urothelial hyperplasia and submucosal lymphohistiocytic and plasmacytic inflammation. HE.

were obtained, with *Salmonella* colonies usually outnumbering non-*Salmonella* colonies. Strains isolated from mesenteric lymph nodes in all cases were identified as *S. enterica* subsp. *enterica* by 16S rDNA sequencing and serotyped as *S.* Dublin.

The pathological findings in these four cases were consistent with septicaemic salmonellosis (Carrique-Mas *et al.*, 2010; Uzal *et al.*, 2016; Pecoraro *et al.*, 2017). An aetiological diagnosis of *S.* Dublin septicaemia was based on bacterial cultures, 16S rDNA sequencing and serotyping in all cases. Additionally, the IHC for *Salmonella* antigen detection was a useful tool to demonstrate the

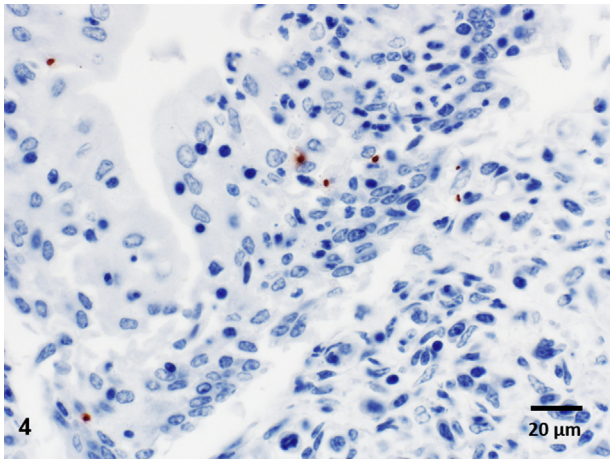

Fig. 4. Urinary bladder, Holstein calf (case 2). Infrequent multifocal anti-*Salmonella* immunoreactivity colocalizes with neutrophil transmigration within the urothelium. IHC.

bacterium intralesionally in the urinary bladder, kidneys and/or intestines of two of the three cases analyzed by this technique.

Although tubulointerstitial nephritis is described occasionally as part of the spectrum of lesions in septicaemia caused by *S.* Dublin in cattle, lower urinary tract lesions have not been described in this species, therefore, this report broadens the spectrum of urinary lesions in this condition. *Salmonella* Dublin has been associated previously with pyelonephritis secondary to unilateral hydronephrosis in a Holstein calf (Taghipur Bazargani *et al.*, 2015). In the cases presented herein, these lesions were ruled out, although there was fibrinosuppurative ureteritis in one of the calves.

Although urocystitis caused by *S. enterica* subsp. *enterica* has been described in man, it is a poorly characterized condition. It has been associated with old age, concomitant diseases such as diabetes mellitus, urolithiasis, urinary tract malformations, chronic disease or immunosuppressive therapy (Tena *et al.*, 2007). *Salmonella* Enteritidis and *S.* Typhimurium are the serotypes most frequently isolated from people with UTI (Tena *et al.*, 2007), although other non-typhoidal salmonellas and *Salmonella* Typhi have been implicated in cases of haemorrhagic cystitis (Na *et al.*, 2013; Polat *et al.*, 2014). Because people act as reservoirs only for *S.* Typhi and *Salmonella* Paratyphi, the occurrence of disease associated with non-typhoidal salmonellas suggests acquisition either from animal reservoirs or from contaminated animal products or foods (Na *et al.*, 2013), so non-typhoidal salmonellosis is of great public health importance. Cattle are a host-adapted species for *S.* Dublin and are considered a good experimental model for non-typhoidal salmonellosis in man (Costa *et al.*, 2012). *Salmonella* Dublin also causes invasive infections in man (Yim *et al.*, 2014).

Unlike human urine, which is a good medium for bacterial growth, the higher osmolarity and pH of bovine urine inhibit bacterial growth (Cianciolo and Mohr, 2016), therefore bacterial urocystitis is uncommon in this species. However, bacteria that have specific virulence factors for adhesion to the urothelium may colonize the urinary tract mucosa and elicit a local inflammatory response. Most commonly, UTI in cattle results from ascending infection, but it can also occur through the descending or haematogenous routes. Ascending infections result from bacteria lodged in the urethra, the origin of which is almost always the rectal microbiota (Cianciolo and Mohr, 2016). In the cases described in this report, disseminated renal and multisystemic lesions suggested that urocystitis and ureteritis may have occurred through descending infections resulting from haematogenous dissemination of *S.* Dublin to the kidneys; however, ascending infection by salmonellae shed through the faeces cannot be ruled out, particularly in heifers.

Salmonella Dublin is highly pathogenic to young calves and septicaemia is the major syndrome caused by this serotype. A recent paper describing the spectrum of lesions induced by *S.* Dublin in <6-month-old Holstein calves in the USA, indicates that inflammatory lesions are frequently found in the lungs (>90% of cases), liver (90% of cases), lymph nodes (62% of cases) and spleen (50% of cases) (Pecoraro *et al.*, 2017). Our results suggest that *S.* Dublin is also capable of causing urocystitis and ureteritis in calves and that the strains involved might have virulence factors that determine uropathogenicity. More extensive molecular and phenotypical studies on *Salmonella* strains isolated from cases of UTI may help understand the mechanisms associated with uropathogenicity of different *Salmonella* serotypes.

Conflict of Interest Statement

The authors declare no conflicts of interest with respect to publication of this manuscript.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcpa.2018.08.005.

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