THESIS

Alina Jil Annika Klewicz

2022

Department of Pathology
University of Veterinary Medicine

Canine Renal Nephroblastoma:

A Literature Review and Case Studies

By
Alina Jil Annika Klewicz

Supervisor:
Dr. Anna Szilasi

Budapest, Hungary
2022

Table of Contents

List of Abbreviations

AFP	Alpha-Fetoprotein
α-SMA	Alpha-smooth muscle actin
CBC	Complete blood count
CKs	Cytokeratins
CT	Computed tomography
FFPE	Formalin fixed and paraffin embedded
H&E	Hematoxylin and eosin
HCC	Hepatocellular carcinoma
HPF	High-power field
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
NWTS	National Wilms tumor study
SCC	Squamous cell carcinoma
SIOP	Societe Internationale D'oncologie Pediatrique
TCC	Transitional cell carcinoma

1. Introduction

Primary renal tumors are uncommon in dogs. They cover only 0,6–1,7% of all canine tumors (Seaman & Patton, 2003). A retrospective study of Bryan et al. (2006) observed that most of the primary renal neoplasia are carcinomas and only a minority of cases are nephroblastomas. For this study multiple medical databases from different animal hospitals were collected between 1986 and 2002.

Nephroblastoma is also referred to as Wilms tumor, embryonal nephroma and embryonal adenosarcoma (Meuten & Meuten, 2017).

In the year 1899, the surgeon Max Wilms published his first monography called "Die Mischgeschwülste der Niere". With his eighty-page monography, based on literature review and seven new cases, he wanted to characterize the mixed tumors of the kidney in humans and define their origin. Which lead to the identification of mesoblastic somite, the mesenchyme and the mesonephros blastema as the leading factors of tumor development. Wilms and his publication greatly contributed to the doctrine of tumorigenesis (Raffensperger, 2015).

Nowadays embryonal nephroma is the most diagnosed primary renal tumor in children, chicken, pigs and fish. The origin of it in human as well as in the veterinary medicine is much more detailed. During nephrogenesis nephron units generally develop from the metanephric blastema. If this differentiation does not go as planned the cells undergo apoptosis. A failure to signal wrongly differentiated cells to undergo apoptosis, can then potentially lead to the development of neoplastic tissue. The nephroblastoma is a triphasic tumour consisting of stromal and epithelial cells in different developmental stages as well as metanephric blastema (Meuten & Meuten, 2017).

Because of the physiological function of the kidney to filter a high amount of blood, the nephroblastoma is prone to metastatic activity and often has malignant properties. However not only the kidney itself can be affected, but the spinal cord can also be prone to development of an embryonal nephroma. In some cases, both locations can be involved. From a histopathological viewpoint both variants are the same (Martin et al, 2014).

As the clinical signs are not pathognomonic and very unspecific (Araujo et al., 2019) the diagnosis is often made very late in its development (Martin et al., 2014).

2. Epidemiology

2.1. Incidence

To characterize the incidences of different primary renal neoplasia, Meuten & Meuten (2017) published a summary of several studies. In total 5 different studies were taken into consideration and analysed (see **Figure 1**). According to the findings only 6% of all primary kidney neoplasia were nephroblastoma.

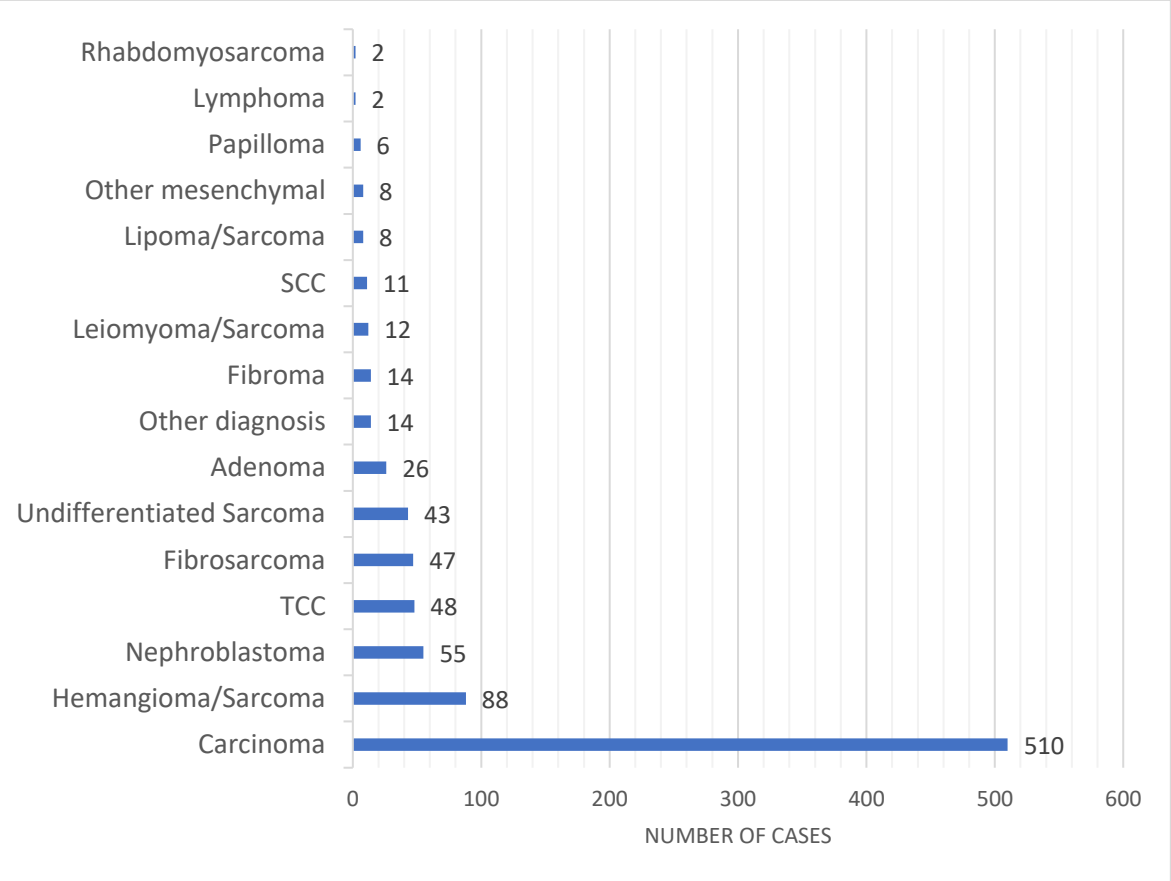

Figure 1: Primary renal neoplasia in dogs (Meuten & Meuten, 2017)

2.2. Age

Although the embryonal nephroma mainly develops during prenatal development, it is often not recognized until it results in clinical manifestation (Meuten & Meuten, 2017).

It is mostly diagnosed in juvenile animals under the age of 2 years nevertheless a few elderly dogs had been reported with malignant embryonal nephroma as well which is shown in **Table 1** (Seaman & Patton, 2003; Martin et al., 2014; Chen et al., 2018; Araujo et al., 2020).

2.3. Breed

Based on case studies, published in veterinary medical literature, nephroblastomas were diagnosed in several small and large breed dogs (see **Table 1**).

However, based on the cases presented in **Table 1**, it is not possible to say unequivocally whether there might be a breed predilection or not, since only 17 cases are included.

Hergt et al. (2019) outlined that large-breed dogs tend to have a predilection for the development of embryonal nephroma.

2.4. Gender

There seems to be no apparent predilection concerning the gender of the dogs, when looking at the 17 cases shown in **Table 1**. Out of these 17 documented cases 8 were male and the remaining 9 were female. But considering further reports Meuten & Meuten (2017) describe a male predisposition.

Table 1: Summary of breed, age and gender of canine renal nephroblastoma in the veterinary literature

Breed	**Age**	**Gender**	**Reference**
Golden Retriever	3 mo	F	Montinaro et al., 2013
Miniature Pinscher	8 y	MC	Chen et al., 2018
Labrador Retriever-Golden Retriever cross	4 mo	F	Chen et al., 2018
Shetland Sheepdog	1 y	F	Chen et al., 2018
Yorkshire Terrier	10 y	M	Chen et al., 2018
Jack Russel Terrier	17 mo	F	Hergt et al., 2017
Shi-Tzu	1 y	F	Lu et al., 1997
Mixed breed	6 y	MC	Araujo et al., 2020
German shepherd	9 y	F	Martin et al., 2014
Labrador Retriever	8 y	MC	Seaman & Patton, 2003
Beagle	8 mo 7 mo	F M	Seaman & Patton, 2003 Takeda et al., 1989
Mixed breed	2 y	FS	Seaman & Patton, 2003
Bernese mountain dog	4 mo	F	Frimberger et al., 1995
Mastiff	8 mo	M	Simpson et al., 1992
American Staffordshire	10 y	M	Recent case – see Chapter 9
Bichon Havanese	5 mo	M	Recent case – see Chapter 9

mo – month, y – years, F – female, M – male, MC – male castrated, FS – female spayed

3. Pathogenesis

It is assumed that the nephroblastoma originates during prenatal development, therefore the embryogenesis plays a central role in the early development of the embryonal nephroma (Chen et al., 2018).

3.1. Embryogenesis

The embryogenesis consists of several stages, the fertilization, cleavage, gastrulation and organogenesis.

3.1.1. Fertilization

During the fertilization, which is the first step of the embryogenesis, a zygote is formed by fusion of a single sperm cell with a single egg cell.

The egg is enclosed by the zona pellucida, which contains glycoproteins and has its purpose in leading the sperm to the egg by releasing chemical-attractors. Underneath is a vitelline layer which separates the egg plasma membrane from the zona pellucida. Once the sperm cell reaches the zona pellucida, the acrosome releases enzymes that help the sperm cell to penetrate the zona pellucida, this process is called the acrosome reaction. Thereupon the species-specific sperm cell binding proteins bind to the species-specific binding receptors of the egg plasma membrane and cause fusion of the sperm and egg plasma membrane. Through a depolarization of the egg plasma membrane a polyspermy can be prevented, this process is referred to as fast block. Hereupon follows the so-called cortical reaction which is triggered by calcium that is released as a result of the depolarization. During the cortical reaction the cortical granules, which are located directly underneath the egg`s plasma membrane, fuse with the egg plasma membrane. Enzymes are released by the cortical granules and cause degradation of the binding receptor proteins. The vitelline layer and the egg's plasma membrane get separated and a fertilization envelope arises, it serves to avoid further sperm cells attaining the egg. This process is also referred to as slow block.

As a result of these steps, the egg gets activated (Georgia Tech Biological Sciences, 2016).

3.1.2. Cleavage

The cleavage is the second stage and starts immediately after the fertilization.

The zygote begins to divide into smaller cells called blastomeres. The course of the cleavage is related to the yolk amount and yolk distribution. Mammals and therefore also canines, have a small amount of yolk, which is also described as oligolecithal, the yolk distribution in these animals is isolecithal. The cleavage in certain animals is also referred to as total

cleavage or holoblastic cleavage. Furthermore, the cleavage in mammals results in blastomeres that are almost equally in size. The within the zona pellucida occurring cleavage, which begins in the oviduct, will finish in the uterus, an early stage of the maturation is the morula. Cell elongation and the formed cavity will then cause the shaping of the next stage, the blastocyst with a fluid filled interior the blastocoel. The later formed chorion- and amnion epithelium develop from the blastocyst, with its outer lining of single trophoblasts and its inner cell mass. From this stage the embryo and its yolk sac will form, as well as the connective tissue of chorion and amnion (Schnorr & Kressin, 2006).

3.1.3. Gastrulation

The following stage is the gastrulation, or also known as the germ layer formation. During this stage the blastula is transformed into a trilaminar structure (Pretzer, 2008). Primary the ectoderm and endoderm develop, which form the outer and inner germ layer. Afterwards, the middle layer, also referred to as the mesoderm, develops between the ectoderm and endoderm (Schnorr & Kressin, 2006).

3.1.4. Organogenesis

Throughout organogenesis the three aforementioned components ectoderm, endoderm and the mesoderm form specific tissues which are as followed:

The endoderm will shape the lining of both gastrointestinal and respiratory tracts.

The ectoderm will convert into neural tissue such as the hypothalamus, and both pituitary lobes as well as the epidermis of the skin. It will also be the base for the development of organs and tissue of reproductive nature such as the mammary glands, the caudal vagina, the vestibule, and the penis or clitoris.

The mesoderm forms the urogenital, circulatory and the muscular skeletal system. Furthermore, gonads and reproductive organs such as the uterus, cervix, cranial vagina, epididymis, and accessory sex glands originate as well from the mesoderm (Pretzer, 2008).

3.1.4.1. Kidney organogenesis

An important factor concerning the genesis of a nephroblastoma is the organogenesis of the kidney (Chen et al., 2018).

For the development of the kidney, the mesoderm first divides into the paraxial, lateral and intermediate mesoderm. The intermediate mesoderm will be the foundation for the three different kidney generations namely the pronephros, mesonephros and the metanephros (Schnorr & Kressin, 2006).

First the pronephros develops and later the mesonephros. Both will regress with time but are essential for the subsequent metanephros (Schnorr & Kressin, 2006; Moritz et al., 2008). The metanephros starts its course of development in parallel with the regression of the mesonephros. Ureter, renal pelvis, renal calices and the collecting tubules are all derived from the ureteric bud. The nephrons later assemble from the metanephrogenic blastema (Schnorr & Kressin, 2006).

Chen et al. (2018) illustrated that remnants of the metanephric blastema are the foundation for nephroblastoma development and thus constitute a defective nephrogenesis. In addition, Chen et al. (2018) described that the components of the nephroblastoma itself, whether they are epithelial, stromal, or blastemal, prove an incomplete transformation of the primitive blastemal cells during nephrogenesis.

4. Diagnosis

The diagnosis of the nephroblastoma proves to be rather difficult, due to the symptoms frequently not being specific. The tumor usually only becomes conspicuous through its expansive growth (Nickel, 2012; Martin et al., 2014).

A physical examination and radiography can help in revealing the presence of an abdominal mass. Subsequently, a laboratory examination together with a diagnostic ultrasound can help to direct the suspicion to the kidneys. The key point for final diagnosis is a histopathological examination (Martin et al., 2014).

4.1. Clinical appearance

The clinical signs can vary greatly and also depend on whether the tumor has metastasized or not. The most common signs illustrated in the veterinary medicine literature, in addition to the abdominal distention, are lethargy, anorexia, emaciation, weight loss, vomiting and haematuria (Martin et al., 2014; Chen et al., 2018). However paraneoplastic polycythaemia, hypoglycaemia, polyuria and polydipsia have also been reported in relation with an embryonal nephroma (Hergt et al., 2019).

Since the nephroblastoma in dogs tend to metastasize in over 50% of the cases (Meuten & Meuten, 2017), extrarenal lesions can also emerge (Araujo et al., 2019).

Chen et al. (2018) describe a case of an eight-year-old castrated Miniature Pinscher where the renal nephroblastoma metastasized to the gingiva and caused a visible swelling. Furthermore, the tumor also commonly metastasizes to the opposite kidney, the lung, the liver, the adrenal, the ovaries, the thymus, the mesentery, the lymph nodes, the thyroid, the spinal cord, and the bone marrow (Martin et al., 2014). In the case of neoplastic involvement of the spinal cord, ataxia and paresis of the hindlimbs can be seen (Nickel, 2012; Araujo et al., 2019).

4.2. Imaging techniques

Nowadays in the practice of veterinary medicine a wide spectrum of imaging techniques is used for diagnostic purpose. The most frequently used in the everyday life of veterinary medicine are the radiography and ultrasonography (Meomartino et al., 2021).

Both of the beforementioned imaging methods can help in identifying the abdominal mass of the embryonal nephroma. (Martin et al., 2014; Chen et al., 2018).

Martin et al. (2014) report a nephroblastoma case of a nine-year-old female German shepherd dog, where radiographic imaging was carried out. An irregular soft tissue density

was seen on the radiography, which took up space in the right abdomen, as well as dislocating the intestinal loops.

During ultrasound examination the tumor mass often appears mixed in echogenicity (Seaman & Patton, 2003; Martin et al., 2014) and in some cases even a cystic or vascular formation has been observed (Martin et al., 2014; Araujo et al., 2020).

Additional diagnostic imaging techniques that might be useful in diagnosing an embryonal nephroma include the excretory urography and computed tomography (CT) (Araujo et al., 2020). During CT scans Montinaro et al. (2013) describe that the tumor mass portrays signs of mixed fluid accumulation and soft tissue density with mild peripheral contrast enhancement. In comparison with normal renal parenchyma the mass shows decreased radiodensity.

4.3. Laboratory examinations

Neoplastic diseases often lead to changes in laboratory values as a result of organ changes or as part of a paraneoplastic syndrome (Moritz, 2012). Several laboratory methods, such as the following: complete blood count (CBC), serum biochemical analysis, urinalysis, urine culture, coagulation panel, fine needle aspiration have been described in veterinary literature to help aid in the diagnosis of nephroblastoma (Seaman & Patton, 2003).

In **Table 2**, five different embryonal nephroma cases can be observed and their respective laboratory results. It has been shown that the majority of the cases reported in **Table 2**, suffered from neutrophilia. In two cases, in addition to the neutrophilia, a microscopic haematuria could be revealed.

Table 2: Abnormal laboratory results of five different nephroblastoma cases

Patient data	Laboratory examinations which had been carried out with their revealing abnormal results	Reference
6-year-old, mixed breed, uncastrated, male dog	**CBC:** lymphopenia **Serum biochemical analysis:** unremarkable	Araujo et al., 2020
9-year-old, German shepherd, intact, female dog	**CBC:** leucocytosis, neutrophilia, **Erythrogram:** the erythrogram revealed anaemia, a low haemoglobin level, a decrease in the volume of packed red blood cells and an increase in erythrocyte sedimentation rate **Serum biochemistry analysis:** Mild increase in total bilirubin, increase in indirect bilirubin, mild increase in alkaline phosphatase, hyperproteinaemia, hyperglobulinaemia **Urinalysis:** proteinuria and microscopic haematuria	Martin et al., 2014
8-year-old, Labrador retriever, neutered, male dog	**CBC:** mature neutrophilia **Serum biochemistry analysis:** hypoglycaemia, increased alkaline phosphatase activity **Urinalysis:** microscopic haematuria	Seaman & Patton, 2003
3-month-old, Golden retriever, intact, female dog	**CBC:** increased neutrophil, lymphocyte and monocyte count **Serum biochemistry analysis:** unremarkable **Urinalysis:** unremarkable	Montinaro et al., 2013
17-month-old, Jack Russell terrier, intact, female dog	**CBC:** erythrocytosis was revealed with an increased red blood cell count, increased haemoglobin, increased haematocrit **Serum biochemistry analysis:** slight hypoglycaemia **Urinalysis:** low urine specific gravity	Hergt et al., 2019

4.3.1. Alpha-Fetoprotein

The alpha-fetoprotein (AFP) belongs to the oncofetal proteins and is found to be prominent in fetal and maternal blood, but also appears in high levels in the blood of patients suffering from particular neoplastic or non-neoplastic diseases (Soltani, 1997).

In human medicine, the AFP is often used for the early diagnosis of hepatocellular carcinomas (HCC), not only in humans but also in dogs, the AFP is remarkably elevated in cases of HCC (Kitao et al., 2006). However, the AFP can also be used to aid in diagnosing a canine nephroblastoma. In the previously described case of the 9-year-old female German shepherd by Martin et al. (2014) the AFP was evaluated and it was found to be increased with a value of 0.89 ng/mL while its reference range is <70 ng/mL.

4.4. Microscopic examinations

4.4.1. Histopathology

The gold standard for a definite diagnosis is a histopathologic examination of the tumor tissue (Nelson et al., 2019).

For the implementation of such an examination, the tissue sample is preserved in a 10% (Martin et al., 2014; Chen et al., 2018) or 20% buffered formaldehyde solution (Araujo et al., 2020) and furthermore cut in sections of 5 μm (Chen et al., 2018). For the staining hematoxylin and eosin (H&E) should be used (Simpson et al., 1992; Lu et al., 1997; Seaman & Patton, 2003; Montinaro et al., 2013; Martin et al., 2014; Chen et al., 2018).

Under microscopic evaluation a mixture of embryonal epithelia encompassing tubules and glomeruli in several stages of differentiation, blastema and mesenchymal tissue can be identified. Even though the predominant cell type is the blastema cells, which are present throughout the whole tumor, they are of no diagnostic value alone. The most helpful concerning a diagnosis are the embryonic glomeruli. These are formed by tufts of epithelium invaginating into a lumen to arrange such glomeruloid structures. Cross-sectioned tufts show a solid mass of cells at the centre of the lumen. So called “Naked nuclei” cells, meaning they have little to no identifiable cytoplasm, line the space and produce a rim. During further examination, with special focus on the area around an embryonic glomerulus, it can be noticed that the glomeruli are encased by irregular tubules with varying sizes of lumens. Cystic structures can be visible, but in most cases to a lesser extent. They are mostly lined by either cuboidal epithelium or squamous epithelium. Mucus, sloughed epithelium or keratin can be present but is not a requisite. The mesenchymal tissue can be described as being loose and areolar as well as myxomatous. In addition, the colour of the mesenchymal tissue is identified as light basophilic (Meuten & Meuten, 2017).

Supplementary to the aspects mentioned above, sometimes necrosis and hemorrhages can be detected as well (Lu et al., 1997).

4.4.2. Immunohistochemistry

With immunohistochemistry (IHC) specific antigens can be identified on the basis of specific bindings between antibody and antigen. Nowadays, IHC is an important component in the field of research and it is also commonly used to help in the classification of neoplasms. Frequently formalin fixed and paraffin embedded (FFPE) tissue samples are used to perform an IHC (Magaki et al., 2019).

Grieco et al. (2006) conducted a study in which they immunohistochemically examined five different porcine nephroblastoma cases. For the preparation the samples were fixed in Carnoy´s fluid. For the examination they used antibodies against vimentin, cytokeratins (CKs), alpha-smooth muscle actin, Factor VIII and laminin.

Vimentin is an intermediate filament protein whose function is to maintain cell integrity. It is considered as a biomarker for cell and tissue development and is frequently expressed in tumors and mesenchymal cells. It serves as a marker for cellular conditions such as inflammation and also for malignant and autoimmune disorders (Pandita et al., 2021).

CKs also belong to the intermediate filaments and can be found in the intracytoplasmic cytoskeleton of epithelial tissue. There are several different CKs that exist. Each of them differs in its expression in the epithelium, therefore with their help different types of epithelial cells can be distinguished which can be useful for classifying tumors.

Furthermore, CKs can be divided into groups of either high- or low-molecular-weight. CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, and CK9 belong to the high-molecular-weight CKs, whereas CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19, and CK20 compromise the low-molecular-weight CKs. Their expression has been found out to be organ specific (Kumar & Jagannathan, 2022).

Alpha-smooth muscle actin (α-SMA) is an actin isoform. It plays an essential role in cell-generated mechanical tension and even though it is mostly confined to vascular smooth muscle cells, it is also found to be expressed in some non-muscle cells like myofibroblasts (Wang et al., 2006).

Coagulation factor VIII, also referred to as anti-hemophilic factor A, belongs to the glycoproteins and is regarded as one of the largest coagulation factors. It is synthesized mostly in hepatocytes as well as in the kidneys, endothelial cells and in the lymphatic tissue (Mazurkiewicz-Pisarek et al., 2016).

Laminins, which also belong to the family of glycoproteins, are major components of the basement membranes. They have their ability in regulating numerous cellular activities and signalling pathways (Aumailley, 2013).

As a result of the study from Grieco et al. (2006) it has been shown that the mesenchymal blastemal cells were positive for vimentin and the stromal cells were positive for vimentin and α-SMA, which indicates myofibroblastic differentiation. All other biomarkers were found to be negative for the mesenchymal blastemal and stromal cells.
The tubuli were all positive for CK19. In addition to the CK19 a few of the tubules showed positive labelling for anti-vimentin, anti-CKs 8-18 and anti-CK AE1/AE3 as well. The last one is also referred to as pancytokeratin, which is a combination of two different clones of anti-CK monoclonal antibodies, that have a broad spectrum since they show reactivity against both high and low molecular weight CKs (Miller, 2003). Furthermore, some tubuli exhibited a strong expression of CK7, these tubuli were found in the stromal septa and were assumed to represent ureteric bud branches. The majority of the tubuli and glomeruli were positive for laminin. The parietal layer of the glomeruli expressed vimentin and half of the parietal epithelial cells supplementary expressed CK19. The visceral layer was positive for vimentin, any CK expression was absent. Some blood vessels, located in the stromal septa, were positive for Factor VIII in addition to α-SMA (Grieco et al., 2006).

In some canine nephroblastoma cases found in the veterinary literature, an immunohistochemical examination has been described (Simpson et al., 1992; Lu et al., 1997).
Simpson et al. (1992) used for an IHC examination of an 8-month-old male Mastiff dog nephroblastoma case the following antibodies: mouse monoclonal anti-porcine vimentin, mouse monoclonal anti-human cytokeratin and mouse monoclonal anti-porcine desmin. Desmin is a cytoskeletal protein and like vimentin and α-SMA, it is expressed by myofibroblasts (Gheissari et al., 2012). The results of the examination of Simpson et al. (1992) revealed, that the blastema cells and stromal spindle cells both expressed vimentin and desmin, but were negative for CKs. The primitive tubuli on the other hand showed only cytokeratin expression and did not express vimentin or desmin. Simpson et al. (1992) also mentioned another case in which the blastema has been positive for vimentin and were the tubuli expressed cytokeratin.

As a conclusion the canine nephroblastoma immunohistochemical results are partly comparable to the porcine nephroblastoma study of Grieco et al. (2006).

4.4.3. Cytopathology

A cytological examination shows a mononuclear cell tumor that often reminds of a lymphoma. Nevertheless, the nephroblastoma can be distinguished from a lymphoma by cells that adhere to each other and form clusters. In the case of a lymphoma, the cells are usually individual.

Another difference that helps to differentiate both tumors based on cytological evaluation, are the exfoliated cells. In the case of a lymphoma these are stationary monomorphic, but in the case of a nephroblastoma they can be monomorphic as well as bimorphic.

The nuclei are often irregular in shape and are large. They have immature vesicular chromatin and fill most of the cytoplasm (Meuten & Meuten, 2017).

5. Prognostic factors

A prognosis can be made based on the evaluation of the histopathological characteristics and by determining the clinical stage (Chen et al., 2018).

5.1. Favourable histopathology

The histopathological features are considered favourable if the glomeruli and tubuli demonstrate differentiation (Chen et al., 2018).

5.2. Unfavourable histopathology

In the case of anaplasia and sarcomatous stroma the histopathology is regarded as unfavourable with a poor prognosis (Chen et al., 2018). Criteria that determine an anaplasia are, on the one hand, the occurrence of atypical multipolar mitotic figures and, on the other hand, nuclei that are remarkably enlarged and exhibit hyperchromasia (Popov et al., 2016).

5.3. Staging

There are two important staging systems in human medicine, the national Wilms tumor study (NWTS) staging system and the Societe Internationale D'oncologie Pediatrique (SIOP) staging system, which can be seen in **Tables 3** and **4**.

The former system is based on surgical and pathological evaluation without a preoperative chemotherapy, the SIOP staging system on the other hand is based on results that have been obtained at post-chemotherapy tumor nephrectomy (Erginel, 2016).

There are a few canine nephroblastoma cases described in the veterinary literature that have performed staging using the NWTS staging system. Dogs diagnosed with an advanced stage of nephroblastoma had a poor prognosis and shorter life expectancy than canine nephroblastoma cases diagnosed as stage 1 (Seaman & Patton, 2003; Montinaro et al., 2013; Martin et al., 2014; Araujo et al., 2020). For instance, in the year 2014, Martin et al. described a case of a stage 3 nephroblastoma with unfavourable histopathology. Despite of that the dog went for surgery and received adjuvant chemotherapy, he died within a few days. On the contrary Araujo et al. (2020) reported a case of a stage 1 canine embryonal nephroma with favourable histopathology, that survived for 33 months without receiving adjuvant therapy following nephrectomy.

Table 3: The NWTS staging system (Erginel, 2016)

Stage 1	a) Tumor is limited to the kidney and can be completely excised b) The tumor is not ruptured before or during surgery c) The vessels of the renal sinus are not involved beyond 2 mm d) No residual tumor is apparent beyond the margins of excision
Stage 2	a) Tumor extends beyond the kidney but can be completely excised b) There is no residual tumor apparent at or beyond the margins of excision c) If there is a tumor thrombus in vessels outside the kidney it is considered as a stage II nephroblastoma if the thrombus can be removed en bloc with the tumor
Stage 3	Residual non-hematogenous tumor is present and confined to abdomen a) Lymph nodes in the renal hilum, the periaortic chains, or beyond are found to contain a tumor b) Diffuse peritoneal contamination by the tumor c) Implants are found on the peritoneal surfaces d) Tumor extends beyond the surgical margins either seen microscopically or grossly e) Tumor cannot be completely resected by reason of local infiltration into vital structures
Stage 4	Hematogenous metastasis or lymph node metastasis
Stage5	Bilateral renal involvement

Table 4: The SIOP staging system (Erginel, 2016)

Stage 1	a) Tumor is limited to kidney and can be completely resected b) The tumor may be protruding into the pelvic system and "dipping" into the ureter, but is not infiltrating their walls c) The vessels of the renal sinus show no involvement d) Intrarenal vessel involvement may be present
Stage 2	a) The tumor extends beyond the kidney or penetrates through the renal capsule and/or the fibrous pseudocapsule into the perirenal fat but can be completely resected b) The tumor infiltrates the renal sinus and/or invades blood vessels and lymphatic vessels outside the renal parenchyma but can be completely resected c) The tumor is infiltrating adjacent organs or is infiltrating the vena cava but can be completely resected
Stage 3	a) Incomplete excision of the tumor, which extends beyond the resection margins b) Any abdominal lymph nodes show involvement c) Tumor rupture before or during surgery d) The tumor shows peritoneal penetration e) Tumor thrombi are present at resection margins of vessels or ureter f) The tumor was surgically biopsied before preoperative chemotherapy or surgery
Stage 4	Hematogenous metastases or lymph node metastases outside of the abdominopelvic region
Stage 5	Bilateral renal involvement

6. Treatment

The treatment in dogs is based on the recommendation of the National Wilms Tumor Study Group, which established a treatment regimen for humans in regard of the tumor stage and its histological characteristics (Martin et al., 2014).

In general, the primary treatment of an embryonal nephroma is the nephrectomy, except in the case of bilateral kidney involvement (Seaman & Patton, 2003).

Furthermore, other treatment options such as chemotherapy or radiotherapy are also used depending on the stage. As an example, for humans that are diagnosed with a stage 2 or higher nephroblastoma a chemotherapy with vincristine or actinomycin D is recommended. In case of stage 3 tumors if there is evidence of a microscopic or macroscopic disease at the site of the surgery a radiotherapy should be implemented (Montinaro et al., 2013).

6.1. Surgical treatment

Surgery not only serves as the basis for determining the tumor stage, but is also the foundation of the treatment (Erginel, 2016). According to the extent, a partial or complete nephrectomy is performed during the surgical resection of the tumor mass (Martin et al., 2014).

6.1.1. Nephrectomy

Nephrectomies have been performed on dogs for centuries, as dogs were previously used experimentally to practice and research the process of nephrectomy before it was officially performed on humans. For instance, in the year 1803 the physiologist Joseph Nicholas Comhaire performed experimental unilateral nephrectomies on a total of 65 dogs. 63 of the dogs died while 2 dogs survived. Only about 66 years later, the first successful nephrectomy has been performed in humans (Goddard & Birks, 2016).

6.1.1.1. Partial nephrectomy

Although partial nephrectomy is commonly performed in human medicine, it is rarely used in veterinary medicine as a preferred method. However, in cases where a unilateral nephrectomy has already been performed beforehand, a partial nephrectomy can be quite helpful. Likewise, if there is a reduced glomerular filtration rate in the remaining kidney or if renal insufficiency is suspected, a partial nephrectomy may be preferable to a complete nephrectomy (Johnston & Tobias, 2018).

There are two different methods to complete a partial nephrectomy, either using open surgery or using laparoscopy (Shariati et al., 2016).

During open surgery the access to the kidney is achieved through a ventral midline incision. For the further course of the surgery the kidney must be detached from its retroperitoneal attachments. It is important to occlude the vascular pedicle temporary. The part of the kidney to be removed must be identified accurately and is thereafter excised by blunt dissection. With the help of overlapping mattress sutures going through both capsule and parenchyma the parenchymal defect is closed (Johnston & Tobias, 2018).

6.1.1.2. Complete nephrectomy

A complete nephrectomy is the treatment of choice in the case of canine nephroblastoma (Martin et al., 2014)

For this kind of surgery, it is important to know in advance that the risk of leaving the kidney inside the body is much greater than the surgery itself and in addition the surgeon needs to make sure that the remaining kidney will be able to keep the patient alive. As in the case of partial nephrectomy, a ventral midline incision is made for the surgical approach. After opening the abdomen, it is crucial that the abdomen is closely inspected and both kidneys are located to ensure that the dog has two kidneys and that the other kidney, which will remain inside the body, shows no gross abnormalities (Johnston & Tobias, 2018).

To perform a nephrectomy a good view of the kidney is required, which can be obtained by retracting the intestines to the opposing side. Furthermore, the kidney should be freed from its retroperitoneal attachment and the renal vein and renal artery should be identified, a ventromedial rotation of the kidneys can help localize them more easily. Thereupon the vessels need to be properly ligated. In general, in the case of a neoplastic change in the kidney it can be of theoretical advantage to ligate the renal vein first before ligating the renal artery. As a result, the arterial flow into the kidney is continued, but no blood escapes from the kidney that could contain potential neoplastic cells. However, from a practical point of view, many surgeons prefer to ligate the renal artery first instead of the renal vein, as this avoids the accumulation of blood in the kidney and the associated risk of pressure rise in the kidney. In addition, by ligating the renal artery first, the blood flow from the kidney will be greatly reduced, which is why the probability that potentially neoplastic cells in the blood exiting the kidney also decreases. After transecting the vessels, the kidney is freed from the remaining retroperitoneal attachments. The renal fossa should be examined for any bleeding, if any is present it should be stopped. Subsequently the ureter is easily dissected down to the bladder and is ligated close to the bladder, followed by transection of it. Before closing the

surgical incision, the renal fossa needs to be examined again and should be lavaged with warm saline (Johnston & Tobias, 2018).

6.1.1.3. Ureteronephrectomy

During a ureteronephrectomy, the kidney and its associated ureter are both removed (Johnston & Tobias, 2018). In the case of kidney tumors, it is highly recommended to perform a ureteronephrectomy, as the tumor can infiltrate the ureter (Schmidt & Findji, 2022).

Not only a possible infiltration of the ureter can be a reason for a it, but a distal ureteral remnant can also be the cause for urinary tract infections as it permits an urinary reflux. Therefore, many surgeons prefer a ureteronephrectomy instead of a nephrectomy alone (Johnston & Tobias, 2018).

6.2. Chemotherapy

The use of chemotherapeutic agents in veterinary medicine was first described in 1946. The report dealt with a dog suffering from a haematopoietic neoplasm which was treated with urethane (Gardner et al., 2016). Nowadays the chemotherapeutic treatment in case of neoplastic diseases in the veterinary medicine is widely distributed and especially in the last few years such treatment has advanced remarkably (Stephens, 2019).

In the veterinary medical literature various chemotherapeutic agents have been used in the treatment of canine nephroblastoma. The five agents which were described were as follows (Chen et al., 2018):

- Vincristine
- Doxorubicin
- Actinomycin D
- Mithramycin
- Cyclophosphamide

6.2.1. Vincristine

Vincristine is derived from the periwinkle plant and belongs to the vinca alkaloids, which are organic compounds consisting of carbon, hydrogen, nitrogen and oxygen. During its mechanism of action, it binds to the protein tubulin and thus inhibits the formation of microtubules which subsequently leads to the arrest of mitosis at the metaphase due to the disruption of mitotic spindle formation. Most importantly during the M and S phases. An

additional mechanism of action is the blocking of glutamic acid utilization and thus interfering with nucleic acid and protein synthesis (Below & Das, 2022).

Treatment with vincristine alone is not common and has not been described in any of the canine nephroblastoma cases found in the veterinary literature (Seaman & Patton, 2003; Montinaro et al., 2013; Martin et al., 2014; Chen et al., 2018). It has been used either in combination with doxorubicin alone (Seaman & Patton, 2003) or in combination with doxorubicin and actinomycin D (Montinaro et al., 2013; Martin et al., 2014). Furthermore, Chen et al. (2018) described a case where vincristine was used in combination with cyclophosphamide.

Its administration should be intravenously, and it is recommended as a 5 to 10-minutes infusion (Below & Das, 2022).

One of the side effects of vincristine in pet animals can be an ileus. It usually occurs 7–14 days after its administration. The symptoms can include general signs such as vomiting and decreased appetite, as well as irregular or absent bowel movements, restlessness or downward dog stretching. Only a few patients suffering from ileus do require hospitalization, as in the majority of affected animals it is self-limiting and can be treated well with the administration of oral drugs (Fullerton, 2018).

6.2.2. Doxorubicin

Doxorubicin is a commonly used and efficient chemotherapeutic drug in the field of veterinary oncology, nonetheless it is also considered as one of the most dangerous ones. It is derived from the streptomyces yeast and belongs to the anthracycline antitumor antibiotic class of chemotherapeutics (Fullerton, 2017).

It is still to be investigated what the exact mechanism of action of doxorubicin is as it is quite complex. By interacting with DNA by intercalation it manages to inhibit macromolecular biosynthesis. This has the effect of inhibiting the enzyme topoisomerase II, and relaxing the supercoils in the DNA for transcription. Doxorubicin stabilizes the Topoisomerase II complex following its breaking of the DNA chain, which has the effect of preventing the double helix of resealing and stopping the replication process. In addition to the before mentioned, doxorubicin furthermore produces free radicals which damage both cell membranes and DNA itself (Rivankar, 2014).

Its administration should be intravenously as a slow infusion, which is recommended to last about 15–20 minutes (Fullerton, 2017).

Furthermore, the use of doxorubicin as a treatment can come along with severe side effects

which include bone marrow suppression, myocardial toxicity and gastrointestinal signs such as nausea, vomiting and diarrhoea (Lori et al., 2010).

The side effects of doxorubicin affecting the heart are both cumulative and irreversible. They can occur regardless of treatment length and especially if administered too quickly. Before the administration of doxorubicin, it may be helpful to perform a cardiologic examination, which includes tests such as echocardiography and electrocardiogram to illustrate any myocardial dysfunction. This is especially useful in breeds that are predisposed to dilated cardiomyopathy in order to reduce the risks of cardiac side effects (Fullerton, 2017).

6.2.3. Actinomycin D

Actinomycin D which is also referred to as dactinomycin is derived from the *Streptomyces parvulus* (Pet Cancer Society, n. d.). It has not only been used in veterinary medicine as a treatment for embryonic nephroma (Montinaro et al., 2013; Martin et al., 2014), it is also commonly used in human medicine as a chemotherapeutic agent for nephroblastoma (Pet Cancer Society, n.d.).

Actinomycin is a cytotoxic agent and acts by binding to DNA and inhibiting the DNA-dependent RNA synthesis. It breaks single-stranded DNA and therefore damages the DNA. As a reason of these events, it causes cell division to be inhibited and helps to stop cancer growth. It can either be administered intravenously as an infusion or it can be directly injected into a specific organ where the tumor is located (Pet Cancer Society, n.d.).

In 1994, Hammer et al. described a study in which 50 dogs with various advanced malignancies were treated with actinomycin D. They not only reported which tumors responded to actinomycin D, but also which side effects occurred and to what extent. Gastrointestinal and hematologic toxicity have been observed, whereas gastrointestinal toxicity was the most common and hematologic toxicity occurred only in a few cases. Furthermore, one of the dogs developed a hypersensitivity reaction and consequently suffered from pruritus and hives. Hammer et al. also described an earlier case in which actinomycin D was used as a treatment, together with surgery and radiotherapy, for a canine nephroblastoma and led to pancytopenia in addition to diarrhoea. Nowadays, further side effects such as mouth ulcers, hair loss, liver disorders, infections, and muscle pain have also been illustrated in connection with the use of actinomycin D (Pet Cancer Society, n.d.).

6.2.4. Mithramycin

Mithramycin, also known as plicamycin belongs to the antibiotics of the aureolic acid group and has antineoplastic properties (Rosol et al., 1994). It is derived from the *Streptomyces argillaceus*, *Streptomyces tanashiensis* and *Streptomyces plicatus* (Schweer et al., 2021). Mithramycin acts by inhibiting the initiation of the RNA-synthesis through binding to the R-C rich regions of the DNA (Rosol et al., 1992). Independently of the tumoricidal activity of Mithramycin, it also has a calcium-inhibition effect. The administration of it is described to be intravenously as an infusion. Side effects that have been reported in dogs in connection with the administration of Mithramycin include gastrointestinal toxicity and hematologic toxicity (Rosol, 1994). Chen et al. (2018) portray a stage 2 canine nephroblastoma case with unfavourable histopathology, in which treatment with mithramycin was carried out in addition to nephrectomy. However, it is not explained and is therefore unclear whether mithramycin was used primarily due to its antineoplastic properties or because of its calcium-lowering effects for example assumed due to neoplasia-associated hypercalcemia.

6.2.5. Cyclophosphamide

Cyclophosphamide has cytotoxic properties and belongs to the alkylating agents (Norris & Withrow, 1984). It is a non-cell-cycle phase-specific drug and acts by crosslinking RNA and DNA and therefore inhibiting the protein synthesis (Ogino & Tadi, 2022). It is activated by hepatic enzymes and for this reason it should not be used in animals suffering from liver insufficiency (Norris & Withrow, 1984).

Cyclophosphamide is either administered orally in form of a capsule or liquid, or it can be given as an injection. In general, in case it is administered per os, it should be given together with food (Gollakner, n.d.). Its administration shows a relatively low toxicity, including sterile haemorrhagic cystitis and bone marrow suppression (Lori et al., 2010). Nevertheless, Gollakner (n.d.) describes further side effects such as alopecia, lack of appetite, vomiting, diarrhoea, depression, difficulty in breathing, dizziness, seizures, and tremors. Cyclophosphamide has not only been used in veterinary medicine as a treatment (Chen et al., 2018) but is also frequently used in human medicine, together with other chemotherapeutics, in case of relapsed Wilms tumor (Erginel, 2016).

6.2.6. Ancillary medications

The administration of ancillary medication can help to inhibit or even avoid the occurrence of serious side effects. Before administering doxorubicin, it is usually recommended to give drugs which have an antiemetic effect such as maropitant, ondansetron or butorphanol. In

the case of administration of vincristine, however, premedication with metronidazole is recommended (Fullerton, 2018).

In a stage 1 nephroblastoma case with unfavourable histopathology described by Seaman and Patton (2003), diphenhydramine was used as a pre-treatment. Seaman & Patton (2003) mentioned that 2mg/kg body weight were injected intramuscularly once 20 minutes before each chemotherapeutic treatment with vincristine and doxorubicin. Diphenhydramine belongs to the H1 receptor antagonists and has antihistaminic and anticholinergic properties (Hofmeister & Egger, 2005).

6.3. Radiotherapy

Intra-abdominal radiotherapy is recommended for stage 3 and stage 4 tumors and furthermore also in the case of stage 2 tumors that show unfavourable histopathologic characteristics (Seaman & Patton, 2003). Montinaro et al. (2013) illustrate a case that was identified as stage 2 canine embryonal nephroma with unfavourable histopathology and was treated by intra-abdominal radiotherapy in addition to nephrectomy and chemotherapy. The dog, however, developed metastases within 8.5 months and had to be euthanized 15 months after the diagnosis.

7. Survival time

The survival time is related to an adequate treatment, without such a treatment a long survival time can usually not be achieved (Hergt et al., 2019).

Based on the prognostic factors, which were previously described in chapter 5, an approximate life expectancy can be given. However, the actual survival times can vary greatly, as demonstrated in **Table 5**. In the cases described in Table 5, a survival time over 2 years could only be reached in some of the stage 1 nephroblastoma cases with nephrectomy as a selected treatment option. None of the other cases included in Table 5 survived more than 2 years. In the two stage 4 nephroblastoma cases illustrated, it is visible that these differ greatly in terms of survival time. Despite favourable histopathology, one case did not survive longer than 4 months while the other case with an unfavourable histopathology survived for 10 months. Accompanying symptoms are probably also decisive as the stage 4 nephroblastoma case with favourable histopathology, described by Chen et al. (2018), showed gingival metastases.

The average time of survival decreases sharply with regard to the respective staging. The mean value of survival time for cases considered as stage 1 in Table 5 is about 24 months while the stage 4 nephroblastoma cases included in Table 5 only showed an average survival time of about 7 months. In addition, it can also be clearly seen that the cases with a favourable histopathology described in table 5 had an average survival time of 17.5 month whereas the cases with unfavourable histopathological characteristics only survived on average for about 10 months.

Johnston & Tobias (2018) report a study that included a total of 82 dogs suffering from a primary renal neoplasm. The study determined the median survival times for dogs with carcinomas, dogs with sarcomas and dogs with nephroblastoma. In the study, dogs with nephroblastoma proved to have a significantly lower median survival time than dogs with carcinomas and sarcomas. The median survival time for nephroblastomas in this study was 6 months, but 12 months for carcinomas and 9 months for sarcomas.

In summary the survival time depends on many factors, such as the tumor stage, the histological characteristics, the selected therapy and the accompanying symptoms of the tumor itself (see **Table 5**).

Table 5: Reported survival times in canine nephroblastoma cases with the associated tumor stage, histopathology, and treatment.

Patient age	Stage	Histopathology	Treatment	Survival time	References
17 mo	1	Fav	Nx	>30 mo	Hergt et al., 2019
6 y	1	Fav	Nx	33 mo	Araujo et al., 2014
8 y	1	Unfav	Nx, Vincristine, Doxorubicin	>24 mo	Seaman & Patton, 2003
8 mo	1	Unfav	Nx	>8 mo	Chen et al., 2018
10 y	2	Fav	Nx	>15 mo	Chen et al., 2018
3 mo	2	Fav	Nx	>19 mo	Montinaro et al., 2013
2 y	2	Unfav	Nx, Actinomycin D, Intra-abdominal radiotherapy	15 mo	Montinaro et al., 2013
8 mo	2	Unfav	Nx, Mithramycin	>1,5 mo	Chen et al., 2018
4 mo	3	Fav	Nx, Vincristine Actinomycin D, Doxorubicin	4 mo	Montinaro et al., 2013
9 y	3	Unfav	Nx, Vincristine, Actinomycin D, Doxorubicin	11 days	Martin et al., 2014
8 y	4	Fav	Nx	<4 mo	Chen et al., 2018
8 y	4	Unfav	Vincristine, Cyclophosphamide	10 mo	Chen et al., 2018

Fav – favorable, mo – months, Nx – Nephrectomy, Unfav – unfavorable, y – years

8. Extrarenal nephroblastoma

A nephroblastoma can also occur extrarenally in dogs. Extrarenal nephroblastomas are frequently found to be localized in the spinal cord (Schmidt & Findji, 2022).

8.1. Spinal cord nephroblastoma

A canine spinal cord nephroblastoma can occur independently of the occurrence of a renal nephroblastoma (Brewer et al., 2011). The assumption for the origin of such kind of a tumor is that ectopic renal tissue gets entrapped in the dura mater during embryogenesis, this assumption is reinforced by the diagnostic aspects such as the clinical features, the histological characteristics and immunohistochemistry staining methods (Terrell et al., 2000). Extrarenal nephroblastomas usually are non-invasive and show low metastatic activity (Schmidt & Findji, 2022).

A canine spinal cord nephroblastoma was first described in 1984 and is a rare canine tumor (Brewer et al., 2011). Although larger dog breeds such as the German Shepherd or Retriever appear to be more predisposed to this tumor, spinal cord nephroblastoma has also been diagnosed in small dog breeds. Mostly dogs younger than 3 years old (Tagawa et al., 2020) are affected and a gender predisposition is not known for this type of tumor (Brewer et al., 2011).

The tumor is predominantly reported as an intradural-extramedullary mass which is localized between the 10th thoracic and 3rd lumbar spinal cord segment. Intramedullary or extradural nephroblastoma can also occur, but usually it does not occur frequently (Tagawa et al., 2020). Due to the compression of the spinal cord, affected dogs often show hindlimb paresis and ataxia (Tagawa et al., 2020), these symptoms are reported to occur bilateral as well as unilateral in some cases. There is also a reported case were the dog suffered from paraplegia and the deep pain perception was no longer present (Brewer et al., 2011). During clinical examination itself, the dogs often show discomfort during palpation of the thoracolumbar vertebrae (Brewer et al., 2011). Radiography can often be unremarkable in the case of spinal cord nephroblastoma (Brewer et al., 2011; Tagawa et al., 2020) therefore for diagnosing the mass in the spinal cord, advanced imaging methods can be used, such as a myelogram, a post-myelogram CT or magnetic resonance imaging (MRI). For further identification of the tumor mass a surgical biopsy is required (Brewer et al., 2011).

From a histological point of view, a spinal cord nephroblastoma has comparable characteristics to a renal nephroblastoma, as it consists of blastemal components which are poorly differentiated, as well as stromal, and epithelial components, which can form tubules

or glomeruloid like structures (Terrell et al., 2000). During histological examination, nonetheless, special care must be taken as the nephroblastoma can resemble an ependymoma or primitive neuroectodermal tumor, so it is important to further distinguish them with the help of IHC (Brewer et al., 2011). A key point during the immunohistochemical examination is that spinal cord nephroblastomas are immunoreactive positive for cytokeratin whereas ependymomas and primitive neuroectodermal tumors are negative (Terrell et al., 2000). Furthermore Brewer et al. (2011) described cerebrospinal fluid analysis as useless for diagnosing a spinal cord nephroblastoma, since the obtained values are often in the reference range, or the changes are non-specific and can also indicate any other central nervous system disorders.

Since the tumor is relatively rare, and the affected dogs are usually euthanized post diagnosis, there are only very few documented therapeutic approaches to be found described in the veterinary literature (Terrell et al., 2000).

In 2011, Brewer et al. reported a study of eleven confirmed canine spinal cord nephroblastomas which were treated in different ways. 6 out of the 11 affected dogs underwent surgery and the tumor was resected using a laminectomy, a hemilaminectomy or a combination of the two surgical techniques. One dog was treated conservatively with administration of glucocorticoids. The remaining 4 dogs did not receive any therapy. Brewer et al. (2011) also mentioned that adjuvant radiation therapy and the administration of polyethylene glycol after surgery might increase the survival time. In the study characterized by Brewer et al (2011), the dogs that underwent tumor resection as part of a surgery had a survival time of 0 to 976 days, the median value was 70.5 days. Nonetheless, there are also a few long-term survival cases to be found in the veterinary medical literature, Nackaichi et al. (2022) reported a canine spinal cord nephroblastoma case in which the dog died 11 years post-surgery from causes unrelated to the tumor. The dog was treated postoperatively with radiation therapy.

9. Case Reports of the University of Veterinary Medicine Budapest

9.1. Nephroblastoma in a 5-month-old intact male

The first case illustrated deals about a 5-month-old intact male bichon Havanese dog who was clinically unremarkable based on a physical examination carried out at the age of 2 months. At the age of 4.5 months the dog was presented to the clinic of the University of Veterinary Medicine in Budapest with symptoms like vomitus and polyuria/polydipsia. A blood examination was carried out and the results were unremarkable. Further ultrasonographic examination revealed a distinct mass in the abdominal cavity, which had the size of about the head of a baby. Due to the mass visible on ultrasound, an exploratory laparotomy was performed at the age of 5 months. The laparotomy revealed an amorphous tumor mass originating from the left kidney caudal pole. Since the tumor had invaded the right liver lobes and the surrounding guts and omentum, a resection of the tumor could not be guaranteed and the tumor was considered inoperable. In addition, the tumor showed a hematoma, which lead to the suspicion of a former rupture of it. The owner decided to euthanize the dog. Two samples were taken from the left kidney and were further examined by the Department of Pathology of the University of Veterinary Medicine Budapest.

9.1.1. Pathological examination

The samples that have been taken had a firm consistency and are illustrated in **Figure 2**. The tumor itself had a gray to tan color and some dark brown components could be seen, it was also observed that the samples had a slightly lobular appearance. After cutting the samples, it became clear that the cut surface presents to be heterogenic. The sections in which the samples were cut after fixation were about 2×2×2.5 cm and 2×3×2 cm.

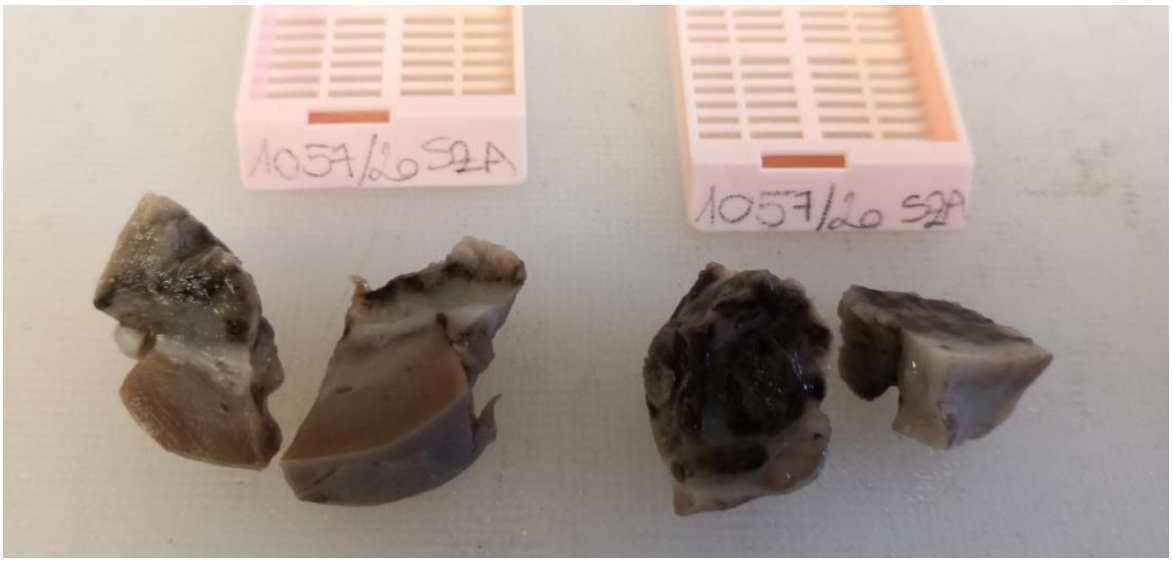

Figure 2: Formalin fixed samples of the left kidney which were given to the Department of Pathology of the University of Veterinary Medicine Budapest.

9.1.1.1. Histopathological examination

For the histopathological examination of the samples, 10 sections in total were used. A well-visible renal tissue, renal cortex and medulla could be detected. A heterogeneous tumor was seen compressing the renal tissue and the tumor appeared well-demarcated but did not show encapsulation. The neoplastic tissue contained embryonic epithelium, as well as poorly differentiated blastema and mesenchymal stroma. In addition, the neoplastic substance showed multifocal haemorrhagic and necrotic areas.

In the **epithelial component** of the neoplastic tissue primitive glomerular and irregular tubular structures (see **Figure 3**) could be detected. The epithelial cells in the neoplastic tissue were cuboidal-to-columnar and had indistinct cell borders. The nuclei appeared to be oval and the chromatin was finely granulated, some prominent nucleoli were detected. The cytoplasm was light basophilic. Furthermore, the number of mitotic figures per high-power field (HPF) was found to be 7-10. In addition, the tubules presented an irregular structure, the lumina varied in diameter and some were almost cystic dilated.

The **stromal component** consisted of loose and disorganized bundles of mesenchymal cells (see **Figure 4**), which showed an elongated shape and had an oval nucleus. Some, however, were more differentiated elongated, and had a cigar-like nuclei. The oval nuclei were euchromatic, and some nucleoli were prominent. The cytoplasm was eosinophilic and 0-2 mitotic figures per HPF were found. The matrix appeared loose, slightly basophilic and it

displayed some myxomatous regions. In larger areas, on the other hand, it appeared acidophilic and reminiscent of collagen fibres.

The **blastema** appeared to be multifocally, like islands and showed poor differentiation (see **Figure 5**). The blastemal cells were either round or polygonal. They each presented a small nucleus which proved to be oval in shape and was euchromatic. Nucleoli were well-visible. Furthermore, the cells demonstrated a scant cytoplasm.
Mitotic figures were counted as well and in total 10–14 mitotic figures per HPF were determined. Besides many diffusely apoptotic cells could be identified.

In this case the epithelial and stromal components were the predominant components of the tumor. Only a small portion of the tumor reflected the blastemal component.

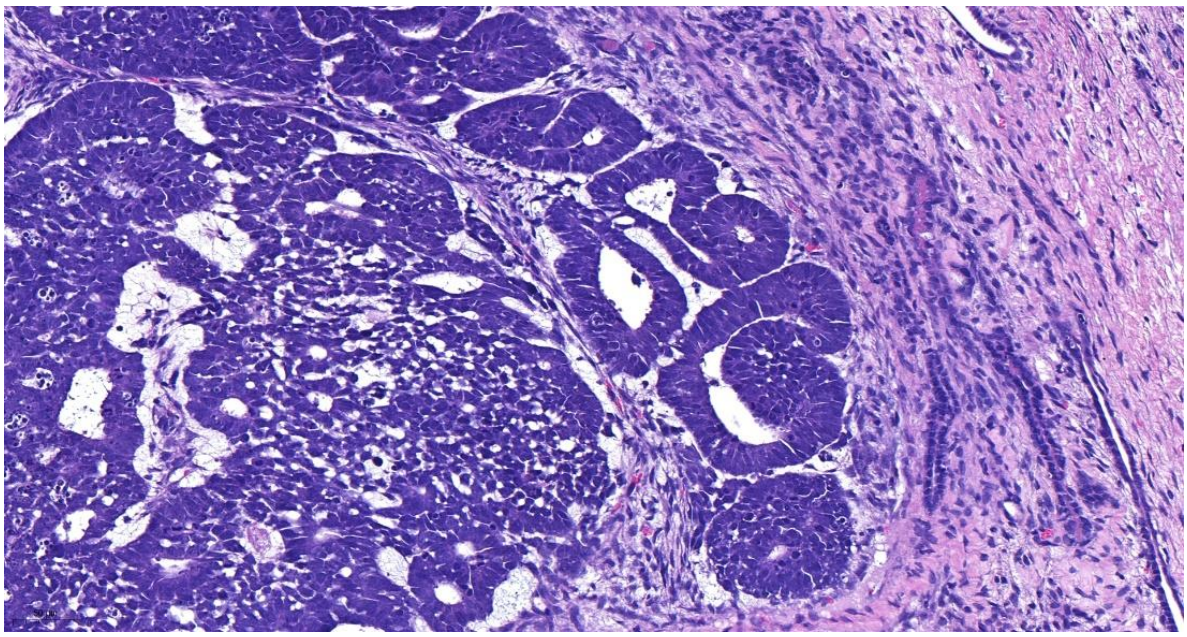

Figure 3: Histopathology of the tumor mass with H&E staining. Irregular tubular structures lined by cuboidal-to-columnar epithelial cells (bar=50 µm, 100× magnification).

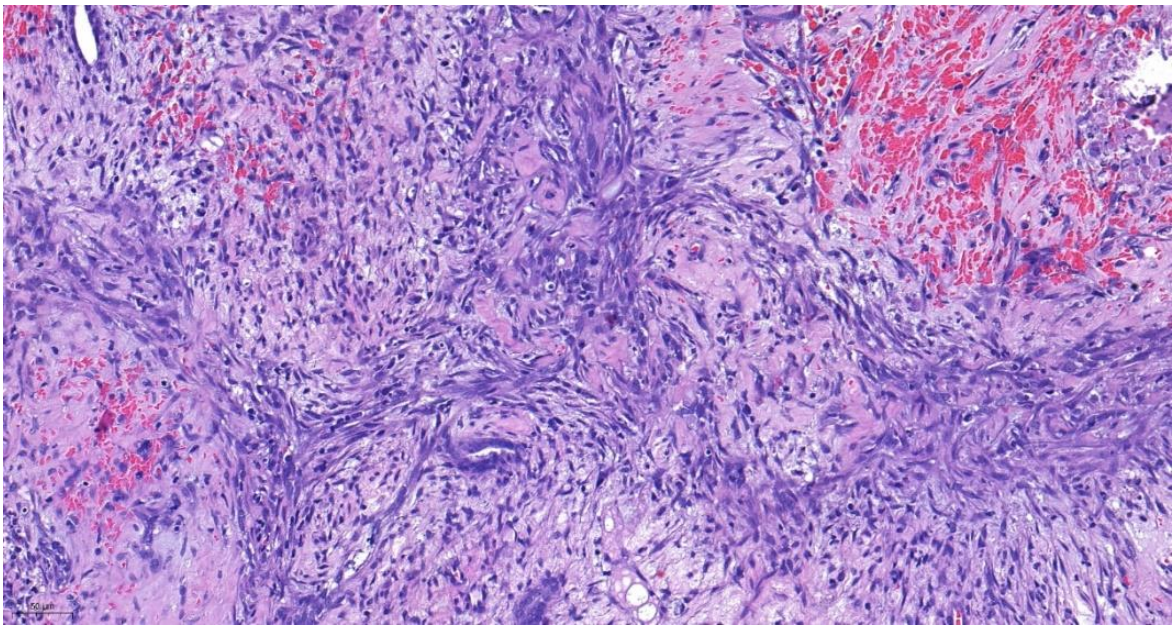

Figure 4: Histopathology of the tumor mass with H&E staining. Multiple mesenchymal cells and multifocal haemorrhages (bar=50 µm, 100× magnification).

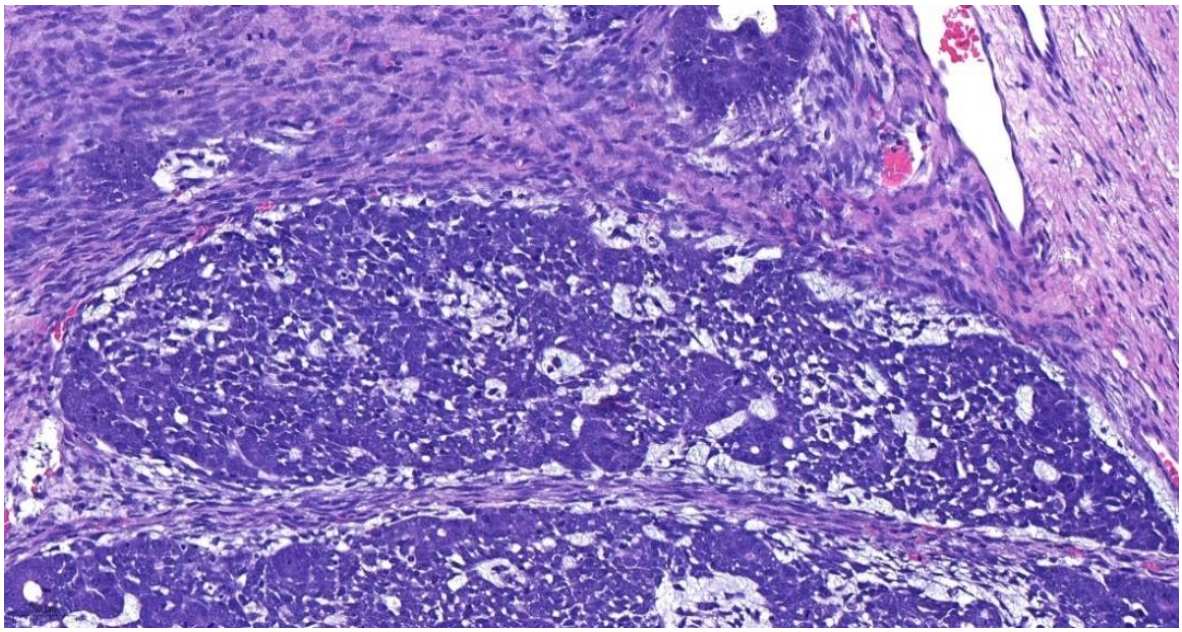

Figure 5: Histopathology of the tumor mass with H&E staining. Blastemal cells subdivided into islands by the stromal component (bar=50 µm, 100× magnification).

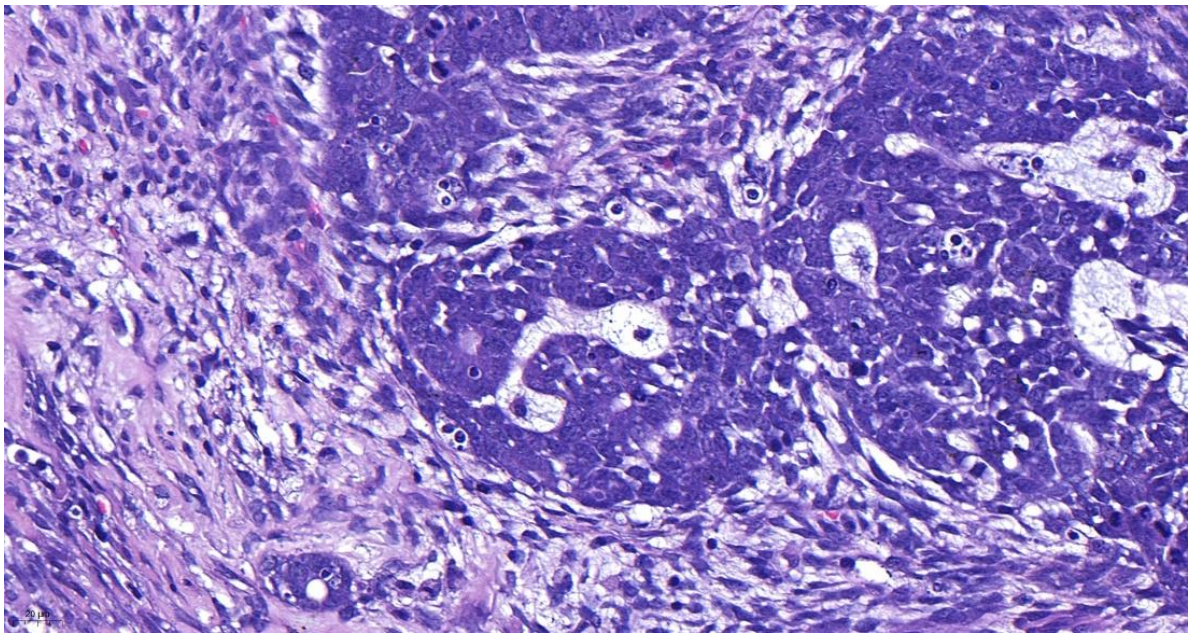

Figure 6: Histopathology of the tumor mass with H&E staining.
Multiple diffusely apoptotic cells are visible (bar=20 µm, 200× magnification).

9.1.1.2. Immunohistochemical examination

In addition to the histopathological examination an immunohistochemical examination of the samples was conducted as well to ensure a more accurate identification of the tumor. The antibodies pancytokeratin, vimentin and desmin were used for this study. The result of the IHC showed that almost all stromal cells and most of the blastemal cells expressed vimentin. A large part of the epithelial cells also showed positivity for vimentin, but some glomerular components were negative (see **Figure 7**). Furthermore, about half of the epithelial cells cytoplasma showed strong, granular staining with pancytokeratin (see **Figure 8**). An expression of both vimentin and pancytokeratin indicates epithelial-to-mesenchymal transition, which demonstrates a physiologic phenomenon during organogenesis. Desmin was only moderately expressed by some of the stromal cells (see **Figure 9**). Therefore, a myofibroblastic transition could not be confirmed.

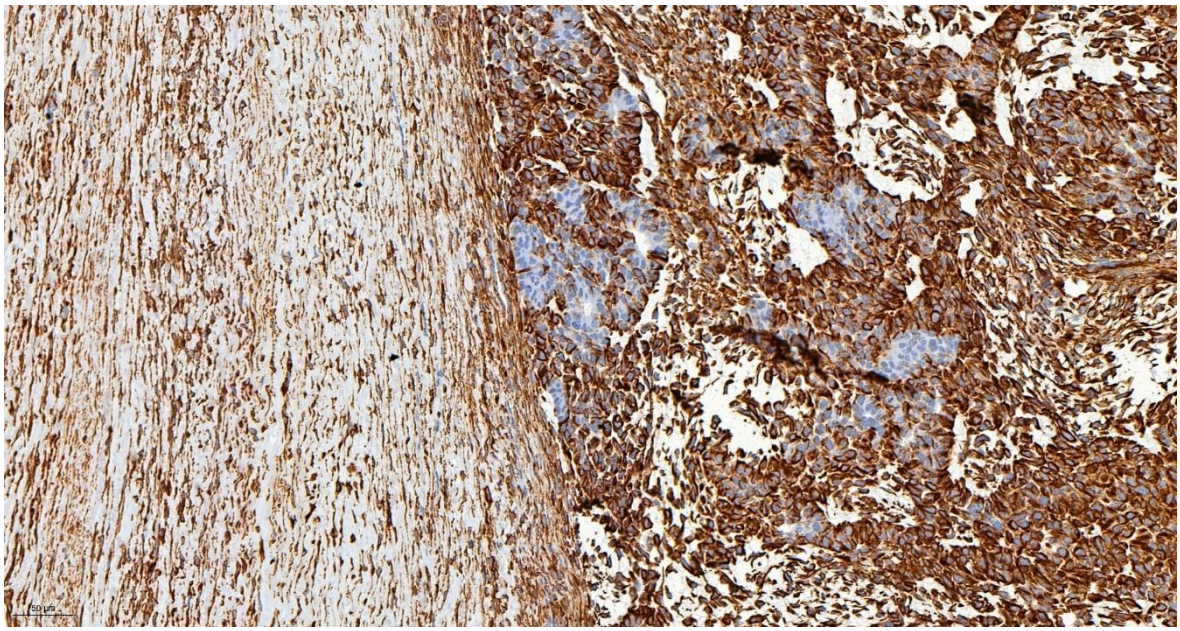

Figure 7: Vimentin antibody staining, IHC.

Mesenchymal type cells are stained with brown chromogen (bar=50 µm, 100× magnification).

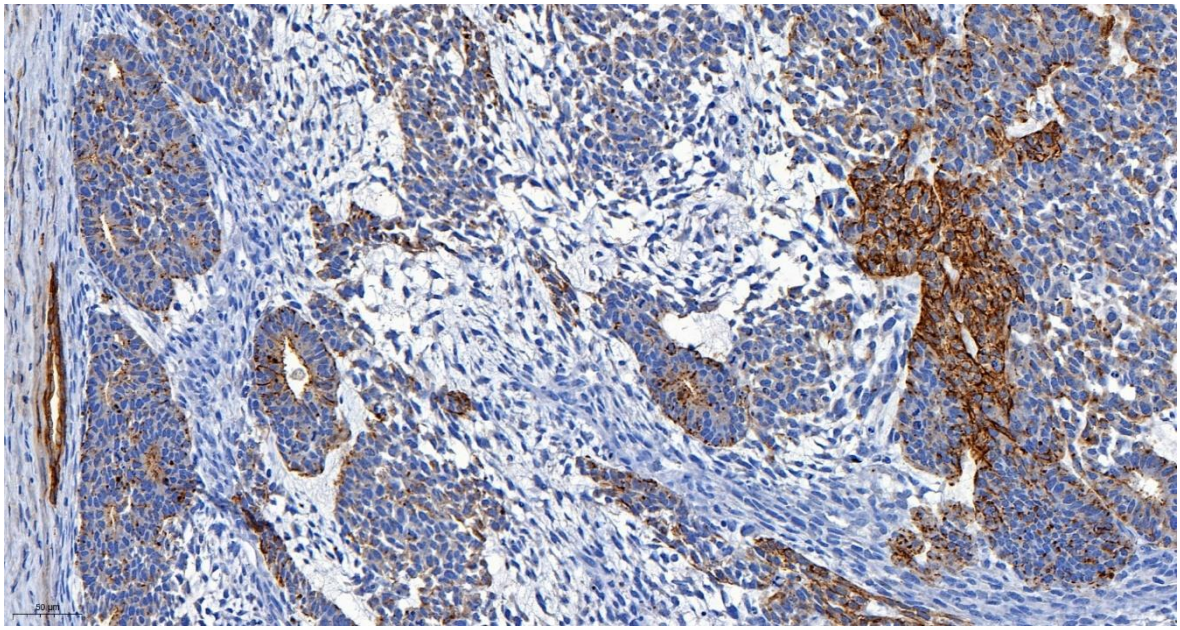

Figure 8: Pancytokeratin antibody staining, IHC.

Epithelial type cells are stained with brown chromogen (bar=50 µm, 100× magnification).

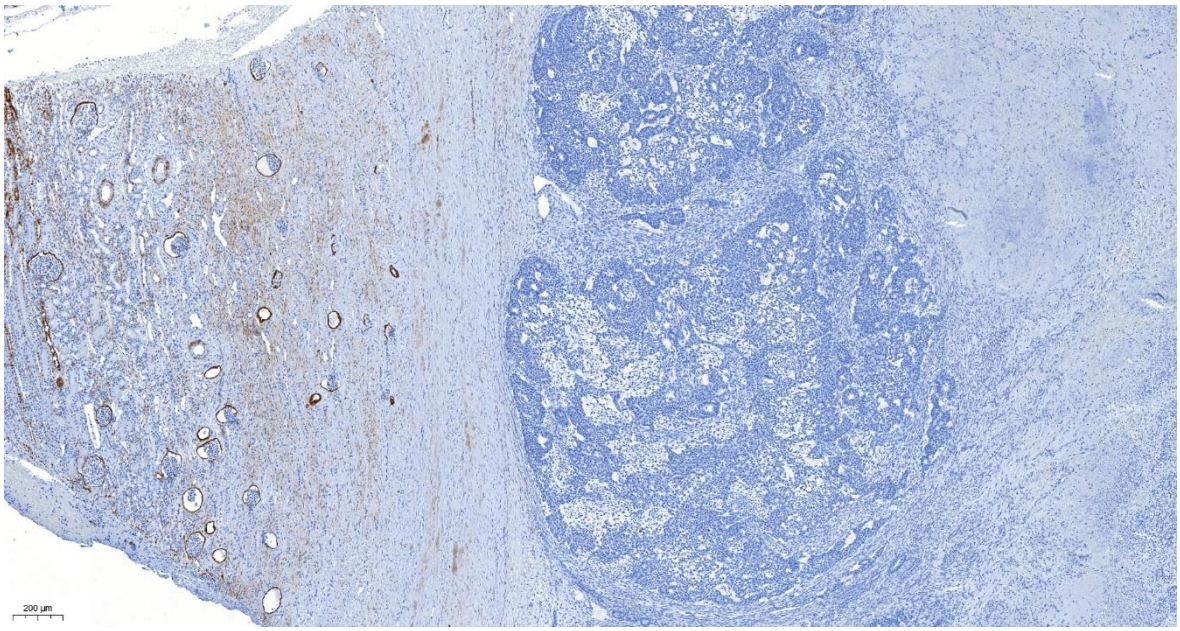

Figure 9: Desmin antibody staining, IHC.
Myofibroblast type cells are stained with brown chromogen (bar=200 µm, 40× magnification).

The Department of Pathology of the University of Veterinary Medicine came to the conclusion that the kidney tumorous mass originating from the left kidney caudal pole can be identified as a nephroblastoma based on the triphasic neoplastic tissue and their characteristics and furthermore based on the findings during IHC.

9.2. Nephroblastoma in a 10-year-old intact male

A 10-year-old intact male American Staffordshire was presented to the clinic of the University of Veterinary Medicine in Budapest due to a palpable abdominal mass. Except for the palpable mass, the dog was doing fine and did not show any further symptoms. A blood test was initiated and revealed mild anaemia and slightly elevated phosphorus levels. Additionally, an ultrasound scan was performed which revealed an abdominal mass measuring 15 centimetres in diameter.

The following day an exploratory laparotomy was performed and the mass was identified as a tumor which originated from the left kidney´s caudal pole. The tumor was amorphous, and the exact size was 15×20 cm. The omentum was already invaded by the tumor. During the surgery, a prostatic cyst has also been discovered, which was opened. A nephrectomy was performed, and the patient was doing well after surgery.

9.2.1. Pathological examination

A total of 5 samples were forwarded to the Pathology Department of the University of Veterinary Medicine Budapest for further examination. The tumor had a firm consistency and consisted of a gray to tan substance with some dark brown regions. Furthermore, it had a slightly lobular appearance. After cutting the samples, it became clear that the cut surface presents to be heterogenic. The sections in which the samples were cut after fixation were about 3×3×1,5 cm, 3×1×1 cm; 4×3×2 cm; 4×2,5×2 cm and 4×2×1,5 cm.

9.2.1.1. Histopathological examination

Subsequently, a histopathological examination was performed on a total of 10 sections with H&E staining. Like in the previous case, in this case there was as well a well-visible renal tissue, renal cortex and medulla. It became apparent that a heterogeneous mass was compressing the renal tissue. The tumor was well-dermarcated but non-encapsulated. The neoplastic tissue appeared to be triphasic and consisted of embryonic epithelium, poorly differentiated blastema and mesenchymal stroma. In this case no haemorrhages and necrotic areas could microscopically be detected.

In the **epithelial component** of the tumor both primitive glomeruli and irregular tubular structures could be seen. Tufts of epithelium invaginated into a lumen to form these glomerular structures. The epithelial cells were cuboidal-to-columnar and showed indistinct cell borders. They had an oval nucleus with finely granulated chromatin. Some nucleoli were prominent. The cytoplasm presented to be pale basophilic. Mitotic figures per HPF were 0–3. The presented tubuli had an irregular structure, their lumina varied in diameter and some were almost cystic dilated.

The **stroma** consisted of loose and disorganized bundles of mesenchymal cells. The cells were elongated and each presented an oval nucleus. Some showed better differentiation and had a cigar-like nucleus. The oval nuclei were finely granulated and some nucleoli were prominent. The cytoplasm presented to be eosinophilic. 0–2 mitotic figures per HPF were detected. The matrix appeared loose, light basophilic and it displayed some myxomatous regions. In larger areas it appeared acidophilic and resembled collagen fibres.

The **blastemal component** was poorly differentiated and appeared to be multifocally, like islands. The majority of the cells presented to be round or polygonal. They each had an oval, small and euchromatic nucleus. The nucleolus was well-visible. Furthermore, the cells

demonstrated a scant cytoplasm. Mitotic figures were counted to be 5–8 per HPF. Besides many diffusely apoptotic cells could be seen.

Like in the previous described case, the epithelial and stromal components were also in this case the predominant components of the tumor. Only a small portion of the tumor reflected the blastemal component.

Furthermore, during the histopathological examination it could be detected that in the intact renal tissue there was a multifocally thickening of the glomerular walls and in the renal interstitium small groups of lymphocytes and plasma cells could be observed.

The Department of Pathology of the University of Veterinary Medicine Budapest came to the conclusion that the tumor originating from the left kidney caudal pole can be identified as a nephroblastoma based on the triphasic appearance and its characteristics. In addition, interstitial nephritis and glomerulonephritis were diagnosed.

9.3. Conclusion

As a conclusion, both case reports showed almost no symptoms except for polyuria/polydipsia in the first case described and a palpable abdominal mass in the second case illustrated. As elucidated in chapter 4, nephroblastoma often show no specific symptoms and usually only become conspicuous through its expansive growth (Nickel, 2012; Martin et al., 2014). This was reflected in both cases as further examination such as ultrasonography identified an abdominal mass which could be localized by exploratory laparotomy as originating from the kidney.

The typical triphasic tissue for a nephroblastoma consisting of embryonic epithelium, blastema and mesenchymal tissue (Meuten & Meuten, 2017) was found in both cases. The embryonic glomeruli described by Meuten & Meuten (2017), which are important for diagnostics, and are recognizable by tufts of epithelium invaginating into a lumen, could also be recognized in both cases. Lu et al. (1997) described that in some cases haemorrhages or necrotic areas can be visible, this was confirmed in one of the two cases. Meuten & Meuten (2017) mentioned that the predominant cell type are mostly blastemal cells, but in the cases described the blastemal component only reflected a small part of the tumor.

An IHC was only carried out in one of the two cases described. Compared to the canine nephroblastoma IHC results illustrated by Simpson et al. (1992), in both cases the blastemal cells and stromal cells expressed vimentin. In the case described by Simpson et al. (1992),

the blastemal and stromal cells additionally showed positivity for desmin. In the case of the 5-month-old intact male bichon Havanese dog of the University of Veterinary Medicine on the other hand, desmin was only moderately expressed by some of the stromal cells. Furthermore, Simpson et al. (1992) reported that the primitive tubuli only showed cytokeratin expression, in the case of the University of Veterinary Medicine however, not only half of the epithelial cells expressed pancytokeratin, a large part also showed positivity for vimentin except for some glomerular components which were negative.

Nonetheless, the IHC results of the two cases are very similar and have many points of agreement. However, more canine nephroblastoma cases with an IHC would be necessary to provide more precise information on the immunohistochemical appearance of a canine nephroblastoma.

10. Abstract

Primary renal tumors are rare in dogs. Only 0.6–1.7% of all tumors are primary renal neoplasia and only about 6% of these are diagnosed as nephroblastomas.

Since the origin of a nephroblastoma is usually a defective nephrogenesis and it develops from remnants of the metanephric blastema, the tumor is commonly diagnosed in dogs under 2 years of age. But there are also case reports in which nephroblastomas were diagnosed at an older age. Commonly, the diagnosis proves to be rather difficult, since the patients often do not show any specific symptoms. Most often, the tumor becomes apparent because of its palpable abdominal mass. In about 50% of the cases, the tumor metastasizes and therefore extrarenal lesions can also occur.

For further examinations, imaging techniques such as ultrasonography and radiography are usually used to visualize the mass more precisely. In some cases, a nephroblastoma can also occur extrarenal, as is often the case in the spinal cord, for example. Here, however, radiography can be less effective in some cases and therefore advanced imaging methods such as a myelogram, a post-myelogram CT or MRI can help for identifying the mass. Laboratory tests can also aid to direct the suspicion to the kidneys. Nonetheless, a clear diagnosis can only be made with the help of a histopathological examination. Histopathological, a triphasic tumor consisting of embryonic epithelia encompassing tubules and glomeruli in several stages of differentiation, blastema and mesenchymal tissue can be recognized. For the diagnosis of important embryonal glomeruli are formed by tufts of epithelium invaginating into a lumen. Furthermore, the immunohistochemical characteristics of a nephroblastoma can also assist diagnosing the tumor. Blastemal cells and stromal cells express vimentin, and in some cases they express desmin as well, while the epithelial cells mostly show positivity for cytokeratin.

The tumor can be staged using the NWTS staging system, which was carried over from human medicine. Treatment options depend on staging, for instance a nephroblastoma classified as a stage 2 or higher stage is advised to have chemotherapy in addition to nephrectomy. The survival times also depend on the tumor stage and there are great variations visible in different nephroblastoma cases.

11. References

Araujo, D. C. C., Silva, M. A., Pessoa, C. C. V., & Fernandes, J. I., 2020: Renal nephroblastoma in an adult dog. *Brazilian Journal of Veterinary Medicine*, 42(1), e1077820. Doi: https://doi.org/10.29374/2527-2179.bjvm107820

Aumailley, M., 2013: The laminin Family. *Cell Adhesion & Migration*, 7(1), pp. 48-55.

Below, J., & Das, J. M. 2022: Vincristine. [online]
URL: https://www.ncbi.nlm.nih.gov/books/NBK537122/. Accessed: 25 August 2022.

Brewer, D. M., Cerda-Gonzales, S., Dewey, C. W., Diep, A. N., Van Horne, K., McDonough, S. P., 2011: Spinal cord nephroblastoma in dogs: 11 cases (1985–2007). *JAVMA*, 238(5), pp. 618-624.

Bryan, J. N., Henry, C. J., Turnquist, S. E., Tyler, J. W., Liptak, J. M., Rizzo, S. A., Sfiligoi, G., Steinberg, S. J., Smith, A. N., & Jackson, T., 2006: Primary renal neoplasia of dogs. *Journal of Veterinary Internal Medicine*, 20(5), pp. 1155-1160.

Chen, B., Li, W., Wang, F., 2018: A blastema-predominant canine renal nephroblastoma with gingival metastasis: case report and literature review. *Journal of Veterinary Diagnostic Ivestigation*, 30(3), pp. 430-437.

Erginel, B. (2016). Wilms Tumor and Its Management in a Surgical Aspect. In: Marry M. van den Heuvel-Eibrink (Ed.): *Wilms Tumour*. Brisbane, Australia, Codon Publications, pp. 43-48.

Frimberger, A. E., Moore, A.S., Schelling, S.H., 1995: Treatment of nephroblastoma in a juvenile dog. *Journal of the American Veterinary Medical Association*. 207(5), pp. 596-598.

Fullerton, E., 2017: Doxorubicin: An Overview. *Today´s veterinary technician*, May/June 2017, pp. 25-34.

Fullerton, E., 2018: Chemotherapy-Induced Side Effects: Prevention and Treatment. *Today´s veterinary nurse*, Fall 2018, pp. 53-56.

Gardner, H. L., Fenger, J. M., London, C. A. 2016: Dogs as a Model for Cancer. *Annual Review of Animal Biosciences*, 4, pp. 199-222.

Georgia Tech Biological Sciences, 2016: Animal Development I: Fertilization & Cleavage. [online] URL:
https://organismalbio.biosci.gatech.edu/growth-and-reproduction/animal-development-i/.
Accessed: 26 July 2022.

Gheissari, A., Taheri, D., Mozafarpour, S., Beigy, H., Samanianpoor, P., Merrikhi, A., Farajzadegan, Z., 2012: The expression of cytoskeletal proteins in kidney specimens of children with primary focal segmental glomerulosclerosis. *Indian Journal of Nephrology*, 22(6), pp. 444-450.

Goddard, J. C., Birks, T. 2016: A short history of nephrectomy. *Urology news*, 21(1). [online] URL: https://www.urologynews.uk.com/features/history-of-urology/post/a-short-history-of-nephrectomy. Accessed: 28 August 2022.

Gollakner, R.: Cyclophosphamide. [online]
URL: https://vcahospitals.com/know-your-pet/cyclophosphamide. Accessed: 03 September 2022.

Grieco, V., Riccardi, E., Belotti, S., Scanziani, E., 2006: Immunohistochemical Study of Porcine Nephroblastoma. *Journal of Comparative Pathology*, 134(2-3), pp. 143-151.

Hammer, A. S., Couto, C. G., Ayl, R. D., Shank, K. A., 1994: Treatment of tumor-bearing dogs with actinomycin D. *Journal of veterinary internal medicine*, 8(3), pp. 236-239.

Hergt, F., Mortier, F., Werres, C., Flatz, K., von Bomhard, W., 2019: Renal Nephroblastoma in a 17-Month-Old Jack Russell Terrier. *Journal of the American Animal Hospital Association*, 55:e555-03. Doi: https://doi.org/10.5326/JAAHA-MS-6664

Hofmeister, E. H., & Egger, C. M., 2005: Evaluation of diphenhydramine as a sedative for dogs. *JAVMA*, 226(7), p.1.

Johnston, S. A. & Tobias, K. M. 2018: Veterinary Surgery: Small Animal, 2nd Edition. Riverport Lane, Elsevier, pp. 2193; 2198-2201.

Kitao, S., Yamada, T., Ishikawa, T., Madarame, H., Furuichi, M., Neo, S., Tsuchiya, R., Kobayashi, K., 2006: Alpha-fetoprotein in serum and tumor tissues in dogs with hepatocellular carcinoma. *Journal of veterinary diagnostic investigation*, 18(3), pp. 291-295.

Kumar, A., & Jagannathan, N., 2018: Cytokeratin: A Review on Current Concepts. *International Journal of Orofacial Biology*, 2(1), pp. 6-7.

Lori, J. C., Stein, T. J., Thamm, D. H., 2010: Doxorubicin and cyclophosphamide for the treatment of canine lymphoma: a randomized, placebo-controlled study. *Veterinary and Comparative Oncology*, 8(3), pp. 188-195.

Lu, D., Taki, R., Uetsuka, K., Nakayama, H., Doi, K., 1997: A Case of Canine Nephroblastoma with Unusual Neuronal and Mesenchymal Components. *Journal of Toxicologic Pathology*, 10(2), pp. 109-110.

Magaki, S., Hojat, S. A., Wei, B., So, A., Yong, W. H., 2019: An Introduction to the Performance of Immunohistochemistry. In: Yong, W. H. (Ed.): *Biobanking*. Methods in Molecular Biology, vol 1897. New York, NY, Humana Press, pp. 289-298

Martin, K. D. J., Sarangom, S. B., Krishna, B. N. D., Nair, N. D., Pillai, U.N., Philip, S.B., & Kankonkar, A.P., 2014: Renal nephroblastoma in an adult dog-a case report. *Veterinarski Arhiv*, 84(1), pp. 85-96.

Mazurkiewicz-Pisarek, A., Płucienniczak, G., Ciach, T., Płucienniczak, A., 2016: The factor VIII protein and its function. *Acta Biochimica Polonica*, 63(1), pp. 11-16.

Meomartino, L., Greco, A., Di Giancamillo, M. Brunetti, A., Gnudi, G., 2021: Imaging techniques in Veterinary Medicine. Part I: Radiography and Ultrasonography. *European Journal of Radiology Open*, 8:100382. Doi: https://doi.org/10.1016/j.ejro.2021.100382

Meuten, D.J., & Meuten, T.L.K., 2017: Tumors of the Urinary System. In: Meuten, D.J. (Ed.): *Tumors in Domestic Animals*. Fifth edition. Ames, Iowa, John Wiley & Sons, pp.646-649.

Miller, R.T., 2003: Cytokeratin AE1/AE3. URL: https://propath.com/cytokeratin-ae1-ae3/. Accessed: 13 August 2022.

Montinaro, V., Boston, S. E., Stevens, B., 2013: Renal nephroblastoma in a 3-month-old golden retriever. *The Canadian Veterinary Journal*, 54(7), pp.683-686.

Moritz, A., 2012: Problemorientierte Diagnostik. In: Kessler, M. (Ed.): *Kleintieronkologie: Diagnose und Therapie von Tumorerkrankungen bei Hund und Katze.* Third edition. Stuttgart, Enke Verlag, p.34.

Moritz, K.M., Wintour-Coghlan, M., Black, M,J., Bertram, J.F., Caruana, G., 2008: Morphological Development of the Mammalian Kidney. In: *Factors Influencing Mammalian Kidney Development: Implications for Health in Adult Life*. Advances in Anatomy Embryology and Cell Biology, vol 196. Berlin, Heidelberg, Springer, pp.1-9.

Nackaichi, M., Iseri, T., Horikirizono, H., Itoh, h., Sunahara, H., Nemoto, Y., Itamoto, K., Tani, K., 2022: A long survival case of spinal nephroblastoma in a dog. *Open Veterinary Journal*, 12(2), pp. 188–191.

Nelson, M.V., Verschuur, A., Dome, J.S., 2019: Wilms Tumor-Nephroblastoma. In: Malouf, G., Tannir, N. (Eds.): *Rare Kidney Tumors*. Springer, Cham, p. 15.

Nickel, R., 2012: Tumore der Harnorgane. In: Kessler, M. (Ed.): *Kleintieronkologie. Diagnose und Therapie von Tumorerkrankungen bei Hund und Katze.* Third edition. Stuttgart, Enke Verlag, pp. 345-346.

Norris, A. M., & Withrow, S. J., 1984: A Review of Cancer Chemotherapy for Pet Animals. *The Canadian Veterinary Journal*, 25(4), p. 154.

Ogino, M. H., & Tadi, P., 2022: Cyclophosphamide. [online]
URL: https://www.ncbi.nlm.nih.gov/books/NBK553087/. Accessed: 03 September 2022.

Pandita, V., Ajila, V., Babu, G.S., Hedge, S., Faizal Asan, M., 2021: Vimentin and its role as a biomarker in health and disease. *Gulhane Medical Journal*, 63(4), pp. 232-237.

Pet Cancer Society: Dactinomycin. [online]
URL: https://petcancersociety.com/types-of-drug/dactinomycin/. Accessed: 02 September 2022.

Popov, S.D., Sebire, N.J., Vujanic, G.M., 2016: Wilms´s Tumour – Histology and Differential Diagnosis. In: Marry M. van den Heuvel-Eibrink (Ed.): *Wilms Tumour*. Brisbane, Australia, Codon Publications, p. 8.

Pretzer S.D., 2008: Canine embryonic and fetal development: A review, *Theriogenology*, 70(3), pp. 300-303.

Raffensperger, J., 2015: Max Wilms and his Tumor. *Journal of Pediatric Surgery*, 50(2), pp. 356-359.

Rivankar, S., 2014: An overview of doxorubicin formulations in cancer therapy. *Journal of Cancer Research and Therapeutics*, 10(4), p. 853.

Rosol, T. J., Chew, D. J., Couto, C.G., Ayl, R. D., Nagode, L. A., Capen, C. C., 1992: Effects of Mithramycin on Calcium Metabolism and Bone in Dogs. *Vet Pathol*, 29(3), pp. 223-229.

Rosol, T. J., Chew, D. J., Hammer, A. S., Ward, H., Peterson, J. L., Carothers, M. A., Couto, C. G., 1994: Effects of Mithramycin on Hypercalcemia in Dogs. *Journal of the American Animal Hospital Association*, 30(3), pp. 244-250.

Schmidt, J. M., & Findji, L., 2022: Tumore der Harnorgane. In: Kessler, M. (Ed.): *Kleintieronkologie: Diagnose und Therapie von Tumorerkrankungen bei Hund und Katze.* Fourth edition. Stuttgart, Georg Thieme Verlag, pp. 540-541.

Schnorr, B., & Kressin, M., 2006: Embryologie der Haustiere. Fifth edition. Stuttgart, Enke Verlag, pp. 47-55;75;180-187.

Schweer, D., McCorkle, J. R., Rohr, J., Tsodikov, O. V., Ueland, F., Kolesar, J., 2021: Mithramycin and Analogs for Overcoming Cisplatin Resistance in Ovarian Cancer. *Biomedicines*, 9, 70, p. 2.

Seaman, R. L., & Patton, C. S., 2003: Treatment of renal nephroblastoma in an adult dog. *Journal of the American Animal Hospital Association*, 39(1), pp. 76-79.

Shariati, E., Bakhtiari, J., Khalaj, A., Molazem, M., Niasari-Naslaji, A. 2016: Clinical and paraclinical evaluation of partial nephrectomy using laparoscopy and open surgery in dogs: new suturing technique. *Iranian Journal of Veterinary Research*, 18(1), pp. 1-5.

Simpson, R. M., Gliatto, J. M., Casey, H. W., Henk, W. G., 1992: The Histologic, Ultrastructural, and Immunohistochemical Features of a Blastema-predominant Canine Nephroblastoma. *Vet Pathol*, 29(3), pp. 250-253.

Soltani, K. M. D., 1979: Alpha-Fetoprotein: A Review. *The Journal of investigative Dermatology*, 72(5), pp. 211-213.

Stephens, T. 2019: The Use of Chemotherapy to Prolong the Life of Dogs Suffering from Cancer: The Ethical Dilemma. *Animals*, 9(7), 441, pp. 1-2.

Tagawa, M., Shimbo, G., Tomihari, M., Yanagawa, M., Watanabe, K., Horiuchi, N., Kobayashi, Y., Miyahara, K., 2020: Intramedullary spinal nephroblastoma in a mixed breed dog. *The Journal of Veterinary Medical Science*, 82(7), pp. 917-921.

Takeda, T., Makita, T., Nakamura, N., Horne, H., 1989: Congenital Mesoblastic Nephroma in a Dog: A Benign Variant of Nephroblastoma. *Vet. Pathol*, 26, pp. 281-282.

Terrell, S. P., Platt, S. R., Chrisman, C. L., Homer, B. L., De Lahunta, A., Summers, B. A., 2000: Possible Intraspinal Metastasis of a Canine Spinal Cord Nephroblastoma. *Vet Pathol*, 37(1), pp. 94–97.

Wang, J., Zohar, R., McCulloch, C.A., 2006: Multiple roles of a-smooth muscle actin in mechanotransduction. *Experimental Cell Research*, 312, pp. 205-207.

12. Acknowledgements

First of all, I would like to thank my supervisor Dr. Anna Szilasi for all her patience and support while writing my thesis, and also for translating the nephroblastoma cases from Hungarian to English which were provided by the Department of Pathology. Therefore, I additionally would like to thank the Department of Pathology for providing me both of these nephroblastoma cases and allowing me to use them for my thesis.

Furthermore, I would like to thank my grandfather Dr. Karl Zierhut and my parents who made it possible for me to study veterinary medicine in Budapest and have supported me all the way through the past years.

HuVetA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Alina Jil Annika Klewicz

Contact information (e-mail): klewi@outlook.com

Title of document (to be uploaded): Canine Renal Nephroblastoma: A Literature Review and Case Studies

Publication data of document: 18.11.2022

Number of files submitted: 1

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA may store more than one copy (accessible only to HuVetA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA as follows (**mark the appropriate box with an X)**:

- [X] I grant unlimited online access,
- [ ] I grant access only through the intranet (IP range) of the University of Veterinary Medicine,
- [ ] I grant access only on one dedicated computer at the Ferenc Hutÿra Library,
- [ ] I grant unlimited online access only to the bibliographic data and abstract of the document.

Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

X	I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetA do not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA in a way that is unlawful.

Date: Budapest, ..14…..day ….11…..month…2022…..year

Alina Klewicz

Author/copyright owner signature

HuVetA Magyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive *is an online veterinary repository operated by the Ferenc Hutÿra Library, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.*

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.
Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

I hereby confirm that I am familiar with the content of the thesis entitled Canine Renal Nephroblastoma: A Literature Review and Case Studies written by Alina Jil Annika Klewicz which I deem suitable for submission and defence.

Date: Budapest, 14 day 11 month 2022 year

.....

dr. Anna Szilasi

Supervisor name and signature

Department of Pathology

.....

Department