



CASE REPORT

Clinical presentations and antimicrobial susceptibilities of *Corynebacterium cystitidis* associated with renal disease in four beef cattle

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Abstract

Background: Renal disease caused by *Corynebacterium cystitidis* in beef cattle may be misclassified as *Corynebacterium renale*, and limited information about *C. cystitidis* infections in beef cattle currently is available.

Objective: To describe clinical presentation, diagnosis, minimum inhibitory concentrations (MICs), and outcome of renal disease caused by *C. cystitidis* in beef cattle.

Methods: Retrospective case series.

Animals: Four client-owned beef cattle.

Results: All affected cattle had anorexia as a primary complaint. Of the 3 that had ante-mortem diagnostic tests performed, all had pyelonephritis based on azotemia in combination with urinalysis and ultrasonographic findings. Cultures yielded *C. cystitidis* which was identified by biochemical testing, 16S RNA sequencing, and mass spectrometry. All affected cattle deteriorated despite aggressive treatment, indicating that *C. cystitidis* infections in beef cattle may carry a poor prognosis. Bacterial isolates collected from the 4 cattle showed similarities in MICs for ampicillin, florfenicol, gentamicin, neomycin, sulfadimethoxine, trimethoprim sulfonamide, and tylosin.

Conclusions and clinical importance: *Corynebacterium cystitidis* should be considered in the differential diagnosis of cattle with renal disease. Definitive diagnosis of *C. cystitidis* as compared to *C. renale* may be challenging.

KEYWORDS

beef cattle, *Corynebacterium cystitidis*, pyelonephritis, renal, urinary

1 | INTRODUCTION

Initially thought to be the same species as *Corynebacterium renale*,¹ *Corynebacterium cystitidis* was identified in 1978 from isolates described as originating from cattle with cystitis. Descriptions of *C. cystitidis* as well as antimicrobial susceptibility data in cattle

Abbreviations: BCS, body condition score; BUN, blood urea nitrogen; FACH, Food Animal and Camelid Hospital; ISU, Iowa State University; MALDI-TOF-MS, matrix-assisted laser desorption ionization-time of flight-mass spectrometry; MIC, mean inhibitory concentration; RI, reference interval; VDL, Veterinary Diagnostic Laboratory.

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currently are lacking in the veterinary literature. Reports of isolates from cases of pyelonephritis in cattle exist,² and a recent survey identified *C. cystitidis* in a single condemned kidney from a cull dairy cow at a slaughter facility.³ We describe 4 cases of *C. cystitidis* associated with renal disease in cattle as well as the antimicrobial susceptibilities of the isolates.

2 | MATERIALS AND METHODS

A retrospective review of medical records was conducted. Medical records from all beef cattle from 2010 to 2020 were reviewed with age, breed, signalment, diagnosis, response to treatment, outcome, and microbiological testing recorded.

3 | RESULTS

Four beef cattle with *C. cystitidis* renal disease were identified between May 2015 and December 2018.

3.1 | Case 1

A 3-year-old, 998 kg, Lincoln Red Shorthorn bull presented in May 2015 to the Food Animal and Camelid Hospital (FACH) at Iowa State University (ISU) for intermittent anorexia. The bull had been examined the day before by the ISU Field Services unit and CBC and serum biochemistry completed which disclosed severe azotemia (blood urea nitrogen [BUN], 104 mg/dL; reference interval [RI], 10-25 mg/dL; serum creatinine concentration, 17.8 mg/dL; RI, 1.1-1.8 mg/dL) with leukopenia ($3.05 \times 10^3/\mu\text{L}$; RI, $4.0\text{-}12.0 \times 10^3/\mu\text{L}$), hyperfibrinogenemia (900 mg/dL; RI, 100-500 mg/dL), and hyperproteinemia (7.9; RI, 6.7-7.5) characterized by presumptive hyperglobulinemia (albumin 3.2 g/dL; RI, 2.5-3.8 g/dL). The bull was current on its vaccinations (bovine respiratory disease complex, and multivalent leptospira bacterin, as well as multivalent clostridial bacterin-toxoid) and intestinal parasite control (previous treatment with fenbendazole). On physical examination, the bull was mildly tachypneic (respiratory rate, 40 breaths per minute), and pink-colored urine was noted. Urinalysis disclosed hematuria, proteinuria, and bacteriuria, and hyposthenuric urine specific gravity (1.006, RI, 1.020-1.050). *Corynebacterium cystitidis* was cultured from the urine (Supplemental Material). Trans-abdominal ultrasound examination indicated no renal abnormalities at the time of initial examination. The bull was hospitalized for 10 days during which time a balanced polyionic fluid was administered IV, as well as 2 doses of parenterally administered ceftiofur crystalline free acid (Excede, Zoetis, Parsippany, New Jersey; 6.6 mg/kg, SC at the ear base, 3 days apart). The azotemia improved with fluid administration over 7 days (BUN, 34 mg/dL; creatinine concentration 9.2 mg/dL), but did not fully resolve. The bull however had resumed normal attitude and appetite. The bull was discharged 11 days after admission at the owner's request with a guarded prognosis for return to full renal function and recommendation for reevaluation in 2 weeks.

Six days later, the bull presented again to ISU FACH for evaluation of reoccurrence of anorexia and lethargy. Physical examination on admission identified an enlarged left kidney with loss of palpable lobulation. Abdominal ultrasound examination showed multiple hyperechoic areas in the renal cortices and pelves. A CBC disclosed leukopenia ($3.6 \times 10^3/\mu\text{L}$), lymphopenia ($1.48 \times 10^3/\mu\text{L}$; RI, $2.5\text{-}7.5 \times 10^3/\mu\text{L}$), and increased plasma protein concentration (8.8) characterized by hyperfibrinogenemia (900 mg/dL). Serum biochemistry showed hypochloremia (78 mEq/L; RI, 100-115 mEq/L), severe azotemia (BUN, 122 mg/dL; serum creatinine concentration, 30.2 mg/dL), hyperphosphatemia (13.8 mg/dL; RI, 5.6-8.0 mg/dL), hypocalcemia (7.2 mg/dL; RI, 8.0-11.4 mg/dL), and increased total protein concentration (7.8 g/dL). Further aggressive treatment with antibiotics and fluids IV was offered but declined by the owners because of poor prognosis. The bull was discharged with instructions to wait out the withdrawal period for salvage slaughter.

3.2 | Case 2

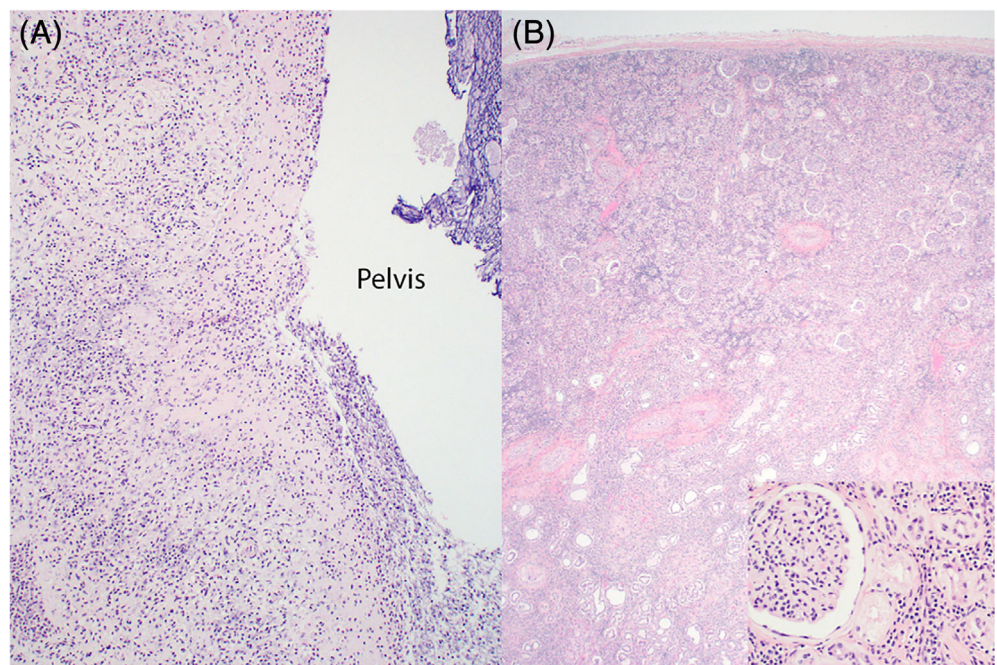
A 7-year-old, unknown weight, commercial beef cow in good body condition (body condition score [BCS] 4, according to the referring veterinarian) that died after a 10-day period of lethargy and anorexia had samples submitted to the ISU Veterinary Diagnostic Laboratory (VDL) in February 2016 for evaluation of kidneys, liver, colon, and small intestine. The submitting veterinarian described icterus and a very full gallbladder. The pathologist observed thick, yellow-to-tan exudate within the renal pelvis and ureter (pyelonephritis).

Histopathology was performed on submitted fixed samples. Mild to moderate autolysis affected all tissues examined. The renal pelvis contained abundant, flocculent cellular, and karyorrhectic debris, degenerate neutrophils, and scant fibrin. The renal pelvic epithelium was obliterated by inflammation with variable fibrosis and infiltrated by lymphocytes, plasma cells, and fewer macrophages and neutrophils. Medullary and cortical tubules were multifocally ectatic and contained mixed cellular and karyorrhectic debris and neutrophils. The cortical tubules frequently had markedly thickened pale eosinophilic basement membranes. Tubules were variably separated by fibrocollagen and low to moderate numbers of lymphocytes and fewer plasma cells. Mild periglomerular fibrosis also was observed. The liver, colon, and small intestine had moderate autolysis, but were otherwise unremarkable. A swab of the renal tissue yielded *C. cystitidis* (Supplemental Material). Histopathology of the kidney is presented in Figure 1.

3.3 | Case 3

An 11-month-old, 408 kg, Charolais cross steer was presented to the ISU FACH after hours on emergency in February 2016 for evaluation of anuria and anorexia. The owner reported an approximately 60-day history of blood-tinged and viscous urine. The owner had unsuccessfully treated on the farm using multiple doses of enrofloxacin and

FIGURE 1 Histopathology of renal lesions from case 2. A, Renal pelvis, $\times 100$ magnification, hematoxylin and eosin. Urothelium is obliterated by an accumulation of degenerate neutrophils, fibrin, and cellular and karyorrhectic debris. B, Renal cortex, $\times 20$ magnification, inset $\times 200$ magnification hematoxylin and eosin. Tubules are separated by lymphocytes and fibrocollagen. Scattered tubules are dilated, primarily in the corticomedullary junction. There is mild to moderate periglomerular fibrosis (inset)



sulfadimethoxine. On initial examination, vital findings were within normal limits with the exception that the steer strained unproductively to urinate. Transabdominal ultrasound examination identified a large, distended bladder, but normal-appearing kidneys. Serum biochemistry performed on admission identified hypochloremia (95 mEq/L), azotemia (BUN, 44 mg/dL), and increased blood pH (7.602; RI, 7.31-7.53). An initial attempt at resolving the urinary obstruction by perineal urethrostomy was unsuccessful, and consequently a vesiculopreputial anastomosis was performed under general anesthesia. During surgery, the bladder was noted to be grossly distended with a diffusely thickened and hemorrhagic bladder wall. After surgery, the patient was managed with a balanced polyionic solution administered IV and meloxicam (1 mg/kg PO q24h); no additional antibiotics were administered because of the history of recent antibiotic administration on farm before admission.

After surgery, a CBC and serum biochemistry profile showed increased plasma fibrinogen concentration (700 mg/dL), and resolving azotemia (BUN, 29 mg/dL; serum creatinine concentration, 3.3 mg/dL). A urine sample taken at the time of surgery yielded *C. cystitidis* (Supplemental Material). The patient's anorexia resolved with fluid therapy and the steer was discharged 6 days after initial hospitalization. Follow-up evaluation 120 days posthospitalization indicated that the steer had continued to lose weight after hospitalization and the animal was found dead acutely approximately 3 months after being discharged from the hospital. No necropsy was performed.

3.4 | Case 4

A 13-year-old, 590 kg Simmental cow presented to the ISU FACH in December 2018 with a 1-month history of weight loss and anorexia. A urinary tract infection had been diagnosed by the referring veterinarian 2 months earlier, and the cow had been treated with a single

dose of ceftiofur crystalline free acid (6.6 mg/kg). Physical examination disclosed increased heart rate (90 beats per minute; RI, 48-80 beats per minute), decreased BCS (3/9, decreased from the owner-reported BCS of 5/9 several months earlier), 8% dehydration, and red-colored urine. Serum biochemistry identified severe azotemia (BUN, 106 mg/dL; serum creatinine concentration, 6.9 mg/dL) as well as leukopenia ($2.8 \times 10^3/\mu\text{L}$) and increased plasma fibrinogen concentration (1100 mg/dL). Urine was hazy and brown in color with a specific gravity of 1.007. Proteinuria as well as red and white blood cells were detected on dipstick urinalysis. A urine sample was submitted for culture and yielded *C. cystitidis* (Supplemental Material). Abdominal ultrasound examination identified bilateral dilation of the renal pelves (Figure 2). Treatment was initiated using a balanced polyionic fluid and penicillin K potassium (Pfizerpen, Pfizer, New York, New York; 22 000 IU/kg, q6h) administered IV. The BUN improved initially to 87 mg/dL after 4 days of fluid therapy, but after 9 days azotemia persisted (BUN, 106 mg/dL; serum creatinine concentration, 6.9 mg/dL) and the animal was euthanized.

Necropsy identified enlarged, pale kidneys. On section, the renal calyces were dilated and contained multifocal cavitory lesions with viscous opaque fluid. The ureters were distended and the mucosa of both the ureters and urinary bladder was hyperemic. Pyelonephritis was confirmed by histopathology.

4 | DISCUSSION

Corynebacterium is a genus of gram-positive aerobic rods, which can be pathogenic, but is thought primarily to be an opportunist in the presence of altered host immunity or damaged tissue rather than being a primary pathogen. Of the species *C. cystitidis*, *C. renale*, and *C. pilosum*, *C. renale* is thought to be the most virulent pathogen.³

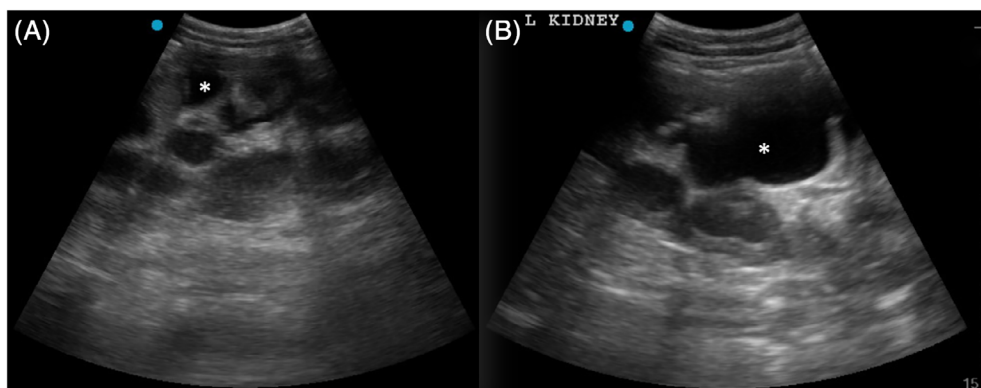


FIGURE 2 Renal ultrasonographic (depth: 18 cm) sagittal sections of the right kidney (A, depth 18 cm) and left kidney (B, depth 15 cm) from case 4. Note the fluid distension of the pelvices as evidenced by the selected anechoic regions (*)

Corynebacterium cystitidis, *C. renale*, and *C. pilosum* preferentially adhere to bladder epithelial cells in contact with the urine and older superficial cells (immediately before shedding), indicating that adhesion probably occurs in senescent cells first.⁴

Routine culture (aerobic and anaerobic) at the ISU VDL initially was utilized to attempt to diagnose the cause of the renal disease in all 4 cases. No strictly anaerobic bacterial organisms were identified from any of the samples. Urine collected by sterile cystocentesis during surgery for case 3 and by catheterization in case 4, yielded moderately heavy pure growth of the bacteria. In case 1 (voided urine), heavy mixed growth was obtained as expected because of skin contamination, but the primary growth was consistent with *Corynebacterium* sp. and no other primary pathogens were identified. For case 2 (kidney swab from necropsy), moderately heavy growth consistent with *Corynebacterium* sp. also was obtained, with a few colonies consistent with contamination during necropsy also noted. A matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI; Bruker) biotyper was utilized in an attempt to speciate the predominant colonies present and yielded a best-match organism of *C. cystitidis* for all cases; but, all MALDI scores were near or below the manufacturer's cutoff of 2.1 for high confidence classification at the species level (2.04 [case 1], 1.94 [case 2], 2.16 [case 3] and 1.96 [case 4]). To determine if the MALDI identification of these isolates was correct, additional biochemical testing and 16S rRNA sequencing was undertaken on isolates saved by the ISU VDL at -80°C for cases 1, 2, and 3 (case 4 failed to grow on reculture). Initial biochemical testing including urease positivity, nitrate reduction, and acid production from various sugars including xylose failed to fully differentiate the isolates as *C. cystitidis* from other renal pathogens such as *C. renale* and *C. pilosum*.⁵ Additional biochemical testing using casein hydrolysis and hydrolysis of Tween 80 compared to a known isolate of *C. renale* thus was undertaken to further aid in differentiation. Results of these additional studies indicated that all clinical *C. cystitidis* isolates had the ability to hydrolyze Tween 80 (Figure 3) but were unable to digest casein (Figure 4); these results match what has been described for *C. cystitidis* and are not consistent with results obtained from known *C. renale* isolates. The 16S rRNA sequencing also was undertaken for the 3 isolates available for further testing; for 2 of the 3 isolates, sequencing confirmed *C. cystitidis* as the most likely match

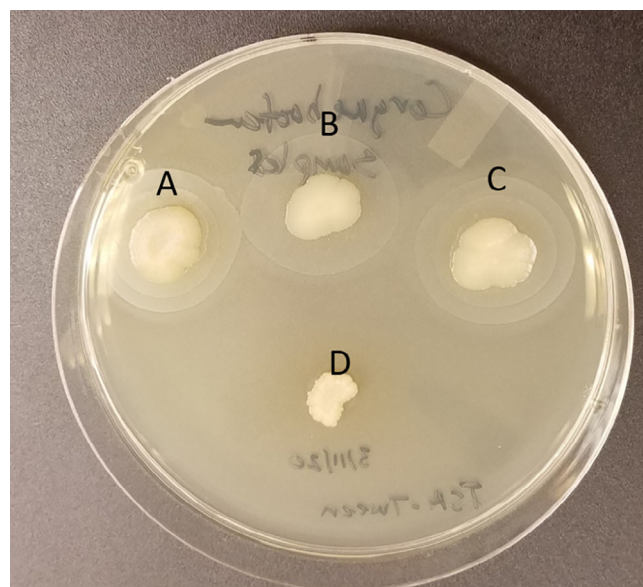
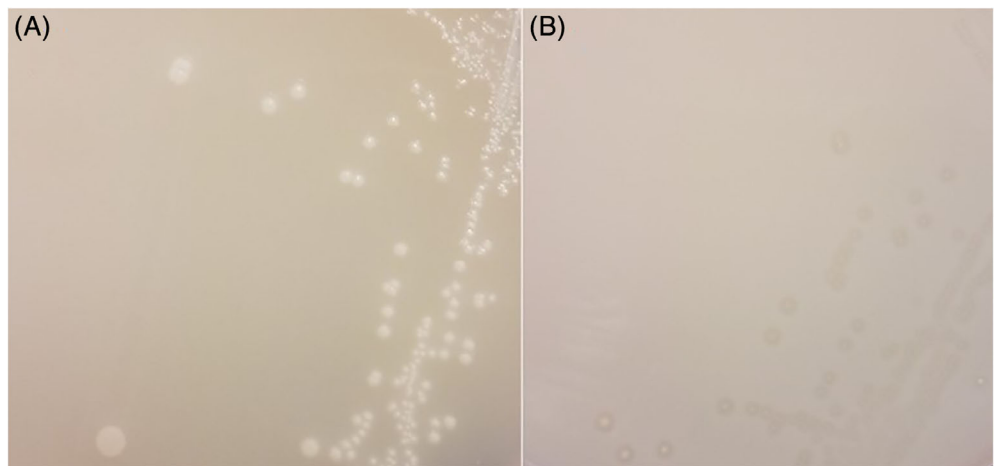


FIGURE 3 Tween-80 hydrolysis of all 3 *Corynebacterium cystitidis* isolates (A-C) compared to known *Corynebacterium renale* isolate (D); image taken after 5 days of incubation at 37°C

(Supplemental Table 1). Sequencing was attempted twice on the third isolate (case 2) and failed to yield a usable sequence for analysis.

The sources of infection in these 4 cases is unclear. Cases of pyelonephritis are thought to be more common in females, and defects such as poor perineal conformation, pneumovagina, and metritis have been linked to development of pyelonephritis in cows, in part because of the short female urethra.⁶ No published information is available about a possible cause of infection in males; and 2 of the cases presented here were male (1 intact and 1 castrated). Bulls are thought to be capable of spreading *C. renale* and *C. cystitidis* to females through sexual contact.⁷ The environment also could represent a source of infection, but in 1 study investigating the survival of *C. cystitidis* from soil in a paddock, survival was only noted for 63 days.⁸ The environment and herd dynamics may play more of a role in maintenance of disease and transmission, because once established in a herd, *C. renale* can be very difficult to eliminate at the herd level.⁹ To the best of our knowledge, no other

FIGURE 4 Casein digestion assay comparing the lack of a reaction displayed by all 3 *Corynebacterium cystitidis* isolates (A) compared to ring of digestion present around known *Corynebacterium renale* isolate colonies (B); image taken after 5 days of incubation at 37°C



cases of renal disease were reported in any animals from the herds involved.

Our 4 cases shared several features. All affected animals showed clinical signs of anorexia, chronic disease, and all had poor clinical outcomes. Three animals initially showed clinical improvement with IV fluids and antimicrobial treatment, but failed to completely recover and relapsed once supportive treatment was discontinued. All affected cattle were azotemic and had macroscopically abnormal urine.

Penicillin traditionally has been considered the first-line antibiotic in cases of pyelonephritis caused by *Corynebacterium* sp. because renal excretion of the drug leads to high concentrations of active drug in renal tissues. Other beta-lactam antibiotics such as ampicillin and cephalosporins also undergo urinary excretion, making them additional options for treatment. Short-term treatment may not be effective, and it has been suggested that, if response is not noted within 4 days, clinicians should consider modifying management, which can present a challenge for long-term treatment.⁶ The mean inhibitory concentration (MIC) profiles of the 4 isolates of *C. cystitidis*, as well as the only 3 *C. renale* isolates obtained from bovine urine or urinary tract specimens submitted to the ISU VDL over a 10-year period (2007-2017) are shown in the supplemental information for comparison. Unfortunately, no specific Clinical Laboratory Standards Institute-approved guidelines are available for resistance determination of these bacteria in cattle. Several breakpoints are available for isolates of *Corynebacterium* sp. from humans and from which break points can be extrapolated,¹⁰ but these breakpoints are not specific for urine and thus should be interpreted with caution. All 7 isolates of *C. cystitidis* and *C. renale* showed similarities for susceptibilities to ampicillin, clindamycin, florfenicol, gentamicin, neomycin, and tiamulin. When *C. cystitidis* MICs were compared to those of *C. renale*, the MICs for ceftiofur for the *C. cystitidis* isolates tended to be higher, whereas the MICs for the fluoroquinolones appeared higher for the examined *C. renale* isolates. The 4 isolates of the *C. cystitidis* from our cases showed similarities in MICs for ampicillin, enrofloxacin, florfenicol, gentamicin, neomycin, sulfadimethoxine, tiamulin, trimethoprim sulfa, tulathromycin, and tylosin. Within both *C. cystitidis* and *C. renale* isolates, wide variation was noted in MICs for tetracycline and macrolide antibiotics. When applying these results, clinicians

should exercise caution, because the use of gentamicin can lead to prolonged tissue residues in cattle.¹¹ Use of enrofloxacin or danofloxacin for this indication in the United States (as given by the owner in case 3) would be extra-label and currently not permissible in the US because of human health and resistance concerns.¹²

As demonstrated in these cases, definitive identification of specific *Corynebacterium* sp. can be challenging, particularly when relying primarily on MALDI-TOF-MS identification. Advanced biochemical testing and 16S rRNA sequencing was utilized in these cases to further aid in the differentiation of *C. cystitidis* from other species of *Corynebacterium*. Additional described diagnostic approaches for *C. cystitidis* include cellular fatty acid composition analysis,¹³ mycolic acid analysis,¹⁴ and amplified rDNA restriction analysis.¹⁵ Such methodologies are limited to research laboratories only and are not readily available in a diagnostic laboratory setting. Future research into the pathogenesis and epidemiology of *C. cystitidis* infection in beef cattle may elucidate risk factors, improved diagnostic techniques, and treatment options. Limitations of our study include the small number of cases, and the loss of isolate from case 4 before further confirmatory 16S rRNA sequencing and biochemical analysis could be performed.

In conclusion, clinicians should consider *C. cystitidis* infection in the differential diagnosis of pyelonephritis in beef cattle with clinical signs of anorexia and weight loss. Although the bacterium does appear to be susceptible to several antimicrobials, cattle may not be presented with clinical signs until infection is advanced and treatment may not be successful.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare extra-label use of ceftiofur and penicillin in this work. This was done in the appropriate manner in the United States.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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