CLINICAL CASE

Mixed type renal nephroblastoma in a three-year-old female dog: Case report

Nefroblastoma renal de tipo mixto en un canino hembra de tres años de edad: Reporte de caso

Nefroblastoma renal tipo misto em cadela de três anos de idade: Relato de caso

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Received: March 17, 2024. Accepted: November 8, 2024

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eISSN: 2256-2958 **Rev Colomb Cienc Pecu**

https://doi.org/10.17533/udea.rccp.v38n3a7

To cite this article:

Rincón-Alarcón DF, Fonseca-Matheus JM, Jaramillo-Chaustre XL. Mixed type renal nephroblastoma in a three-year-old female dog: case report. Rev Colomb Cienc Pecu. Year, Vol, number, and pages pending. DOI: https://doi.org/10.17533/udea.rccp.v38n3a7

Abstract

Anamnesis: Renal nephroblastoma is a rare neoplasm in dogs. This case corresponds to a 3-year-old female Golden Retriever who was treated for bilateral abdominal distention and weight loss. **Clinical and laboratory findings:** There were no alterations in the hemogram or blood biochemistry (AST, AP, BUN, and creatinine). Radiographic findings were enlarged left kidney with a cystic center. **Treatment approach:** A median laparotomy was performed; the intestines and spleen were displaced to expose the left kidney, then this was dissected from the abdominal roof, the ureter, renal artery, and vein were ligated and sectioned, and the affected kidney was excised. Macroscopically, it presented a mass of 15 × 10 × 8 cm with a central cavitation of 4 × 5 cm. Mixed-type nephroblastoma grade II (SIOP and NWTSG) was diagnosed by histopathology. Immunohistochemistry was performed to confirm neoplasm and describe proliferating cell portions using cytokeratin AE1-AE3, Pax-8, and WT-1. **Conclusion:** Related to the rare presentation of this neoplasm, it is very important to describe prognostic indicators in dogs with nephroblastoma. In this case, the use of these markers was useful in supporting the diagnosis**.**

Keywords: *canine; immunohistochemistry; kidney; nephrectomy; nephroblastoma; radiographic findings.*

Resumen

Anamnesis: El nefroblastoma renal es una neoplasia rara en los caninos. El caso corresponde a un canino hembra, Golden retriever de 3 años de edad, que fue atendida por distención abdominal y pérdida de peso. **Hallazgos clínicos y de laboratorio:** El hemograma y las bioquímicas sanguíneas no mostraron alteraciones. El hallazgo radiográfico fue aumento de tamaño en riñón izquierdo con centro quístico. **Aproximación terapéutica:** se realizó una laparotomía media, el bazo y los intestinos fueron desplazados para exponer el riñón izquierdo, luego este se disecó del techo

abdominal, se ligaron y seccionaron el uréter, arteria y vena renales, y se escindió el riñón afectado. Este último macroscópicamente presentó una masa de 15 × 10 × 8 cm con una cavitación central de 4 × 5 cm. En la histopatología se diagnosticó nefroblastoma tipo mixto grado II (SIOP y NWTSG). Se realizó inmunohistoquímica para confirmar la neoplasia y caracterizar las porciones celulares que proliferan usando citoqueratina AE1-AE3, Pax-8 y WT-1. **Conclusiones:** Relacionado con la rara presentación del nefroblastoma, es muy importante describir indicadores pronósticos en perros. En este caso el uso de marcadores fue útil para confirmar el diagnóstico.

Palabras claves: *canino; hallazgo radiográfico; inmunohistoquímica; nefrectomía; nefroblastoma; riñón.*

Resumo

Anamnese: O nefroblastoma renal é uma neoplasia rara em cães. Este caso corresponde a uma fêmea Golden Retriever de 3 anos de idade, que foi tratada devido a distensão abdominal bilateral e perda de peso. **Achados clínicos e laboratoriais:** Não houve alterações no hemograma ou na bioquímica sanguínea (AST, FA, BUN, e creatinina). Os achados radiográficos foram rim esquerdo aumentado com centro cístico. **Abordagem e tratamento:** foi realizada laparotomia mediana, os intestinos e o baço foram deslocados para expor o rim esquerdo, depois este foi dissecado do teto abdominal, o ureter, a artéria e a veia renais foram ligados e seccionados e o rim afetado foi excisado. Macroscopicamente apresentava uma massa de 15 × 10 × 8 cm com cavitação central de 4 × 5 cm. Nefroblastoma tipo misto grau II (SIOP e NWTSG) foi diagnosticado por histopatologia. A imunohistoquímica foi realizada para confirmar a neoplasia e descrever porções celulares em proliferação usando citoqueratina AE1-AE3, Pax-8 e WT-1. **Conclusão:** Relacionado à rara apresentação desta neoplasia é muito importante descrever indicadores prognósticos em cães com nefroblastoma. Neste caso a utilização destes marcadores foi útil no apoio ao diagnóstico.

Palavras-chave: *achado radiográfico; canino, imuno-histoquímica; nefrectomia; nefroblastoma*; *rim.*

Introduction

Nephroblastoma is a rare neoplasm resulting from poor differentiation of the metanephrogenic blastema (Baskin and De Paoli 1977); in humans, it is known as Wilms tumor (Kaste *et al.,* 2008); These are the most common renal neoplasms of pigs and chickens and are usually recognized as incidental findings at slaughter. They are the second most common primary renal tumor in dogs and occur less frequently in cattle (Zachary, 2022).

Under normal conditions, the differentiation of the metanephrogenic blastema is induced in an epithelial component, forming nephrons and the stromal component that makes up the connective tissue of the kidney (Brown and malik 2001); it is considered an embryonal cancer of the developing kidney (Pode-Shakked and Dekel, 2011). It is speculated that these neoplasms result from malignant transformation during normal nephrogenesis or from neoplastic transformation of nests of embryonic tissue that persists postnatally. At necropsy, nephroblastomas can be solitary or multiple masses that often reach a great size, obscuring recognizable renal tissue. Because nephroblastomas arise from primitive pluripotential tissue, histologic features vary but are morphologically similar to the developmental stages of embryonic kidneys. Characteristically, three components are identified in various ratios undifferentiated loose myxomatous mesenchymal tissue, primitive tubules and structures that resemble primitive glomeruli, and scattered nests of cells resembling the metanephric blastema (Zachary, 2022).

The mean age of presentation in canines in a study with five nephroblastomas is 5.2 years (Bryan *et al.,* 2006); other reports describe this neoplasm in young animals less than 6 months (Baskin and De Paoli, 1977; Montinaro *et al.,* 2013). In humans, it is the most common pediatric renal neoplasia in North America (Kaste *et al.,* 2008). There are some cases of extrarenal nephroblastoma in the spinal cord of dogs (Brewer *et al.,* 2011). Some reports of cases have been described in other animal species, such as nephroblastoma renal in a Buffalo calf (*Bubalus bubalis*) (Rama Devi *et al.,* 2011), in a mare (*Equus caballus*) (Jardine and Nesbit, 1996) African hedgehog (*Atelerix albiventris*) (Ueda *et al.,* 2019) and nasopharyngeal nephroblastoma in a Boer goat (*Capra aegagrus hircus*) (Athey *et al.,* 2021).

The most important signs of canine renal neoplasms are hematuria, lethargy, loss of appetite, palpable abdominal mass, and polyuria/polydipsia (Bryan *et al.,* 2006). Since it is a rare neoplasm in veterinary medicine, there are no clear prognostic indicators, and some reports mentioned several

factors can influence (Chen *et al.,* 2018). The histological classification is based on human documents with NWTSG classification (National Wilms Tumor Study Group) and SIOP (Société internationale d´oncologie pediatrique) (Kaste *et al.,* 2008). The prognosis is variable; recent studies using NWTSG classification described some cases with metastases and malignant behavior (Chen *et al.,* 2018); and some with good prognosis after surgery, especially low grades (Hergt *et al.,* 2019; Richardson 2020; Seaman *et al.,* 2003) In dogs with renal neoplasms, surgery is the only treatment that improves survival (Bryan *et al.,* 2006).

Case presentation

Anamnesis

A 3-year-old Golden Retriever, with 30 kg of body weight, was presented for consultation due to severe bilateral abdominal distention with progressive weight loss.

Clinical findings

During clinical examination, an abdominal mass was noted by palpation. The mass had a firm consistency and was attached to the abdominal roof.

Diagnostic aids used

There were no alterations in the hemogram and blood biochemistry, specifically aspartate aminotransferase (AST), alkaline phosphatase (AP), blood urea nitrogen (BUN), and creatinine. The radiographic findings were enlargement of the left kidney with a greater radiopacity appearance concerning the normal parenchyma and the center portion of the kidney with a radiolucent cystic appearance measuring approximately 3 × 4 cm (Figure 1). An abdominal ultrasound study was made (Mindray BC56 ultrasound machine) with a micro-convex transducer 5MHz; the kidney had abnormal architecture with a heterogenous echo-texture with a 4 cm diameter cavity in the caudal portion of kidney parenchyma.

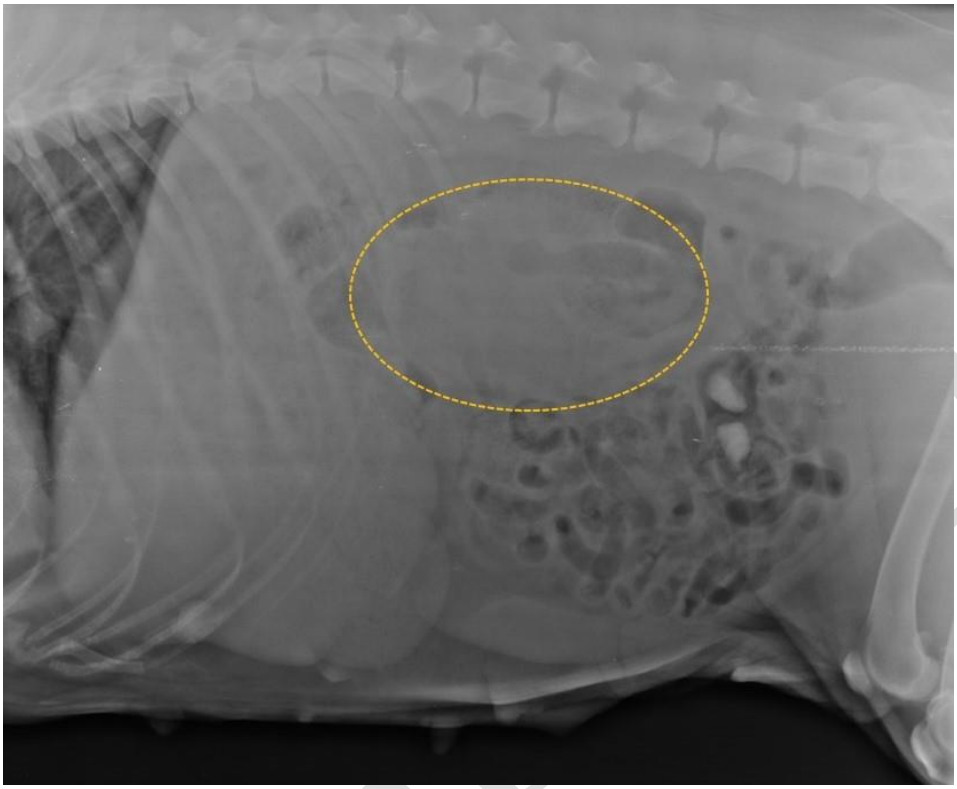

Figure 1. Left lateral X-ray showing the marked size increase of the left kidney (orange arrows) with displacement of intestines and stomach.

Treatment approach

The affected kidney was excised by performing a median laparotomy, the intestines and spleen were displaced to expose the left kidney, then this was dissected from the abdominal roof. The ureter, renal artery, and vein were ligated and sectioned before removing the kidney.

In the macroscopic examination of the left kidney, a mass of 15 × 10 × 8 cm was observed, on the longitudinal section it presented a central cavitation of 4 × 5 cm with yellow serous fluid, the mass had a hard consistency and presented whitish coloration with few reddish foci smaller than 1 cm (Figure 2).

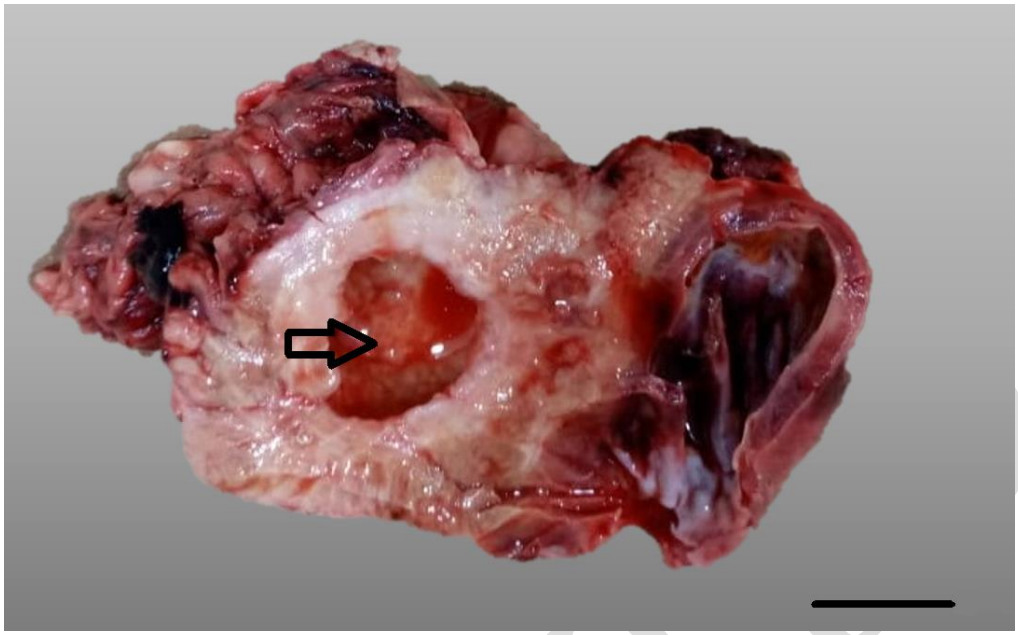

Figure 2. Section of the left kidney in which the cavitation of 3 × 4 cm is evidenced (black arrow), and the deformation is observed with respect to the normal parenchyma. Bar 3 cm.

Histopathology revealed a mixed neoplastic proliferation, with an epithelial component characterized by multiple structures with glomerulus-like morphology, some of this with small diameter and others dilated in Bowman's space (Figure 3). Additionally, several tortuous and small diameters tubules with flattened epithelium were observed. Tissue portions of mesenchymal origin composed of spindle cell tracts with the appearance of connective tissue were evidenced. In some nodular-looking portions of interstitial small groups of round cells, with an approximate diameter of 10-12 µm, and intense basophilic nuclei correspond to undifferentiated blastema cells (Figure 4).

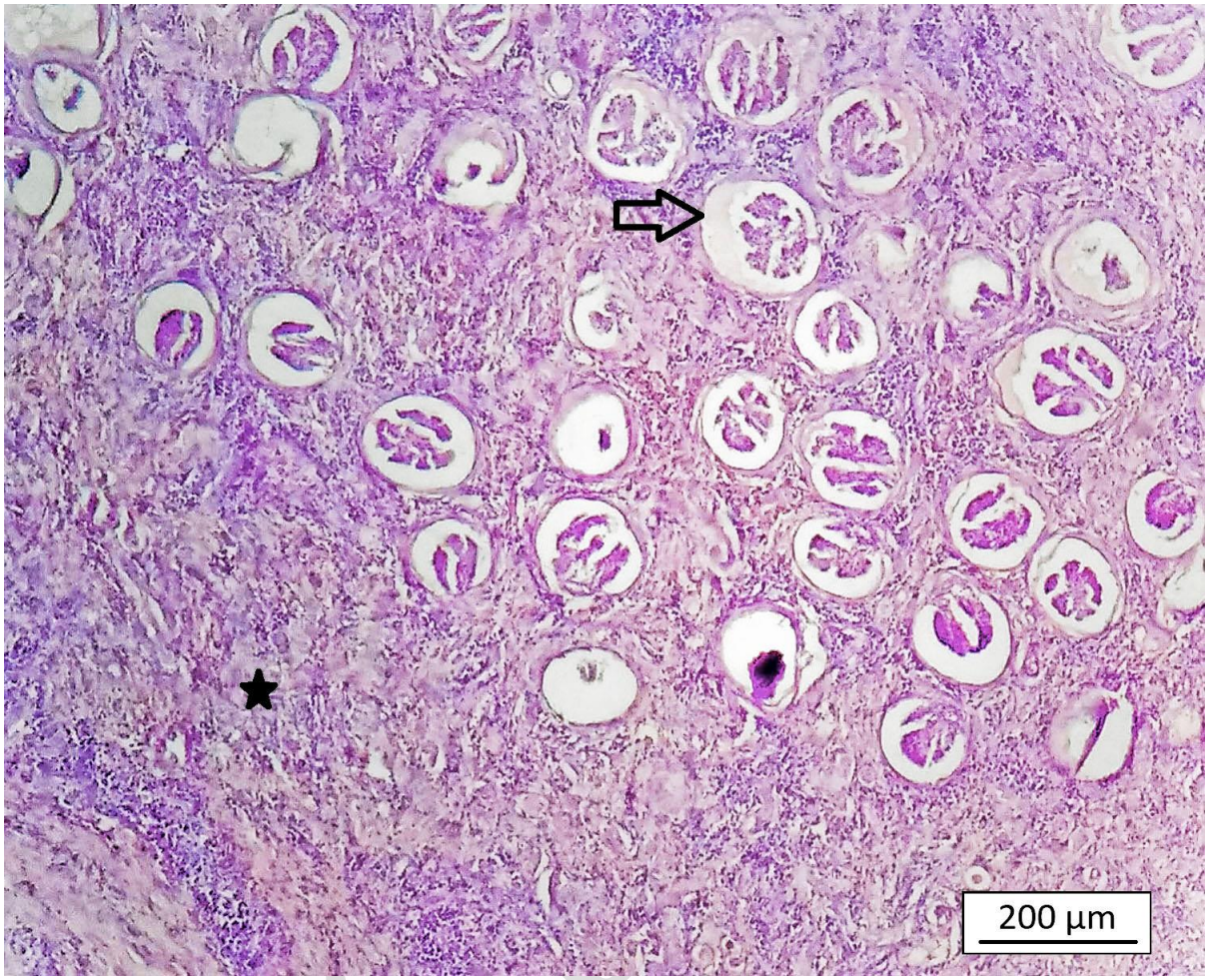

Figure 3. Histopathology H&E. In this neoplasm portion is evident the normal kidney morphology; the glomeruli proliferate (black arrow), separated by connective tissue similar to the portion identified with the asterisk; there are few tubules in this portion. 40x magnification.

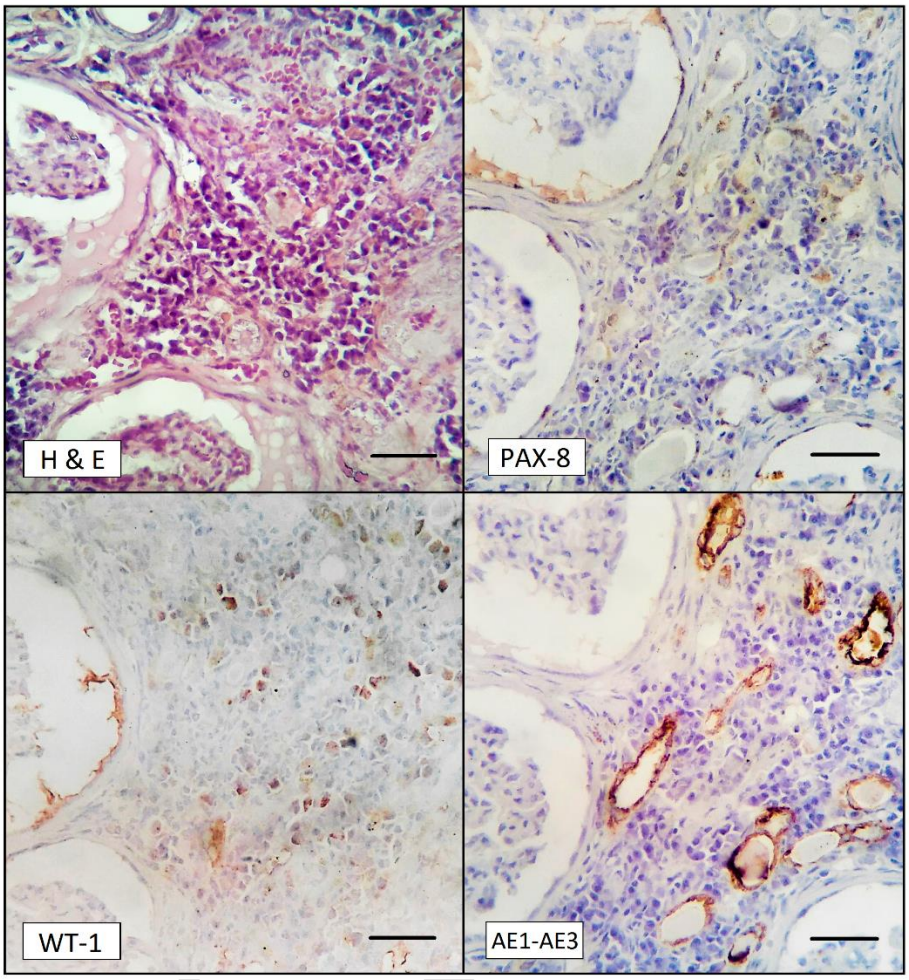

Figure 4. Immunohistochemistry H&E: In this magnification, in the interstitium, the basophilic round-shape blastema cells of 10-12 µm in diameter proliferate, with few tortuous and small diameter tubules. Pax-8: shows mild multifocal immunostaining in tubular epithelial cells and blastema cells. WT-1: mild multifocal immunostaining in blastema cells. AE1-AE3: severe multifocal immunostaining in renal tubule epithelial cells. Bar 50 µm.

Immunohistochemistry, cytokeratin AE1 / AE3, PAX 8 and WT1 were performed in the neoplasm tissue. Immunostaining for Cytokeratins (AE1/AE3), PAX8, and WT1 were performed on tissue-block sections of the kidney with the following methodology based on antibody supplier recommendations. Briefly, the cell-block sections were deparaffinized and rehydrated and then treated with 3% hydrogen peroxide in methanol for 5 minutes to block endogenous peroxidase activity. Tissue sections were incubated for 30 minutes with normal nonimmune serum to eliminate

nonspecific staining. All reagent incubations were at room temperature. Heat-induced epitope retrieval (HIER) with a decloaker was used for all three markers (citrate buffer [pH, 6.0] for Cytokeratins AE1/AE3 and PAX-8; EDTA buffer [pH, 8.0] for WT-1) (Table 1). Visualization of the immunoreaction was achieved using diaminobenzidine tetrahydrochloride and hydrogen peroxide (0.03% in 50 mM Tris-HCl, pH 7.6) for 10 min. Sections were rinsed in tap water, counterstained with hematoxylin, dehydrated through graded alcohol, cleared in xylene, and mounted using DPX.

Table 1. Immunostaining methodology for Cytokeratins (AE1/AE3), PAX8 and WT1 performed on tissue-block sections of kidney

Antibody	Type Antibody	Dilution	Incubation	Pretreatment	Source Antibody
Cytokeratins (AE1/AE3)	Monoclonal-Mouse	1:100	60 min.	HIER- Buffer citrate-pH 6.0	Dako
WT1	Monoclonal-Mouse	1:100	90 min.	EDTA-Buffer-pH 8.0	Dako
PAX-8	Monoclonal-Mouse	1:100	90 min.	HIER- Buffer citrate-pH 6.0	Sigma - Aldrich

HIER: Heat Induced Epitope Retrieval.

WT1 mild immunostaining is evidenced in blastema cells and scarce in epithelial cells; PAX-8 showed moderate immunostaining in cells of the blastema and mild in cells of the renal tubules; Cytokeratin AE1 / AE3 showed severe multifocal immunostaining in epithelial cells and scarce in glomeruli (Figure 4).

Discussion

This case was based on the morphological characteristics, and the use of immunohistochemistry was diagnosed as a mixed-type nephroblastoma due to the triphasic component composed of epithelial, mesenchymal, and blastema-derived cells. Another pattern described in canine nephroblastoma is the blastemal type (Simpson *et al.*, 1992; Chen *et al.*, 2018); other previous

reports do not mention the subtype (Montinaro *et al.,* 2013; Araujo *et al.,* 2020; Richardson 2020). The usual appearance of nephroblastoma in humans is the mixed type, in which the stromal, blastemal, and epithelial portions are proportioned (Al-Hussain and Akhtar 2014), in humans other patterns described are epithelial, stromal, regressive, and anaplastic mainly (Vujanic 2009), a great limitation in dogs with nephroblastoma is related to the fact that it is a rare neoplasm, and it does not allow to relate morphological subtype respect the prognosis (Chen *et al.,* 2018). Additionally, kidney neoplasms are rare in dogs (Bryan *et al.,* 2006). For the previous reasons, this type of neoplasm is limited to case reports, and it is not possible to determine the age of usual presentation; some studies include animals older than 5 years (Bryan *et al.,* 2006; Araujo *et al.,* 2020). Other reports describe this neoplasm in young animals less than 6 months (Baskin and de Paoli, 1977; Montinaro *et al.,* 2013).

In this case, immunohistochemistry was used to confirm the diagnosis due to the rare presentation in dogs and additionally characterize the cell types involved in this neoplasm, demonstrating renal origin of this neoplasm. Cytokeratin AE1-AE3 was used since they are useful in diagnostic pathology, this is called pancytokeratin because it has low and high molecular weight cytokeratins in epithelial cells (Shen *et al.,* 2012). Cytokeratins have already been used in the diagnosis of nephroblastoma, describing marking in the epithelial component (Simpson *et al.,* 1992; Chen *et al.,* 2018). In this case, severe immunostaining in renal tubule epithelial cells was presented.

WT-1 is a transcription factor described in Wilms tumor or nephroblastoma in humans, it is required in kidney development (Kreidberg *et al.,* 1993). In spinal nephroblastoma of dogs, WT-1 was used previously, marking mainly in the cells of the blastema and occasionally in other cells in 9 of 11 cases (Brewer *et al.,* 2011). In this case, mild multifocal immunostaining was evidenced in blastema cells showing the usefulness of this marker in this type of neoplasm in dogs. Pax-8 and Pax-2 are transcription factors that participate in the embryonic development of the kidney (Lechner and Dressler, 1997). Immunostaining in this case with Pax-8 was mild multifocal, especially in epithelial and blastema cells. This marker has already been used in dogs with renal cell carcinoma, showing immunostaining in up to 98% of the cases reviewed (Peat *et al.,* 2017), and this marker is useful in nephroblastoma (Chen *et al.,* 2018).

Based on the SIOP classification, this case is grade II or intermediate risk, in the NWTSG classification it also corresponds to grade II. In humans, this type of classification with complete

resection has a good prognosis even with chemotherapy treatment for a short time (Kaste *et al.*, 2008). In dogs with nephroblastoma, no correlation between histological grades and prognostic is evident (Chen *et al.*, 2018). In humans, surgery is the primary effective treatment with a possible cure in select patients (Kaste *et al.*, 2008). In dogs with renal neoplasms, surgery is the only treatment that improves survival (Bryan *et al.*, 2006). At the date of this report, the patient is still alive with no clinical evidence of metastasis or local recurrence 48 months after nephrectomy, based on radiology and ultrasound examination.

Conclusion

This report describes the usefulness of cytokeratin AE1-AE3, WT-1, and PAX-8 immunohistochemistry in dog nephroblastoma and additionally uses the SIOP, NWTSG classification based on morphology diagnosis (mixed type nephroblastoma). It is necessary to use these classifications in dogs, related to the rare presentation of this neoplasm, and determine possible prognostic indicators in dogs with nephroblastoma for improvement of clinical decisions, early diagnosis, and treatment. No previous reports of this neoplasm in dogs were found in Colombia with the use of complementary immunohistochemistry supporting the final diagnosis.

Declarations

Acknowledgments

The authors express their gratitude to the staff of *Clínica Veterinaria de Pequeños Animales* of *Universidad de Pamplona*.

Funding sources

This case report does not received external funding or any specific grant from funding agencies. This study was funded by the authors.

Conflicts of interest

The authors declare no conflict of interest with respect to the case report, authorship and/or publication of this article.

Author contributions

Clinical examination, diagnosis, surgery, and patient management: Johanna M. Fonseca-Matheus and Xavier L. Jaramillo-Chaustre. Histopathology, literature review, and manuscript writing: Diego F. Rincón-Alarcón. All authors revised the final version.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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