Surgical correction of pyelonephritis caused by multidrug-resistant *Escherichia coli* in a dairy cow

Chirurgische correctie van pyelonefritis veroorzaakt door multiresistente Escherichia coli *bij een melkkoe*

[1]E. Put, [1]B. Valgaeren, [1]B. Pardon, [2]J. De Latthauwer, [2]D. Valckenier, [1]P. Deprez

[1]Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[2]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Bonnie.Valgaeren@UGent.be

ABSTRACT

A four-year-old red Holstein Friesian cow was admitted to the clinic with fever and milk drop. Blood analysis revealed the presence of a chronic infection, and the diagnosis of pyelonephritis of the right kidney was made after repeated ultrasound examinations. The animal was treated with procaine benzylpenicillin, sulfadoxine trimethoprim, oxytetracycline and enrofloxacine but this therapy was not successful. Nephrectomy was performed after the left kidney proved to have a normal function. The removed right kidney was greatly enlarged and filled with pus. *Escherichia coli* was isolated from the kidney. The strain was multidrug-resistant, including resistance to aminopenicillins, streptomycin, sulfonamides and trimethoprim. The cow was treated with amoxicillin and clavulanic acid after the operation. Postoperatively, an abscess developed and a tube drain was placed during a second surgery to enable daily rinsing with a chlorhexidine solution. After a postoperative care period of two months, the animal was sent back to the farm, where it returned to an acceptable level of milk production. This case demonstrates that with basic surgical skills, a good preparation and knowledge of anatomy, nephrectomy is attainable for a first-opinion veterinarian, with an acceptable economic prognosis for the farmer.

SAMENVATTING

Een vier jaar oude rode holstein-friesiankoe werd aangeboden met koorts en een plotse, sterke daling van de melkproductie. Het bloedonderzoek toonde een chronische infectie aan en na herhaalde echografische onderzoeken kon de diagnose van pyelonefritis van de rechternier worden gesteld. Het dier werd zonder effect met procaïne-benzylpenicilline, sulfadoxine-trimethoprim, oxytetracycline en enrofloxacine behandeld. Aangezien de linkernier onaangetast was, kwam het dier in aanmerking voor een nefrectomie van de aangetaste rechternier. De verwijderde rechternier was opvallend groot en gevuld met etter. Hieruit werden kolonies *Escherichia coli* geïsoleerd die resistent bleken tegen aminopenicillinen, streptomycine, sulfonamiden en trimethoprim. De koe werd verder behandeld met amoxicilline en clavulaanzuur op basis van het antibiogram. Postoperatief ontstond een retroperitoneaal abces. Er werd een buisdrain geplaatst tijdens een tweede operatie om dagelijkse spoeling met een chloorhexidineoplossing mogelijk te maken. Hierna bleef het dier koortsvrij en herstelde goed. De koe werd uit de kliniek ontslagen en was op het bedrijf in staat om een goede melkproductie te halen. In deze casus wordt aangetoond dat nefrectomie, mits een goede voorbereiding, een haalbare ingreep is voor de eerstelijnsdierenarts in geval van unilaterale pyelonefritis, met een acceptabele economische prognose voor de landbouwer.

INTRODUCTION

Pyelonephritis is one of the most common renal pathologies in cattle (Divers et al., 1982; Markusfeld et al., 1989; Rosenbaum et al., 2005). It involves infection and inflammation of the renal sinus, with only non-specific symptoms of an acute infection. These symptoms include milk drop, anorexia, fever and depression. Pyelonephritis is most frequently due to ascending infections, but in rare cases, it may also have a hematogenous origin. Female animals are predisposed and most cases are related to recent calving, abortion or urinary catheterization (Yeruham et al., 2006). *Corynebacterium renale* (*C. renale*) and *Escherichia coli* (*E. coli*) are the most frequently isolated causative agents from pyelonephritis cases in cattle (Braun et al., 2008; Moore et al., 2010). Both bacteria have fimbriae, which enable them to bind to the epithelial cells of the urogenital tract (Hayashi et al., 1985; Gyles and Fairbrother, 2010).

Treatment of pyelonephritis consists of fluid therapy, anti-inflammatory therapy and administration of antimicrobials, which are active against the involved pathogens (Van Metre, 2009). In different countries, formularies to enhance responsible antimicrobial use have recently been initiated. In the Netherlands, procaine benzylpenicillin or sulfadoxine trimethoprim are proposed as first-choice treatments for pyelonephritis. Second-choice drugs are ampicillin, amoxicillin and the combination of procaine benzylpenicillin and dihydrostreptomycin (Werkgroep Veterinair Antibioticum Beleid, 2012). The antibacterial therapy should be maintained for at least three to four weeks (Kahn and Line, 2010). Whereas *C. renale* is commonly susceptible to penicillins, *E. coli* is naturally resistant (Prescott, 2013). *E. coli* is a species which easily acquires resistance to various antimicrobial agents, either by mutation in chromosomal DNA or by plasmid transfer (Okusu et al., 1996; Rogers et al., 2011). In recent years, an overall increase in (multi)resistance of *E. coli*, associated with antimicrobial use, has been reported worldwide (Levy and Marshall, 2004; Rogers et al., 2011). In this case report, a case of pyelonephritis caused by a multidrug-resistant *E. coli* strain is described, in which unilateral nephrectomy resulted in clinical recovery.

CASE REPORT

A four-year-old red Holstein Friesian cow was admitted to the Faculty of Veterinary Medicine of the Ghent University (Belguim) with symptoms of milk drop, fever and anorexia, one month after calving. At arrival, the cow was alert and in good body condition. No other abnormalities were found during the physical examination. Also the rectal palpation and ultrasonic examination were normal except for a slightly enlarged right kidney, visible on the transcutaneous ultrasound.

Venous blood analysis showed dehydration, causing an increased blood urea nitrogen (BUN) and total protein concentration and leukocytosis (Table 1). Urinary analysis only revealed a slight positive protein reaction on urine stick but a negative nitric acid test. The animal was hospitalized with an acute infection of unknown origin and treated by administration of procaine benzylpenicillin (16 mg/kg IM, Duphapen®, Zoetis Belgium s.a., Belgium). Fluid therapy was performed by intravenous perfusion and oral drenching. The fever persisted and after four days, also sulfadoxine trimethoprim (12.5 mg/kg and 2.5 mg/kg, respectively IV, Borgal® 24%, Virbac, France) and oxytetracycline (7.2 mg/kg IM, Duphacycline®, Zoetis Belgium s.a., Belgium) were administered for three days each, together with flunixin meglumin (2.2 mg/kg

Table 1. Hematology, biochemistry and electrophoresis conducted on the serum of venous blood on different times during the stay in the clinic.

|	Day 1[1]	Day 4	Day 8	Day 11	Day 19	Day 34	Reference values
Leukocytes	4.2	12.2	10.6		2.0	4.8	6.0 - 9.0 x 10^9/l
Packed cell volume	314	268	237				250 - 350 ml/l
Neutrophils	43	40	83		20	39	15 - 45 %
Lymphocytes	57	32	17		50	29	45 - 75 %
Monocytes		28			25		2 - 7 %
Eosinophils		1			7		0 - 10 %
Thrombocytes	193	201	151		467	576	100 - 800 x 10^9/l
Total protein	87	69		82	87	77	60 - 80 g/l
Urea (BUN)	15.7			5.3			3 - 8 mmol/l
Creatinine	141			92			88 - 172 µmol/l
Gamma glutamyltransferase	28			33			<30 mU/ml
A/G ratio	0.61						0.70 - 0.95
Albumin	38						30 - 40 %
Alpha globulin	18						12 - 16 %
Beta globulin	10						9 - 13 %
Gamma globulin	34						31 - 44 %

[1] Day 1 = arrival at the clinic
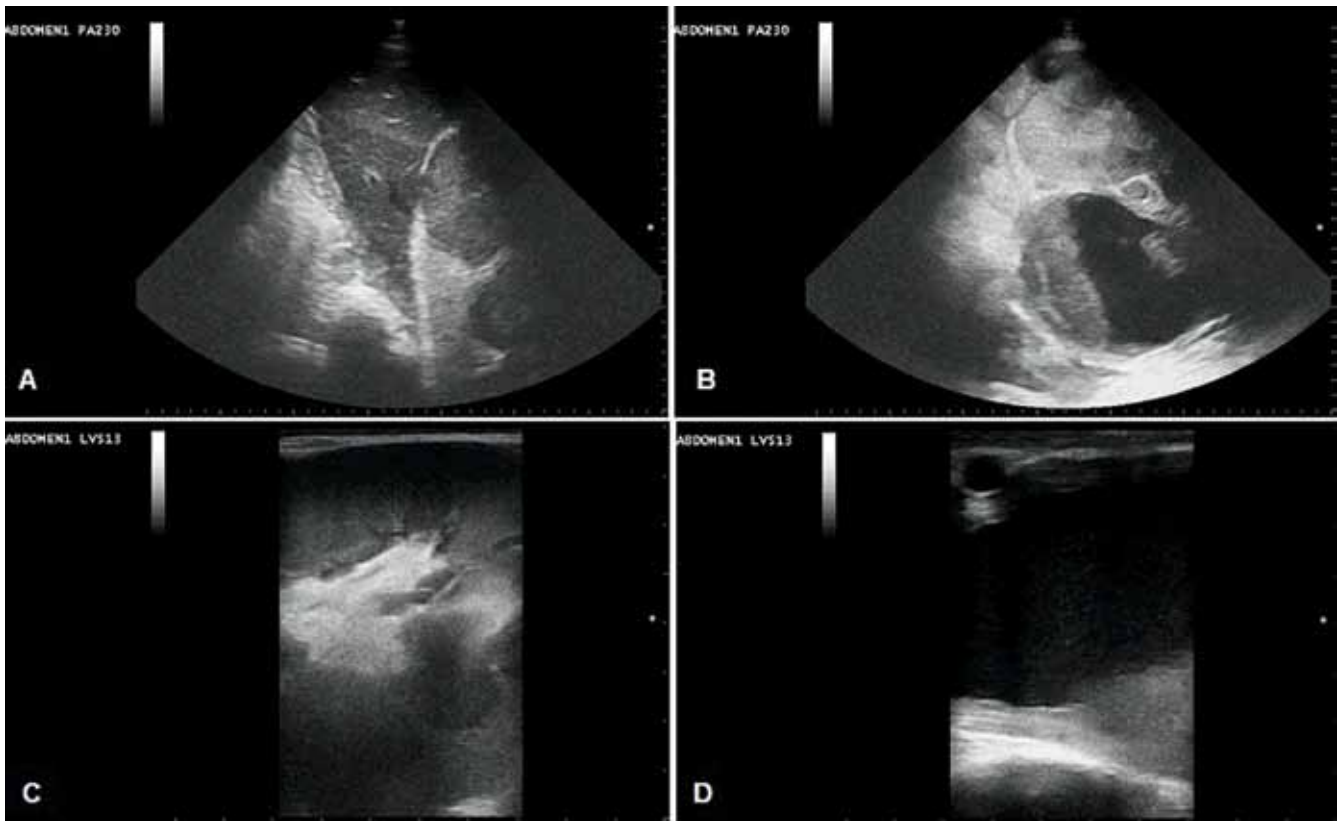
Figures 1A and B. Ultrasound images made with a 3.5 MHz sectorial probe with a depth of 27 centimeters after 12 days of hospitalization. Figures 1C and D. Transrectal ultrasound images made with a 7.5 MHz linear probe with a depth of 8 cm after 12 days of hospitalization. Figure 1A shows the normal liver and on the right side, a part of the right kidney. Figure 1B shows the right kidney with vesicles filled with fluid. The kidney is greatly enlarged. Figure 1C visualizes the normal-sized left kidney with the renal sinus in the center and medulla and cortex surrounding it. Figure 1D shows the bladder with echogenic particles swirling in the urine and irregular lining of the bladder wall on the bottom right side.

IV, Emdofluxin, Emdoka, Belgium) in every fever episode. Repeated clinical and ultrasonographic examination, 12 days later, revealed a marked dilatation of the right kidney. The enlarged right kidney consisted of several vesicles filled with hypoechogenic fluid, consistent with pyelonephritis (Figure 1). The bladder had an irregular lining of the wall and echogenic particles swirling in the urine. Blood examination at that time showed a chronic infection (leukocytosis, neutrophilia, lymphopenia, increased total protein), but normal BUN and creatinine.

Since the additional treatment with enrofloxacin (3.1 mg/kg IV, Floxadil, Emdoka, Belgium) for three days was not successful and given that the renal values were still within normal reference values, it was opted to perform a unilateral nephrectomy after 12 days of hospitalization. Before the surgical procedure, the animal received procaine benzylpenicillin combined with neomycin (14.0 mg/kg and 7.0 mg/kg, respectively IM, Neopen, Intervet, the Netherlands), together with flunixin meglumin (2.2 mg/kg IV, Emdofluxin, Emdoka, Belgium) as part of the standard surgical protocol. The surgical procedure was performed standing, with epidural, distal paravertebral and linear infiltration anesthesia on the right side and flank us-

ing procaine hydrochloride (Procaïne hydrochloride 4% + Adrenaline, VMD n.v., Belgium). Additionally, the animal was sedated with xylazine hydrochloride (0.012 mg/kg IV, Xyl-M® 2%, VMD n.v., Belgium). On the right side, parallel with the last rib, an incision was made through the skin and abdominal muscles (Figure 2A). Figure 2B shows that the right kidney covered the whole of the right flank, instead of expanding normally from the last rib until the second or third lumbar vertebra (Budras et al., 2003b). First, the renal vascular structures and ureter were identified through blunt dissection at their site of insertion in the renal hilus. The renal artery was ligated three times, using absorbable braided multifilament suture material (Surgicryl® polyglycolic acid, SMI, Belgium). The thickened ureter was followed approximately 15 cm caudally, where it was ligated two times and cut between these ligatures to prevent leakage of fluids. The renal vein was not found before removal and ruptured. Subsequently, the vein was clamped and ligated until there was no more visible blood loss. The affected kidney weighed 10 kg instead of the normal 600 - 750 g (Budras et al., 2003b). As shown in Figure 3A, large vesicles were visible on the surface. The kidney, including the vesicles, was filled with fluid and pus
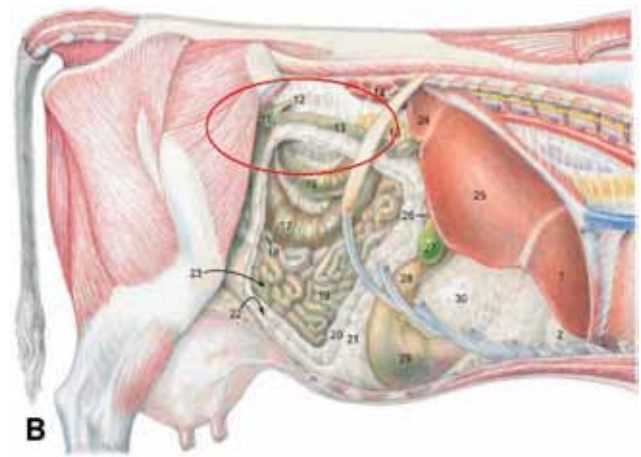
Figure 2A. View after incision of the skin and abdominal muscles parallel and caudal to the last rib. The arrow indicates the right kidney inside the renal capsule immediately visible after the opening of the retroperitoneal cavity. The asterisk shows the location of the last rib. Figure 2B. Schematic of the organs in the abdominal cavity on the right side of a cow. Number 14 shows the normal size of the right kidney in its normal place. The red circle shows the position of the affected kidney in this case. This means that the other structures (11-16), the intestines, were replaced more ventrally (adapted from Budras et al., 2003a).


Figure 3A. The right kidney after nephrectomy. The kidney was enlarged and large vesicles on the surface are visible. It weighed 10 kg. Figure 3B. The kidney was filled with pus and cloudy urine, visible after longitudinal transection. The normal structures of the kidney, namely cortex, medulla, sinus and lobes are still recognizable but the papillae and collecting ducts are dilated.

(Figure 3B). A swab was taken from the content of one of the vesicles in the kidney and submitted for bacterial culture. *E. coli* colonies were isolated and an antibiogram using the disc diffusion method was made. In this method, the correlation of the diameter of the zone of growth inhibition with the minimal inhibitory concentration (MIC) is used. The method is consistent in 71 – 90% of the cases. The disk diffusion method is clinically more useful as it is cheaper and quicker than determining the MIC (Dickert et al., 1981). The *E. coli* strain in the present case was resistant to ampicillin, amoxicillin, streptomycin, sulfonamides and trimethoprim (Table 2). Since the bacterium was susceptible to amoxicillin and clavulanic acid (8.4 mg/kg and 2.1 mg/kg, respectively IM, Noroclav Inject®, Norbrook Laboratories, United Kingdom) and amoxicillin is excreted via the urine, this combination was chosen to continue the treatment and a daily dose was given for the next 32 days (BCFI, 2011).

Post-surgically, the rectal temperature returned to normal within one day. However, five days later the fever returned (Figure 4). Ultrasound follow-up revealed the presence of cloudy fluid inside the subperitoneal cavity, which was created after the nephrectomy. Since the formation of a postoperative abscess was suspected, a second operation was performed. After epidural and linear infiltration anesthesia, the cavity was opened again with a right flank incision. Large fibrin strands were removed and a turbid yellow-orange fluid was present in the retroperitoneal cavity. Analysis showed this was exudate rather than transudate by the presence of large amounts of protein and white blood cells. A tube drain with fenestrations was placed in the retroperitoneal cavity between two openings in the abdominal wall (Figure 5). This enabled rinsing of the abscess twice a day with 0.07% chlorhexidine solution for 23 days after the second operation. Figure 4 shows that four days after the second

Table 2. The results of the antibiogram of the isolated colonies *Escherichia coli*, using the disk diffusion method.

Sensitivity	Antibacterial agents
Sensitive	Amoxicillin and clavulanic acid Cephalosporins: ceftiofur, cefquinome Aminoglycosides: spectinomycin, gentamicin Fluoroquinolones Tetracyclines
Intermediary	Aminoglycosides: neo-, kana-, fram-, paromomycin, apramicin
Resistant	Aminopenicillins: ampi-, amoxicillin Streptomycin Sulfonamides Trimethoprim
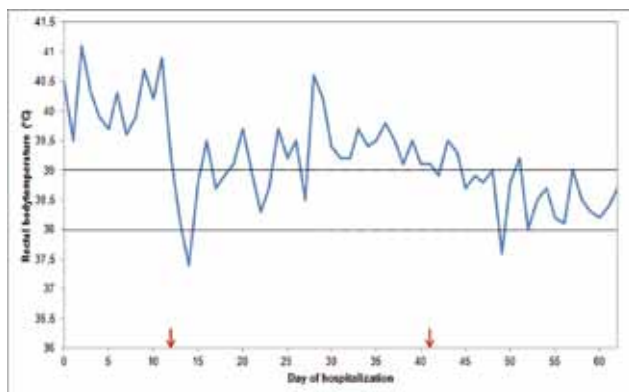
Figure 4. The development of the body temperature of the animal during hospitalization. Day 1 is the day of arrival at the clinic. The red arrows indicate the two surgeries that were performed on day 12 and 41 of hospitalization, respectively. The dotted lines between 38 en 39 °C indicate the range of the normal body temperature of a cow.

surgery, the fever subsided and the body temperature remained within normal limits. Ultrasonic follow-up revealed that the retroperitoneal cavity was smaller. The cow recovered well and the farmer reported that the cow returned to acceptable production levels in the following lactation.

DISCUSSION

In this case report, the challenge is emphasized of making a diagnosis based on vague clinical signs. Milk drop and fever may point to an infectious process somewhere in the body but ancillary diagnostics are often needed to find the exact source. To distinguish pyelonephritis from other chronic infections, urinary analysis may prove useful (Sink and Weinstein, 2012). In this case, no abnormalities were found, probably due to blockage of the affected kidney or dilution of present protein or white blood cells with fluids from the other kidney. The negative result of the urinary biochemical analysis and the size of the removed kid-

ney suggest the presence of an obstruction of the ureter, but no evidence of this was found. Other useful tools are blood analysis and ultrasound examinations. These enable the clinician to make a definite diagnosis and can also be used in daily practice. In the present case, blood analysis revealed an increase in total protein, globulin and neutrophil concentrations and a decrease of lymphocytes. These results and the ultrasonic observations, cystic lesions and a dilated sinus, are consistent with findings in the literature and other reported cases (Yeruham et al., 2006; Floeck, 2007, Braun et al., 2008; Van Metre, 2009).

Starting with first-choice antimicrobials, several agents were empirically used before the definitive diagnosis was made. Since urine examination revealed no abnormalities, urine was not cultured before the echographic diagnosis of pyelonephritis and following nephrectomy. The causative *E. coli* strain, which was cultured from the pus of the affected kidney, was multidrug-resistant by the time the antibiogram could be made. The isolate was, amongst others, resistant to antimicrobial agents used during the hospitalization (Table 2). Antimicrobial resistance can predominantly be selected by the use of antibiotics (Chantziaras et al., 2014). Since no urine culture was done before the antimicrobial treatment in the present case, it was not possible to determine whether the *E. coli* isolate had already been resistant on day 1 or the resistance was acquired due to the intensive antimicrobial use during therapy. Therefore, the therapy failure observed in this case cannot be attributed with absolute certainty to the resistant strain. After final diagnosis and availability of the antibiogram, the duration of the therapy was four weeks, as described in the literature (Kahn and Line, 2010). Little information is available on the resistance profile of *E. coli* strains adapted to colonization of the urinary tract. In humans, multidrug-resistant *E. coli* strains in urinary tract infections have been described (Manges et al., 2001). To the authors' knowledge, this is the first case report to illustrate that a multidrug-resistant *E. coli* strain may be involved in pyelonephritis in cattle. This case is particularly interesting since sulfonamides with trimethoprim are

recommended as first-choice treatment for pyelonephritis, but the current strain was resistant to these drugs (Werkgroep Veterinair Antibioticum Beleid, 2012). More information on the resistance profile of isolates of the urinary tract is warranted. In this case report, the treatment was continued with amoxicillin and clavulanic acid, since the *E. coli* strain was resistant to the other second-choice antimicrobials, streptomycin and ampicillin (Werkgroep Veterinair Antibioticum Beleid, 2012). Serious adverse effects of amoxicillin and clavulanic acid, including renal toxicity, are rare in human patients (Todd and Benfield, 1990; Mrvos et al., 2013). No information was found on effects in cattle but in studies in humans, the combination is suggested to be a good second choice in the treatment of pyelonephritis.

Important to mention is that therapy failure is common in cases of pyelonephritis, even when the bacteria are susceptible to the used antimicrobial agent. In this case for example, the isolate was sensitive to fluoroquinolones but clinically, there was no improvement after injections with enrofloxacin. A severe infection with bacterial biofilm formation and pus, may prevent the penetration of the antibiotic to the causative agents and the removal of the infectious agents (Miesner and Anderson, 2008). Reduced diffusion of the antimicrobial drugs in the dilated kidney might result in subtherapeutic drug levels. In combination with antimicrobial drug resistance, this can be the cause of treatment failure. If the other kidney is healthy, a unilateral nephrectomy can be performed (Braun et al., 2008). This surgery can be economically feasible, as most cattle return to normal production levels and animals with high economic and/or genetic values can be saved (Miesner and Anderson, 2008). As described in the present case report, the operation can be performed standing via a right flank incision. If removal of a left kidney is necessary, this is possible via the left flank after fasting (Braun et al., 2008).

During surgery, it is important to prevent penetrating the peritoneum to minimize the chance of contaminating the abdomen and the development of peritonitis (Vogel et al., 2011). In the present case, the peritoneum was damaged during surgery, despite using careful blunt dissection as a precaution. However, the kidney was removed without leaking fluids, which reduced the risk of peritonitis. Other possible complications are blood loss and leakage of urine. This can be prevented by ligating the ureter, renal artery and vein multiple times (Miesner and Anderson, 2008). During the operation in the clinic, the artery and ureter were ligated before removing the kidney, minimizing the risk of exsanguination and leakage of possibly infected fluids. Spreading of infection from a kidney to the surrounding tissues has been described in non-surgical cases, so the risk of abscess formation is always present (Van der Kolk et al., 1991). When there is a suspicion of infection in the retroperitoneal cavity, the incision can be left open to drain, or a passive

Figure 5. Placing of a fenestrated tube drain during the second surgery. The tube was placed in the retroperitoneal cavity between a dorsal and ventral opening in the abdominal wall to allow daily rinsing with chlorhexidine. The arrow points towards the incision site of the first surgery and the asterisk shows the location of the last rib.

drain can be placed. In the current case, this might have prevented the formation of the retroperitoneal abscess and the need for a second surgery (Fubini, 2004).

CONCLUSION

This case report demonstrates that also *E. coli* isolates involved in pyelonephritis in cattle can be multidrug-resistant, as has already been seen for other diseases. The use of different antimicrobial drugs, in combination with the inaccessibility of the infection, may lead to therapy failure. In order to use antimicrobials responsibly, isolation of the causative pathogen by urinary analysis or renal puncture is advisable to avoid unnecessary antimicrobial use. Also in this severe case, nephrectomy turned out to be a feasible procedure with acceptable prognosis, since the infection was unilateral. It is recommended to allow drainage of infected fluids by placing a drain to avoid a postoperative abscess. The surgery in this case report was performed by non-specialized veterinarians. This demonstrates that with basic surgical skills, a good preparation and knowledge of anatomy, this surgery is attainable for a first-opinion veterinarian.

REFERENCES

BCFI (2011), Antimicrobiële middelen. In: Belgisch Centrum voor Farmacotherapeutische Informatie (Editors). *Gecommentarieerd Geneesmiddelenrepertorium voor Diergeneeskundig Gebruik*. Edition 2011, Faculté de Médecine Vétérinarie, Liège, p. 1-53.

Braun U., Nuss K., Wehbrink D., Rauch S., Pospischil A. (2008). Clinical and ultrasonographic findings, diagnosis and treatment of pyelonophritis in 17 cows. *The Veterinary Journal 175*, 240-248.

Budras K.-D., Habel R.E., Wünsche A., Liebich H.G., Bragulla H., König H. (2003a). Abdominall wall and abdominal cavity. In: Budras K.-D. (Editor). *Bovine Anatomy: An Illustrated Text*. 1st Edition, Schlütersche GmbH & Co., Hannover, 66-77.

Budras K.-D., Habel R.E., Wünsche A., Liebich H.G., Bragulla H., König H. (2003b). Pelvic Cavity and Inguinal Region, including Urinary and Genital Organs. In: Budras K.-D. (Editor). *Bovine Anatomy: An Illustrated Text*. 1st Edition, Schlütersche GmbH & Co., Hannover, 78-95.

Chantziaras I., Boyen F., Callens B., Dewulf J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. *Journal of Antimicrobial Chemotherapy 69*, 827-834.

Dickert H., Machka K., Braveny I. (1981). The uses and limitations of disk diffusion in the antibiotic sensitivity testing of bacteria. *Infection 9*, 18-24.

Divers T.J., Crowell W.A., Duncan J.R., Whitlock R.H. (1982). Acute renal disorders in cattle: a retrospective study of 22 cases. *Journal of the American Veterinary Medical Association 181*, 694-699.

Floeck M. (2007). Sonographic application in the diagnosis of pyelonephritis in cattle. *Veterinary Radiology & Ultrasound 48*, 74-77.

Fubini S.L. (2004). Surgery of the kidney. In: Fubini S.L., Ducharme N.G. (Editors) *Farm Animal Surgery*, Saunders, St. Louis, 419-422.

Gyles C.L., Fairbrother J.M. (2010). *Escherichia coli*. In: Gyles C.L., Prescott J.F., Songer J.G., Thoen C.O. (Editors). *Pathogenesis of Bacterial Infections in Animals*. 4th Edition, Blackwell Publishing, Ames, 279-284.

Hayashi A., Yanagawa R., Kida H. (1985). Adhesion of *Corynebacterium renale* and *Corynebacterium pilosum* to the epithelial cells of various parts of the bovine urinary tract from the renal pelvis to urethra. *Veterinary Microbiology 10*, 287-292.

Kahn C.M., Line S. (2010). Urinary system. In: *The Merck Veterinary Manual*. 10th Edition, Merck & Co., Inc., Whitehouse Station, p. 1379 – 1418.

Levy S.B., Marshall B. (2004). Antibacterial Resistance Worldwide: Causes, Challenges and Responses. *Nature Medicine 10*, 122-129.

Manges A.R., Johnson J.R., Foxman B., O'Bryan T.T., Fullerton K.E., Riley L.W. (2001). Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *Journal of Medicine 345*, 1007-1013.

Markusfeld O., Nahari N., Kessner D., Adler H. (1989). Observations on bovine pyelonephritis. *The British Veterinary Journal 145*, 573-579.

Miesner M.D., Anderson D.E. (2008). Unilateral nephrectomy of cattle. *Veterinary Clinics of North America Food Animal Practice 24*, 497-500.

Moore R., Miyoshi A., Pacheco L.G.C., Seyffert N., Azevedo V. (2010). *Corynebacterium* and *Arcanobacterium*. In: Gyles C.L., Prescott J.F., Songer J.G., Thoen C.O. (Editors). *Pathogenesis of Bacterial Infections in Animals*. 4th Edition, Blackwell Publishing, Ames, p. 279-284.

Mrvos R., Pummer T.L., Krenzelok E.P. (2013). Amoxicillin renal toxicity: how often does it occur? *Pediatric Emergency Care 29*, 641-643.

Okusu H., Ma D., Nikaido H. (1996). AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (mar) mutants. *Journal of Bacteriology 178*, 306-308.

Prescott J.F. (2013). Beta-lactam antibiotics: Penam penicillins. In: Giguère S., Prescott J.F., Dowling P.M. (Editors). *Antimicrobial Therapy in Veterinary Medicine*. 5th Edition, Wiley Blackwell, Oxford, 133-152.

Rogers B.A., Sidjabat H.E., Paterson D.L. (2011). *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *Journal of Antimicrobial Chemotherapy 66*, 1-14.

Rosenbaum A., Guard C.L., Njaa B.L., McDonagh P.L, Schultz C.A., Warnick L.D., White M.E. (2005). Slaughterhouse survey of pyelonephritis in dairy cows. *Veterinary Record 157*, 652-655.

Sink C.A., Weinstein N.M. (2012). Routine urinalysis. In: Sink C.A. (Editor). *Practical Veterinary Urinalysis*. 1st Edition, John Wiley & Sons, Inc., West Sussex, 19-112.

Todd P.A., Benfield P. (1990). Amoxicillin/clavulanic acid. *Drugs 39*, 264-307.

Van der Kolk J.H., Van Niel M. H., Sloet Van Oldruitenborgh-Oosterbaan M.M. (1991). Peritonitis as complication of cysto-uretero-pyelonephritis in a cow. *Tijdschrift voor Diergeneeskunde 15*, 628-932.

Van Metre D.C. (2009). Diseases of the renal system. In: Smith B.P. (Editor). *Large Animal Internal Medicine*. 4th Edition, Mosby Elsevier, St. Louis, p. 961-963.

Vogel S.R., Desrochers A., Babkine M., Mulon P.Y., Nichols S. (2011). Unilateral nephrectomy in 10 cattle. *Veterinary Surgery 40*, 233-239.

Werkgroep Veterinair Antibioticum Beleid (2012). Formulary dairy cattle. Publication Koninklijke Nederlandse Maatschappij voor Diergeneeskunde, Houten, July 2012.

Yeruham I., Elad D., Avidar Y., Goshen T. (2006). A herd level analysis of urinary tract infection in dairy cattle. *The Veterinary Journal 171*, 172-176.