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Original Article
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Bilateral pyelonephritis due to *Escherichia coli* infection in a captive jaguar (*Panthera onca*)[1]

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ABSTRACT.- Wronski J.G., Argenta F.F., Raiter J., Ehlers L.P., Sala R.D.V., Siqueira F.M., Cardoso D.F., Sonne L. & Pavarini S.P. 2020. **Bilateral pyelonephritis due to *Escherichia coli* infection in a captive jaguar (*Panthera onca*).** *Pesquisa Veterinária Brasileira 40(7):554-558*. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: juliawronski@gmail.com

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a highly diverse pathotype of *E. coli* which colonizes the intestine, and it is considered an important etiological agent associated with bacteremia and other systemic infections, among them urinary tract infection. Retrospective studies evaluating morbidity and mortality of nondomestic felids have demonstrated that urinary tract diseases are among the main causes of death for geriatric animals. Also, mesenchymal neoplasms of the uterus are common in wild felids, and they possess variable morphologic characteristics related to invasiveness and malignancy. This report describes a case of bilateral pyelonephritis due to extraintestinal uropathogenic *E. coli* infection in a captive jaguar (*Panthera onca*). The diagnosis was confirmed through pathological, bacterial and immunohistochemical findings. According to molecular analysis, this *E. coli* strain was classified in the phylogroup F, possessing the following virulence-associated genes: *usp, cnf-1, hlyA, papC* and *sfa.* Additionally, this *E. coli* was highly resistant to β-lactams and first-generation cephalosporin. This jaguar also presented a uterine leiomyoma with distinct distribution, and severe degenerative articular disease, both of them described as frequently seen lesions in geriatric animals from the *Panthera* genus.

INDEX TERMS: Bilateral pyelonephritis, *Escherichia coli*, infection, captive jaguar, *Panthera onca,* extraintestinal pathogenic *E. coli*, uropathogenic *E. coli*, pyelonephritis, uterine leiomyoma, wildlife animals.

RESUMO.- [**Pielonefrite bilateral por *Escherichia coli* em uma onça-pintada (*Panthera onca*).**] *Escherichia coli* extraintestinal patogênica (ExPEC) é um patotipo altamente diverso de *E. coli* que coloniza o intestino e é considerada um agente etiológico importante, associado com bacteremia e outras infecções sistêmicas, dentre elas infecções do trato urinário. Estudos retrospectivos avaliando morbidade e mortalidade de felídeos não domésticos demostram que doenças do trato urinário estão entre as principais causas de morte de animais geriátricos. Ainda, neoplasias mesenquimais uterinas são comuns em felídeos de cativeiro e possuem características morfológicas variáveis relacionadas a invasividade e malignidade. Neste relato é descrito um caso de pielonefrite bilateral por *E. coli* extraintestinal uropatogênica em uma onça-pintada de cativeiro (*Panthera onca*). O diagnóstico foi confirmado através dos achados patológicos, bacteriológicos e imuno-histoquímicos. A partir da análise molecular, esta cepa de *E. coli* foi classificada no filogrupo F, possuindo os seguintes genes associados a virulência: *usp, cnf-1, hlyA, papC* and *sfa.* Adicionalmente, a bactéria isolada foi altamente resistente a β-lactâmicos e cefalosporinas de primeira geração. Foi observado ainda um leiomioma uterino com distribuição distinta e doença articular degenerativa severa, ambas

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descritas na literatura como comumente observadas em animais geriátricos do gênero *Panthera.*

TERMOS DE INDEXAÇÃO: Pielonefrite bilateral, *Escherichia coli*, onça-pintada, *Panthera onca*, *E. coli* patogênica extraintestinal, *E. coli* uropatogênica, pielonefrite, leiomioma uterino, animais selvagens.

INTRODUCTION

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a highly diverse pathotype of *E. coli* which colonizes the intestine, and it is considered an important etiological agent associated with bacteremia and other systemic infections, among them urinary tract infection (Johnson & Russo 2005, Smith et al. 2007). Frequently, ExPEC are classified into groups according to the anatomical site of infection, with uropathogenic *E. coli* (UPEC) being associated with urinary tract infection (UTI); these are considered the most common bacteria identified in UTI from animals and humans (Johnson & Russo 2005, Liu et al. 2015).

The capability of UPEC to lead to symptomatic UTI is related to expression of numerous virulence-associated genes (Liu et al. 2015). Among the main virulence factors (VF) of UPEC isolated from dogs and cats, studies highlight type 1 fimbriae (*fim*), pilus associated with pyelonephritis (*pap*), S fimbriae (*sfa*), afimbrial adhesion (*afa*), α-hemolysin (*hly*), aerobactin, cytotoxic necrotic factor 1 (*cnf1*), and cytolethal distengin toxin (*cdt*) as frequently responsible for acute UTI (Tramuta et al. 2011, Liu et al. 2015). Additionally, phylogenetic studies show that *E. coli* strains can be assigned to different phylogenetic groups (A, B1, B2, C, D, E, F and *E. coli* cryptic clade I), and the profile of virulence associated-genes are extremely diverse between each group and among isolates from the same group (Clermont et al. 2013, Liu et al. 2015). The predominant group in several studies is B2, presenting isolates with the most robust virulence factors and causing clinical sings considered severe (Johnson & Russo 2005, Smith et al. 2007, Tramuta et al. 2011, Clermont et al. 2013, Liu et al. 2015).

Retrospective studies evaluating morbidity and mortality of nondomestic felids have demonstrated that urinary tract diseases are among the main causes of death for geriatric animals (Hope & Deem 2006, Junginger et al. 2015). Also, felids from the genus *Panthera* are prone to the development of uterine neoplasia, among them, leiomyoma (Chassy et al. 2002, Junginger et al. 2015).

The aim of this study is to report a case of bilateral pyelonephritis due to UPEC strain infection in a 19-year-old captive jaguar and to characterize a uterine leiomyoma with atypical presentation in the same animal.

MATERIALS AND METHODS

Clinical and epidemiological data from the jaguar were obtained directly with the veterinary responsible for the zoo. During the necropsy procedure, multiple sections of organs were collected, fixed in a 10% neutral buffered formalin solution, routinely processed for histology, and stained with hematoxylin and eosin (HE). Samples of the main organs were refrigerated and submitted to microbiological evaluation.

Fragments of the kidney were incubated for 24h at 37°C in blood agar 5% and MacConkey agar and biochemistry characterized according to MacFaddin (2000). Subsequently, the isolated bacterium was tested for antibiotic susceptibility against fifteen antimicrobials by disc diffusion method: amoxicillin (30mcg), ampicillin (10mcg), amikacin (30mcg), ceftazidime (30mcg), cephalothin (10mcg), ciprofloxacin (5mcg), enrofloxacin (5mcg), flumequine (30mcg), florfenicol (30mcg), gentamicin (30mcg), imipenem (10mcg), nalidixic acid (30mcg), nitrofurantoin (300mcg), penicillin (30mcg), and sulfazotrim (25mcg). Bacterial DNA was extracted by boiling and PCR assays were performed to detect ExPEC classical virulence genes, following the phylogenetical classification as previously described by Clermont et al. (2013) as a quadriplex PCR assay.

Sections of kidney and uterus were submitted to immunohistochemistry (IHC) for smooth muscle actin (SMA), vimentin, and *E. coli*. Fragments of uterus were incubated with SMA monoclonal antibody (Dako, 1A4 clone, dilution of 1:100, antigen retrieval with Tris EDTA pH 9.0 on pressure pan) and vimentin monoclonal antibody (Zimed, V9 clone, dilution of 1:200, antigen retrieval with citrate buffer pH 6.0 on pressure pan). Fragments of kidney were incubated with *E. coli* polyclonal antibody (Virostat, Portland/ME, USA, dilution of 1:200, antigen retrieval with citrate buffer pH 6.0 on microwave oven). The amplification signal for all three IHC was achieved by using the peroxidase-labeled universal polymer method (MACH 4 Universal HRP-Polymer, Biocare Medical, Pacheco/CA 94553, USA). The reaction was revealed with 3-amino-9-ethylcarbazole (AEC, Dako North America, Carpinteria/CA 93013, USA), followed by counterstaining with Harris hematoxylin. Positive and negative controls were used in all IHC tests.

RESULTS

A 19-year-old jaguar (*Panthera onca*) was submitted for necropsy. The animal was born and remained its whole life in captivity, having three pregnancies during this period. For approximately 15 days before death, the jaguar presented clinical signs of apathy, dyspnea, diarrhea, lameness, prostration, and refusing movement. Antibiotic therapy with benzathine penicillin was applied (1.200.000IU each 48h for eight days), however the animal evolved to recumbency and death.

At necropsy, alterations were observed in multiple organ systems. At external examination, the jaguar was obese, and presented multiple swollen joints. The pelvis of both kidneys was distended due to marked accumulation of green purulent material (Fig.1A). Multiple white striations extending from the cortex to the medulla and reaching the pelvis were noted. The ureters and urinary bladder were unremarkable. Moreover, the uterus was markedly enlarged, measuring 12.0 x 4.0 x 4.0cm (Fig.1B), with multiple firm nodules disseminated in the organ, expanding the uterine wall and reducing almost completely the lumen at both uterine horns and uterine body. On cut surface, these nodules were nondelimitated, firm, and white (Fig.1B inset). The ovaries presented several cysts measuring up to 2.0cm in diameter. Additionally, multiple joints presented severe erosion of articular surfaces, with associated exposition of subchondral bone and deposition of fibrin, as well as marked osseous proliferations (osteophytes), and severe proliferation of articular capsule. In the lungs, multiple red to gray areas were noted in the caudal lobes.

Histologically, located mainly in the renal pelvis but extending also into the adjacent medullary parenchyma, there was marked inflammatory infiltrate of neutrophils, lymphocytes and macrophages, associated with multifocal areas of moderate necrosis, fibrin deposition, cellular debris

and coccobacilli bacterial aggregates (Fig.1C). At the renal cortex, there were multifocal to coalescing areas of interstitial fibrosis, mild inflammatory infiltrate of lymphocytes and plasma cells, mild multifocal glomerulosclerosis and hyaline cylinders inside tubules. The urinary bladder presented mild mononuclear inflammatory infiltrate associated with multifocal areas of hemorrhage in the submucosa.

The uterus had a neoplastic proliferation of mesenchymal cells expanding the myometrium and markedly reducing the uterine lumen, constituted by spindle-shaped cells with abundant eosinophilic cytoplasm. The cells were arranged in interlacing bundles mixed with an abundant stream of connective tissue. There was mild pleomorphism and no mitotic figures. In the ovaries, multiple cystic structures were located in the periovaric region.

Additional histological findings included severe proliferation of the articular capsule due to proliferation of synoviocytes and fibrous connective tissue (degenerative joint disease) with associated chondrocyte loss and myelofibrosis, mild keratitis and anterior uveitis, and moderate parathyroid hyperplasia. No relevant microscopic alterations were seen in the other organs.

The immunohistochemical technique demonstrated mild intracytoplasmic immunostaining for vimentin and SMA in the uterine neoplasia, and marked multifocal granular labeling for *E. coli* predominantly inside macrophages in the renal pelvis as well as associated with cellular debris, free in the renal pelvis (Fig.1D).

Pure *E. coli* colonies were isolated and identified through biochemical and molecular analysis. The strain was characterized as uropathogenic *E. coli* (UPEC) belonging to the phylogroup F and the following virulence factors (VF) were detected: *usp, cnf-1, hlyA, papC* and *sfa.* The strain was resistant to penicillin, cephalothin, and amoxicillin, and showed intermediary susceptibility to ampicillin.

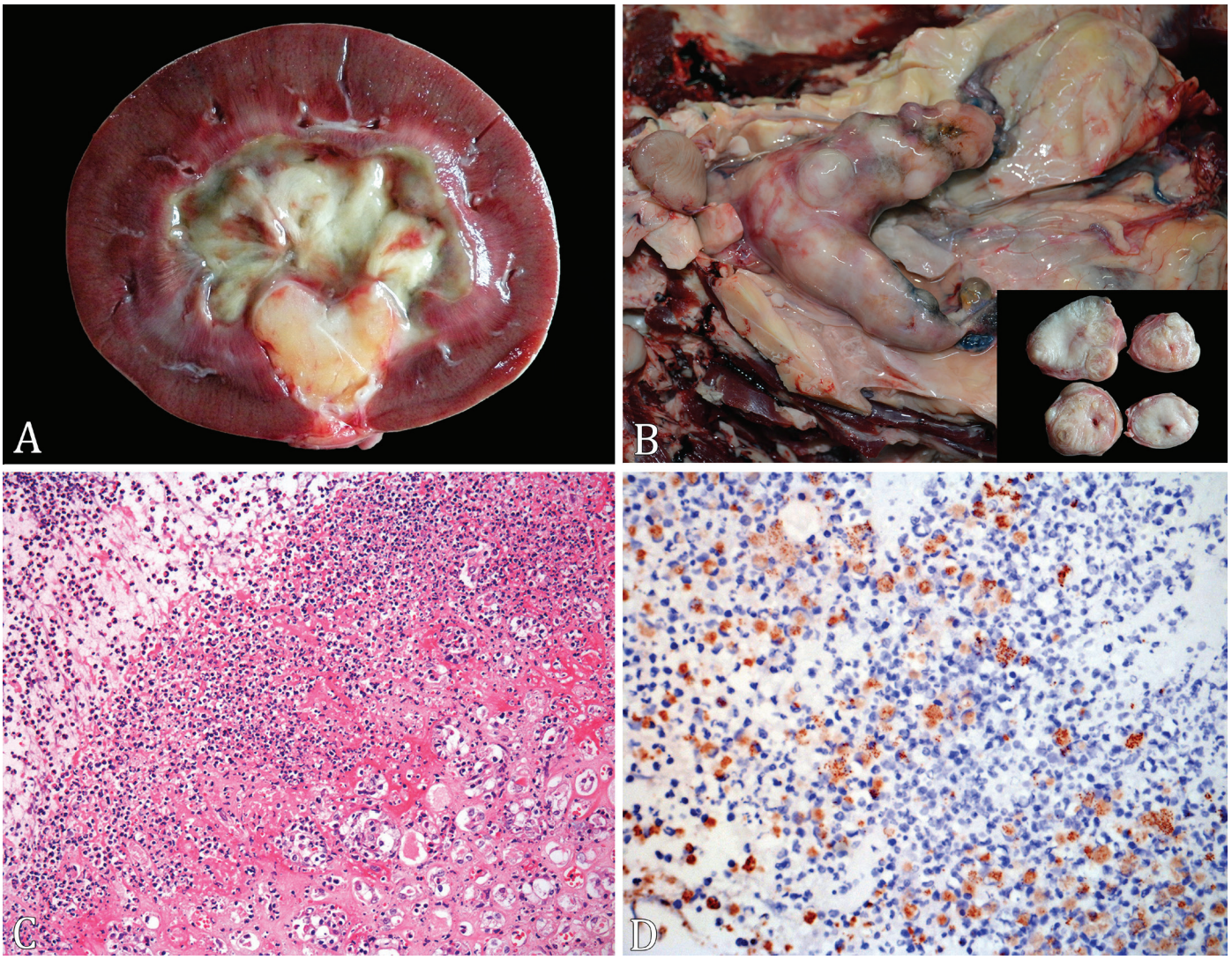

Fig.1. Bilateral pyelonephritis due to Escherichia coli infection and uterine leiomyoma in a jaguar. (**A**) Longitudinal section of kidney showing marked accumulation of green purulent material in the distended pelvis. (**B**) The uterus is markedly enlarged due to multiple coalescing nodules expanding the uterine wall. Inset: transverse sections of the uterus, with multiple nondelimitated white nodules reducing the uterine lumen. (**C**) Renal pelvis showing marked mixed inflammatory infiltrate, associated with necrosis, fibrin deposition, and cellular debris. HE, obj.10x. (**D**) Marked multifocal granular immunostaining for E. coli predominantly inside macrophages in the renal pelvis. Immunohistochemistry anti-E. coli, 3-amino-9 ethylcarbazole, Harris hematoxylin counterstain, obj.20x.

DISCUSSION AND CONCLUSION

The diagnosis of bilateral bacterial pyelonephritis due to uropathogenic *Escherichia coli* was based on pathologic, bacteriological and immunohistochemical findings. The causes of death in captive nondomestic felids vary in the literature, including chronic renal disease (Junginger et al. 2015), trauma (Metz et al. 2017), and stillbirths or neonatal (Hope & Deem 2006) as the most important. The neoplastic disease was considered an important cause in most studies, mainly for geriatric animals (Hope & Deem 2006, Junginger et al. 2015, Metz et al. 2017).

Extraintestinal infections due to ExPEC are described for several species, although reports of it causing disease in wild felids are scarce (Carvallo et al. 2010, Carvalho et al. 2012). In general, they are considered opportunistic pathogens who colonize the intestine. When it infects immunocompromised animals or in the presence of specific risk factors, bacteria can reach the urinary tract and cause UTI (Foxman 2014, Singer 2015). Among predisposing factors listed in the literature (Foxman 2014), obesity, female sex, anatomic abnormalities, and trauma/manipulation were identified in the case here described. Another important risk factor is the pathogenicity of the isolate, mainly related to its VF, since some can cause disease even in immunocompetent individuals (Johnson 2003, Hutton et al. 2018).

Isolates of ExPEC, in general, have multiple VF, and extensive profiles promote more invasive infections; inversely, no single VF is sufficient for the pathogenesis of UTI (Johnson 2003). Different studies performed by several researchers state that VF such as *papA, papC, sfa/sfc, afa/draBC, iutA, hylA Cnf-1,* and *kpsMT II* are related to acute infections of the urinary tract for dogs, cats, and humans, although the virulence profile is highly diverse according to the strain (Smith et al. 2007, Tramuta et al. 2011, Liu et al. 2015, Hutton et al. 2018). Additionally, the combination of *papC, sfa, hlyA,* and *cnf1* appears frequently (Tramuta et al. 2011), as observed in the jaguar from this case, which presented all four, in addition to *usp*.

Although single virulence genes cannot cause disease, as mentioned above, some VF can aggravate the disease. Adhesin-associated genes, such as *papC* and *sfa,* may be critical for illness severity (Hutton et al. 2018). P fimbriae, or pilus associated with pyelonephritis (*pap* type *C* in the described *E. coli*), show strong epidemiologic association with pyelonephritis, sepsis, prostatitis (Johnson 2003, Liu et al. 2015), which may explain the pyelonephritis without evidence of infection in other sites, mainly urinary bladder. Additionally, the expression of toxin genes, such as *cnf1, hlyA* and *usp* identified in this *E. coli*, may lead to more extensive tissue damage in the host, causing release of host nutrients, disabling of immune effector cells and, consequently, facilitating bacterial dissemination (Paniagua-Contreras et al. 2017).

The *E. coli* isolated was classified as phylogroup F. The predominant phylogenetic group described to UPEC strains is the B2, being the phylogroup F usually the least observed (Liu et al. 2015, Hutton et al. 2018). Group F was firstly described in a phylogenetic study performed in human samples (Jaureguy et al. 2008), and further differentiation from the other groups was achieved through a quadriplex PCR proposed by Clermont et al. (2013). Due to genetic similarities and because they are genetically closely related, phylogroup F is referred as a sister of B2 by some authors (Jaureguy et al. 2008, Clermont et al. 2013). Notably, studies performed in samples from dogs and cats have shown both phylogroups presenting a high number of virulence associated-genes (Liu et al. 2015) and the presence of extensive profiles tend to cause more aggressive infections (Johnson 2003).

Antibiotic resistance is a widely discussed topic in ExPEC literature, mainly in UPEC strains. A study performed in the United States demonstrated most strains from urinary tract of domestic felids were resistant to at least one antibiotic, being 98% and 62% of strains resistant to cephalothin and ampicillin, respectively (Liu et al. 2015). In the case presented here, the isolated *E. coli* was resistant to β-lactams and first-generation cephalosporin, explaining the negative response of the jaguar to the treatment adopted by veterinarians of the zoo. Moreover, these findings are similar to the antibiotic resistance profile of some *E. coli* strains from domestic and wild felids, as well as for humans (Smith et al. 2007, Carvalho et al. 2012, Liu et al. 2015).

In addition to the renal lesions, the jaguar from this case presented also a widespread uterine leiomyoma, periovaric cysts, degenerative joint disease in multiple joints, keratitis and anterior uveitis, and hyperplasia of parathyroid glands. Most of these lesions were reported for geriatric animals in previous studies of pathology of wild felids (Hope & Deem 2006, Junginger et al. 2015, Metz et al. 2017).

Reproductive tumors are among the most common types of neoplasia in captive *Panthera* species (Junginger et al. 2015, Kloft et al. 2019). Smooth muscle neoplasms are commonly reported in zoo felids, they involve mainly the uterus, and leiomyomas are considered the most common among all reproductive neoplasia (aside from mammary tumors) in most studies (Chassy et al. 2002, Owston et al. 2008, Junginger et al. 2015, Kloft et al. 2019). However, they are almost always not related to the cause of death, as they are common spontaneous tumors of felids (Junginger et al. 2015, Kloft et al. 2019), similarly to what is observed in the case here described. Despite, there seems to be no reports in the veterinary literature of leiomyomas with such widespread distribution as noted in this case.

Considering the characteristics of the isolated strain of *E. coli* identified in the jaguar from this study and the severity of the reported case, in addition to the predisposition of felids from the genus *Panthera* to develop uterine neoplasia, we highlight also the importance of the characterization of conditions as in this case to improve clinical approaches for captive wild felids, mainly for endangered animals.

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Conflict of interest statement.- The authors have no competing interests.

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