Ciência Rural, Santa Maria, v.52:9, e20210441, 2022
http://doi.org/10.1590/0103-8478cr20210441
ISSNe 1678-4596
PATHOLOGY

Granulomatous nephritis and uremia associated with *Klossiella equi* in a horse from Rio Grande do Sul, Brazil

Eryca Ceolin Lamego[1] Stella Maris Pereira de Mello[1] Roberta Wilborn[2] Marcos Eduardo Neto[2] Carlos Eduardo Nogueira[2] Mariana Martins Flores[1*]

[1]Programa de Pós-graduação em Medicina Veterinária, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), 97105900, Santa Maria, RS, Brasil. E-mail: marianamflores@yahoo.com.br. *Corresponding author.
[2]Hospital de Clínicas Veterinárias, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brasil.

ABSTRACT: *Klossiella equi is an unusual protozoan of equids. In most cases, it does not cause renal injury. A case of K. equi-associated granulomatous nephritis leading to uremia in a brazilian mare is described. The animal presented colic, and among the complementary exams, increased urea and creatinine was observed. The kidneys were unremarkable on gross exam; however, a multifocal granulomatous nephritis with tubular degeneration associated with intraepithelial and intraluminal protozoa was observed histologically. The large intestines were edematous and hemorrhagic. The importance of including K. equi infection among the differential diagnosis of horses with kidney disease in southern Brazil is highlighted.*
Key words: *kidney, protozoa, uremia, histopathology.*

Nefrite granulomatosa e uremia associada a *Klossiella equi* em um equino no Rio Grande do Sul, Brasil

RESUMO: *Klossiella equi é um protozoário incomum de rim de equídeos, geralmente considerado de pouca importância clínica. Descreve-se um caso de klosselliose associada a lesão renal e uremia em uma égua. O animal foi atendido com quadro de cólica, e dentre os exames, foi constatado aumento de ureia e creatinina. Na necropsia, os rins não apresentavam lesões macroscópicas, no entanto, na histopatologia, observou-se nefrite granulomatosa com degeneração tubular associada a protozoários intraepiteliais e intraluminais. A parede do intestino grosso estava edemaciada e hemorrágica. Se destaca a importância da inclusão de infecção por K. equi nos diagnósticos diferenciais de equinos com doença renal no sul do Brasil.*
Palavras-chave: *rim, protozoário, uremia, histopatologia.*

Klossiella equi is a coccidian parasite that infects kidneys of horses, ponies, zebras and donkeys (CIANCIOLO & MOHR, 2016). Despite being known for more than 50 years, the prevalence and life cycle of this protozoan are still poorly understood (BALLWEBER et al., 2012; CIANCIOLO; MOHR, 2016). *K. equi* infection is classically considered of little clinical importance, as it does not cause impaired renal function in most animals (LEE & ROSS, 1977; AUSTIN & DIES, 1981; CIANCIOLO& MOHR, 2016). Despite this, rare scientific reports describe *K. equi*-associated nephritis and uremia in horses (BALLWEBER et al., 2012; Baker et al., 2018). In addition; although, histopathology is the main form of diagnosis, most reports present old and/or low-quality histologic images of the parasite (LEE; ROSS, 1977; AUSTIN; DIES, 1981; ANDERSON et al., 1988). The most recent literature on the subject does not describe histological findings in detail (BALLWEBBER et al., 2012; BAKER et al., 2018), and the main veterinary pathology textbooks do not contain images of all phases of the protozoan (CIANCIOLO; MOHR, 2016; BRESHEARS; CONFER, 2017). In addition, as it is an infectious agent with a poorly understood cycle and with potential of infecting other horses, reporting individual cases in distinct geographic regions is of major importance on the epidemiologic surveillance of the disease. This note described a case of *K. equi*-associated granulomatous nephritis and uremia in a mare from southern Brazil.

Received 06.07.21 Approved 09.22.21 Returned by the author 11.12.21
CR-2021-0441.R2
Editor: Rudi Weiblen

A two-year-old Crioulo mare presented to the Hospital de Clínicas Veterinárias da Universidade Federal de Pelotas (UFPel) with a complaint of apathy. No history of previous diseases was reported. It had tachycardia (100 bpm), tachypnea (64 mpm), congested mucous membranes and mild dehydration (6%) upon exam. The following parameters were unremarkable: hematocrit (0,516 l/l, reference value [RV]: 0.32-0.53 l/l), plasma protein (80 g/l, RV: 58-87 g/l), fibrinogen (4 g/l, RV: 1-4 g/l), platelet count (250 x 10^3/µl, RV: 100,000-350,000), total leukocyte count (9,400/µL, RV: 5,400-14,300/µL), segmented cell count (6,768/µL, RV: 2,260-8,580/µL) and lymphocyte count (2,350/µL, RV: 1,500-7,700/µL). Serum biochemistry revealed uremia (creatinine: 380.13 µmol/L, RV: 106.8-167.96 µmol/L; and urea: 18.73 µmol/L, RV: 1.67-4 µmol/L). No findings suggestive of immunosuppression were observed. The mare was submitted to euthanasia and necropsy.

On necropsy, the intestinal loops were congested and distended by gas. The colon was diffusely edematous, hemorrhagic and ulcerated. The remaining abdominal and thoracic organs, including kidneys, were unremarkable. Organ fragments were fixed in 10% formalin and sent to the Laboratório de Patologia Veterinária (LPV) of the Universidade Federal de Santa Maria (UFSM), where they were processed and stained with Hematoxylin and Eosin (HE). Multiple intraepithelial protozoa morphologically compatible with *K. equi* were observed in the kidney tubules (Figure 1A). Micro and macrogametes (Figures 1B, 1C), trophozoites (Figure 1C) and sporonts (Figure 1D) were identified within the epithelial cells of proximal convoluted tubules. In addition, there were free intraluminal sporoblasts (Figure 1E) in the collecting and distal convoluted tubules. Swelling, vacuolation (degeneration) and desquamation of tubular epithelial cells – including those infected by protozoa – were also observed. Additional findings included interstitial inflammation constituted of lymphocytes, plasma cells, macrophages and multinucleated giant cells (Figure 1F) and rare intratubular protein casts. Glomeruli were characterized by mild increase in cellularity and podocyte hypertrophy. The gross intestines had severe transmural edema and moderate to severe multifocal submucosal hemorrhages. Additionally, a cyathostomine-associated eosinophilic colitis was observed.

Information regarding *K. equi* prevalence in equids are scarce in the literature. Important textbooks do not mention prevalence data (CIANCIOLO; MOHR, 2016), and most articles report individual cases. Despite that, the parasite has been reported in North America (AUSTIN; DIES, 1981), Europe (REZAIE et al., 2013), Africa (KARANJA et al., 1995) and Oceania (REPPAS; COLLINS, 1995). Although, the parasite is known to occur in South America, reports are scarce to absent. The authors of this manuscript were unable to find scientific reports of its occurrence in Brazil.

A definitive diagnosis of kidney injury associated with *K. equi* was established based on histopathology. The histological visualization of the protozoan is sufficient, since *K. equi* is the only known renal coccidian of horses (BAKER et al., 2018; RAZIE et al., 2013). Genomic sequencing and urinalysis can be used in live animals (BALLWEBER et al., 2012; BAKER et al., 2018). However, these techniques are limited when compared to histopathology, as it allows visualizing parasite-associated lesions (ANDERSON et al., 1988). Detecting the protozoan in urine can be difficult because sporocysts do not settle easily, and flotation with saline results in sporocyst rupture (REPPAS; COLLINS, 1995).

Differential histologic diagnoses include other parasites and fungi that might infect the equine kidney, among which *Coccidioides immitis* should be mentioned. However, this organism is histologically characterized by spherules, and it induces pyogranulomatous nephritis, which differs from the purely granulomatous lesions observed in klossiellosis (CIANCIOLO; MOHR, 2016). Although, *K. equi* is similar to other intestinal coccids of domestic animals, this is the only equid coccid infecting the kidney (REZAIE et al., 2013).

The complete life cycle and pathogenesis of *K. equi* infection are still poorly understood (CIANCOLO; MOHR, 2016). Different stages of development of the protozoan have already been identified in the kidney; however, the extra renal cycle remains unknown. It is speculated that the animals are infected by ingesting sporocyst-contaminated pasture (GARDINER et al, 1998). Once ingested, trophozoites travel through the bloodstream until they reach the kidneys, where they infect tubular epithelial cells and form schizonts and merozoites (BAKER et al., 2018). Thereafter, gametogenesis occurs, where micro and macrogametes are fertilized and give rise to the sporont, which will mature and release sporoblasts (GARDINER et al, 1998). Sporoblasts become sporocysts, which contain multiple sporozoites; these are released into the tubular lumen and eliminated in the urine, to contaminate the environment. Epidemiologic factors favoring the

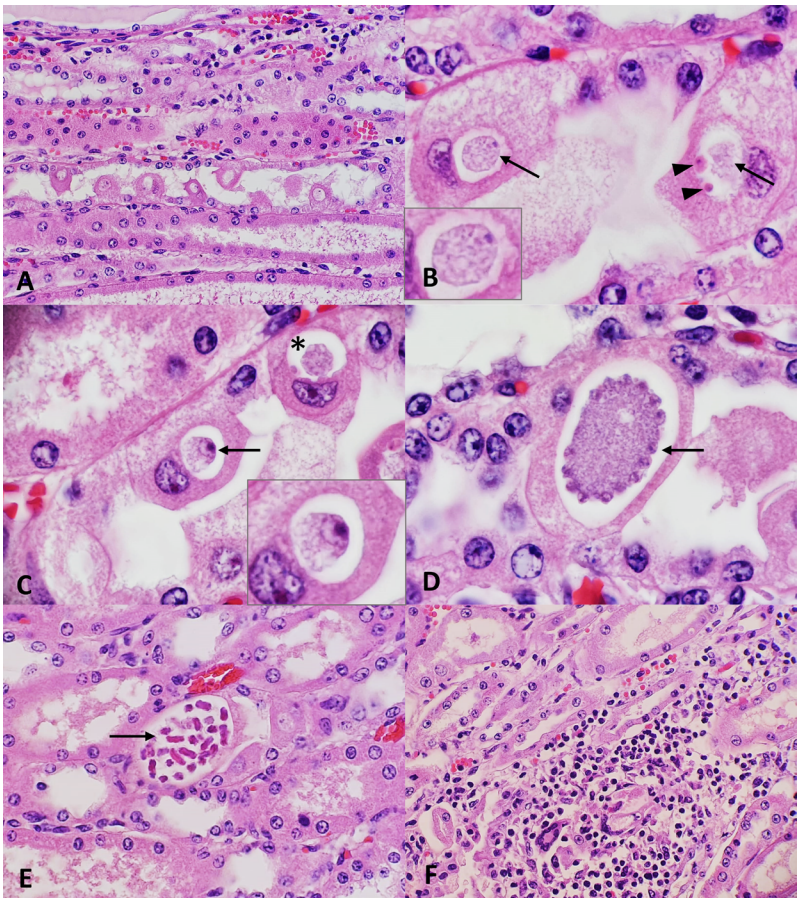

Figure 1 - *Klossiella equi* infection in horse kidney. (A) Tubular epithelial cells are swollen, with parasite-containing vacuoles (Hematoxylin and eosin [HE], Obj 20X). (B) Intraepithelial round, approximately 25 µm structures containing a faded nucleus (macrogametes, arrow) and smaller round to oval, 2-3 µm structures (microgametes, arrow head). Inset: macrogamete in a greater detail (HE, Obj 100X). (C) Intraepithelial round, 20 µm structure with a prominent nucleus (trophozoite, arrow). A macrogamete and a microgamete are seen within another cell (asterisk) (HE, Obj 100X). (D) Intraepithelial oval, 70 x 50 µm structure with multiple peripheral sporoblasts budding from the central mass (sporont) (arrow) (HE, Obj 100X). (E) Intratubular 2-4 µm rod-shaped eosinophilic structures (free sporoblasts) (arrow) (HE, Obj 20X). (F) Interstitial granulomatous inflammation with tubular loss is observed (HE, Obj 20X).

infection of horses with sporocysts in contaminated pastures are not known (Baker et al., 2018).

K. equi infection is classically asymptomatic and generally does not cause secondary renal damage, being considered incidental (LEE; ROSS, 1977; AUSTIN; DIES, 1981). Regardless, there are rare reports of horses that developed clinical signs – among which, hematuria (BALLWEBER et al., 2012; Baker et al., 2018) –, and parasite-associated histologic lesions (BALLWEBER et al., 2012). Even among these reports, uremia is uncommon (Baker et al., 2018). The kidneys are reportedly normal on gross exam, and varying degrees of interstitial inflammation and tubular degeneration are described on histological analysis (BALLWEBER et al., 2012). Granulomatous inflammation is probably incited by tubular rupture in heavily parasitized kidneys (ANDERSON et al., 1988). Some studies suggested that severe infections might be associated with some degree of immunosuppression (ANDERSON et al.,

1988; BALLWEBBER, 2018). However, *K. equi* infection without associated immunosuppression has also been described (Baker et al., 2018). The mare from this report had its death associated with colic probably associated with both uremic colitis and cyathostomin infestation, the latter possibly indicating some degree of immunosuppression. Regardless, it was not possible to confirm that this animal was immunosuppressed, and it is well known that healthy adult animals are also at risk for cyathostomin-related disease (UZAL et al. 2016). The clinical and histopathological findings described confirmed *K. equi*-associated nephritis and uremia in a horse from southern Brazil. Klossiellosis should be included among differential diagnoses in horses with kidney disease.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

ANDERSON, W. I. et al. *Klossiella equi* induced tubular nephrosis and interstitial nephritis in a pony. **Journal of Comparative Pathology**, v.98. n.3. p.363-366, 1988. Available from: <https://www.sciencedirect.com/science/article/abs/pii/0021997588900448>. Accessed: Mar. 14, 2021. doi: 10.1016/0021-9975(88)90044-8.

AUSTIN, R. J.; DIES, K. H. Klossiella equi in the kidneys of a horse. **The Canadian Veterinary Journal,** v.22, n.5, p.159-161, 1981. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1790026/>. Accessed: Apr. 15, 2021.

BAKER, R. E. et al. *Klossiella equi* infection in a cheval canadian mare. **Veterinary Clinical Pathology**, v.47. p.654-659, 2018. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/vcp.12660>. Accessed: Mar. 14, 2021. doi: 10.1111/vcp.12660.

BALLWEBER, L. R. et al. *Klossiella equi* infection in an immunosuppressed horse: evidence of long-term infection. **Case Reports in Veterinary Medicine**, v.2012, p.4.id230398, 2012. Available from: <https://www.hindawi.com/journals/crivem/2012/230398/#abstract>. Accessed: Mar. 10, 2021. doi: 10.1155/2012/230398 (Eletronic publication).

BRESHEARS, M. A; CONFER, A. W. The Urinary System. In: Zachary, J. F. **Pathologic Basis of Veterinary Disease**. 6th ed. St Louis: Elsevier, 2017. Cap.11, p.672.

CIANCIOLO, R. E.; MOHR, F. C. Urinary System. In: MAXIE, M. G. **Jubb, Kennedy, and Palmer's pathology of domestic animals**. Saint Louis: Elsevier, 2016. v.2. Cap.4, p.443.

GARDINER, C. H. et al. Apicomplexa. In: _____. **An Atlas of Protozoan Parasites in Animal Tissues**. 2. ed. Washington: Government Printing Office, 1988. P.61-62.

KARANJA, D.N.R. et al. Donkey klossiellosis in Kenya. **Veterinary Parasitology**, v.59, p.1-5, 1995. Available from: <https://www.sciencedirect.com/science/article/abs/pii/030440179400739Y>. Accessed: Aug.14, 2021.

LEE, C. G.; ROSS, B. V. Renal coccidiosis of the horse associated with *Klossiella equi*. **Australian Veterinary Journal**, v.53. n.6, p.287-288, 1977. Available from: <https://doi.org/10.1111/j.1751-0813.1977.tb00219.x>. Accessed: Apr. 15, 2021. doi: 10.1111/j.1751-0813.1977.tb00219.

REPPAS, G.P.; COLLINS, G.H. *Klossiella equi* infection in horses; sporocyst stage identified in urine. **Australian Veterinary Journal**, v.72, n.8, p.316-318, 1995. Available from: <https://doi.org/10.1111/j.1751-0813.1995.tb03564.x >. Accessed: Mar. 10, 2021. doi: 1751-0813.1995.tb03564.

REZAIE, A. *Klossiella equi* in a donkey – a first case report from Iran. **Tropical Biomedicine**, v.30, n.3, p.543-546, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/24189684/>. Accessed: Mar. 18, 2021.

UZAL, F. et al. Alimentary system. In: MAXIE, M. G. **Jubb, Kennedy, and Palmer's pathology of domestic animals.** Saint Louis: Elsevier, 2016. v.2. Cap.1, p.217.