A case of triphasic nephroblastoma with lung metastases in an angora rabbit

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SUMMARY

Kidneys, liver, lung, and heart of a 1-year-old male Angora rabbit from a commercial facility were the material of this case. Macroscopically, an amorphous and elastic mass with cysts was observed on the left kidney. The mass weighed 875 grams and was 11x12x4.5 cm in dimensions. At the cross section, a tumor with lobular appearance, covering the pelvis and medulla of the kidney was observed, which distinguished from the atrophic kidney tissue via a distinctive capsule. Microscopically, blastemal, epithelial, and stromal areas containing polygonal neoplastic cells with pleomorphic hyperchromatic nuclei and with narrow cytoplasm were noticed. Primitive avascular glomeruluses and undeveloped tubules along with neoplastic cells lined up around vessels were seen in many areas. Occasional cystic forms, mitotic figures, and intensive necroses were present in these areas. Stromata was hemorrhagic and was full of intensive loose and myxomatous vessels. In addition, metastatic cartilage near capsule was found in the cortex. Immunohistochemically, a strong immunoreactivity was detected with cytokeratin staining only in primitive tubular epithelia and vimentin staining only in blastemal and stromal areas. This is the first report that, a triphasic nephroblastoma (tumor) was diagnosed in an Angora rabbit using histopathological and immunohistochemical examinations.

Keywords : Rabbit, nephroblastoma, immunohistochemistry, vimentin, cytokeratin.

RéSUMÉ

Un cas de néphroblastome triphasé avec des métastases pulmonaires chez un lapin Angora.

Les reins, le foie, les poumons et le coeur d’un lapin Angora de 1 an provenant d’une animalerie commerciale font l’objet de cette étude. Macroscopiquement, une masse amorphe et élastique, avec des kystes, a été observée sur le rein gauche. Cette masse pesait 875 grammes et mesurait 11x12x4.5 cm. A la coupe, il a été observé une tumeur d’aspect lobulaire, recouvrant le cortex et la médullaire rénale, et distinctement démarquée du parenchyme rénal atrophique par une capsule. Microscopiquement, des zones blastiques, épithéliales et stromales contenant des cellules néoplasiques polygonales, avec un noyau pleomorph et hyperchromatique et un cytoplasme restreint, ont été notées. Dans de nombreuses zones, des gloméruluses avasculaires primatifs, des tubules non développés et des cellules néoplasiques alignées autour des vaisseaux ont été observés. Dans ces zones, d’occas ionnelles formes kystiques, des figures de mitoses ainsi que des foyers de nécrose étaient présents. Le stroma était hyperhémique et contenait de nombreux vaisseaux lâches et myxoides. De plus, des foyers de cartilage métaplasique étaient présents près de la capsule. En immunohistochimie, une très forte réactivité a été détectée contre la cytokeratine dans les structures épithéliales tubulaires primitives alors qu’un marquage de la vimentine n’était présent que dans les zones blastiques et stromales. Ce cas représente la première description d’un néphroblastome triphasique chez un lapin Angora, avec des investigations histopathologiques et immunohistochimiques.

Mots-clés : Lapin, nephroblastome, immunohistochimie, vimentin, cytkératine.

Introduction

Embryonal nephroma (also known as Wilm’s tumor, nephroblastoma, or renal or embryonal adenocarcinoma) are embryonal tumors that stem from the primitive nephrogenic blastem [1,6,23]. Although nephroblastoma is very common in pigs and poultry, and to some extent in calves and dogs, it is very rare in other species [3,12,13,16,22]. Although nephroblastoma is very rare in rabbits, it can spontaneously occur in males younger than one year old [23]. Embryonal nephroma may result from retroviral infections or some carcinogenic substances in various laboratory animals [11]. Nephroblastomas can be unilateral or bilateral filling up the abdominal cavity and exerting pressure on the adjacent organs. Tumors histologically consist of primitive avascular glomerulus, tubules, and different intensity of myxomatous mesenchymal elements, and contain neoplastic cells, tubules- and glomerulus-like forms [2,19,20]. In some cases, besides epithelial tissues, smooth or striated muscle fibers, cartilage, bone, and fat tissue are also found in the stroma of tumors [14]. Metastases are unusual except in dogs [15,19,25,27].

This report describes a nephroblastoma with macroscopic, microscopic, immunohistochemical findings in an Angora rabbit.

Materials and Methods

ANIMAL

A 1-year old Angora male rabbit was included into study. Two weeks before euthanasia, the rabbit at a commercial facility had exhibited restricted movements, decreased libido, anorexia and decreased appetite. A hard mass was palpated in the abdominal region.

MACROSCOPIC EXAMINATION

A tumor mass and organs (kidneys, liver, lung, and heart) of a euthanized 1-year-old male Angora rabbit from a commercial facility was examined.

MICROSCOPIC EXAMINATION

Tissue samples were formalin-fixed, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE), and examined under a microscope.
**IMMUNOHISTOCHEMICAL EXAMINATION**

Histological sections of formalin-fixed tissues from the tumors were immunohistochemically stained using commercially available mouse monoclonal anti-cytokeratin (Pan Ab-1 clone AE1/AE3, Neomarkers). Mouse monoclonal anti-vimentin (Ab-2 clone V9, Neomarkers) primer antibodies was used. These two staining techniques were carried out according to Avidin-Biotin-Peroxidase complex (ABC) method [9,13].

**Results**

**MACROSCOPICAL EXAMINATION**

Upon euthanasia and necropsy by the veterinarian of the commercial facility, a mass of 875 grams and 11x13x4.5 cm in dimension was noticed on the left site of the kidney. The mass was amorphous and elastic consistency with cystic forms on it (Figure 1a). Lobular appearance at the cut surface of the tumor was present covering the pelvis and medulla of the kidney, and the tumor was separated from atrophic kidney tissue via a distinctive capsule. The mass had white grayish and yellowish areas and also dark red and darkish necrotic and hemorrhagic areas (Figure 1b). Dark red foci embedded in parenchyma were present with 0.2x0.3 cm in dimension in the left cranial lobe of the lung.

**MICROSCOPICAL EXAMINATION**

Microscopically, tumor consisted of blastemal, epithelial, and mesenchymal stromas, which were in appearance lined next to one another in the same area. Epithelial elements of the tumor were well differentiated, rounded and squamoused with rudimentary tubular structures. These tubules formed through lining up of cells in a row with basophilic and narrow cytoplasm and hyperchromatic nuclei around lumen. In addition, duplications with different degrees in tubular epithelia and shed epithelial cells with eosinophilic materials in the lumen were seen (Figure 1c). Blastemal areas were neoplastic cell clusters with round and squamouse shape including eosinophilic materials along with narrow cytoplasm, and basophilic nuclei. Periphery of these areas was tubular structures transformed from blastemal forms (Figure 1d). Occasional primitive glomerulus-like areas were observed with blastemal sliding into tubules without vascularization (Figure 1e). Mesenchymal parts consisted of fibroblast-like spindle shaped cells in clusters. These cells covered the tumor with wide sarcomatous stroma, and were divided into several lobes by thin irregular septa, and in swirl-like appearance and with no borders (Figure 1f). Mesenchymal elements were mixed between blastemal cells and epithelial tissues. Mitoses was distinctly present in blastemal areas but to some extent in other areas as well. Intensive necroses at the center and around these areas were noticed. Intensive hemorrhage in the necrotic areas, and proteinaceous materials in the lumen of cystic and tubular structures were noticed. Stroma of tumor was well vascularized, hyperemic, and with hemorrhage. Metaplastic cartilage was also seen in stroma near cortex and capsule (Figure 2a). Mononuclear cell infiltrations between dilated tubules and interstitial cortex were observed in adjacent areas of the tumor and other kidney tissues of medulla with hyaline cylinder in the lumen. Foci of metastatic tumors containing primitive tubules and areas of mostly blastemal and myxoid stroma were seen in the lung (Figure 2b). No pathological findings were present in the heart or liver.

**IMMUNOHISTOCHEMICAL EXAMINATION**

A strong immunoreactivity was observed only with cytokeratin in primitive tubular epithelia (Figure 2c) and only with vimentin in blastemal and stromal areas, using anti-cytokeratin and anti-vimentin antibodies, respectively (Figure 2d, e, f).

**Discussion**

Wilm’s tumor or nephroblastoma is most common in pigs and chickens [14]. To a lesser extent, nephroblastoma has also been reported in cattle, horses, sheep, dogs, cats, deer, and rats [4,5,10-12,14]. Spontaneous nephroblastoma is very rare in rabbits [5,9,26].

No clinical sign of embryonal nephroma are usually detected and is observed as a coincidental finding in slaughterhouses and at necropsy [12,14]. In pigs, when nephroblastoma is located only in one kidney, the other kidney compensates the entire function of both kidneys and becomes hypertrophic, therefore, no sign of renal impairment abdominal distention are observed [18]. Abdominal distention due to a large palpable sublumbar mass in cats and dogs is also an important clinical sign [18,20,24]. In addition, nonspecific clinical signs such as reduced appetite and weight loss as well as macroscopic and microscopic hematocrit can be seen [18]. Another clinical sign is hypoglycemia which is reported to return to normal following removal of an embryonal nephroma [2,3]. It has been reported that a dog with a ruptured embryonal nephroma might die due to hemoperitoneum [19]. Secondary absolute polycythaemia resulted from excessive production of erythropoietin has been reported in a case of nephroblastoma in rabbits [16]. In our case, comparisons with the literature could not be made in terms of clinical findings of nephroblastoma, [5,16,25,27] since no other information on the clinical signs except restricted movements, decreased libido, anorexia, decreased appetite, abdominal distention, and a general but fast corruption in the whole body were available.

Nephroblastoma is a congenital tumor and is formed during neonatal or fatal stage [23]. It is unnoticeable in the later stages of life unless there is a clinical sign. It is more common in young animals (<1 year), and rare in older animals [14]. No sex or breed predispositions were found, although embryonal nephroma has been reported to be more common in males than in females [18]. Due to limited findings of the history and assessing of the only one animal in the present report, our study was in accordance with those of the studies investigating tumour in young and male animal [7,12,24]. Nephroblastomas are real embryonal tumors that develop from primitive nephrogenic blastems and foci of kidney dysplasia [2,11]. The neoplasm is composed of multipotent and undifferentiated rudimentary vestigial renal tissue that retains its primitive characteristics [2,9]. Therefore, it has been suggested that there is a morphological relationship between embryonal nephroma and embryonic kidney development, and that the tumor develops either in early neonatal stage.
Figure 1. Nephroblastoma observed on the right kidney of an Angora rabbit: Macroscopic appearance (1a, 1b), Microscopic appearance of the cut section (1c, 1d, 1e, 1f).

1a. Right kidney. Appearance of the tumor mass with amorphous and elastic consistency with cystic forms weighing 875 grams and 11x12x4.5 cm in dimension on the tissue of left kidney.

1b. Tumor with lobular appearance covering the pelvis and medulla of the kidney, and separated from atrophic kidney tissue via a distinctive capsule with necroses of dark red-darkish in color along with hemorrhage at the cut surface of the left kidney.

1c. Duplications with different degrees in tubular epithelia and shed epithelial cells with eosinophilic materials in the lumen. HE, X400.

1d. Blastemal areas with neoplastic cell clusters including eosinophilic along with narrow cytoplasm, and basophilic nuclei round and oblate. Periphery of these areas with tubular structures transformed from blastemal forms. HE, X400.

1e. Primitive glomerulus-like areas. HE, X400.

1f. Mesenchimal parts consisting of fibroblast-like spindle shaped cells in clusters. These cells covered the tumor with wide sarcomatos stroma, and were divided into several lobes with thin septums irregular, without borders, and swirl-like appearance HE, X200.
Figure 2. Nephroblastoma observed on the right kidney of an Angora rabbit: Microscopic appearance of the cut section (2a, 2b, 2c, 2d, 2e, 2f).

2a. Metaplastic cartilage placing in stroma near cortex and capsule of the left kidney. HE, X200.
2b. Foci of metastatic tumors containing primitive tubules (T) and areas of mostly blastemal and miczoid stroma in the lung. HE, X400.
through neoplastic transformations or during nephrogenesis [7,9,18,24]. Some carcinogenic substances are reported to cause neoplastic transformations in rabbits [7,9]. It was reported that administrating N-ethylnitrosourea intraperitoneally to rabbits (breed of IIIVO/J) at 18th day of pregnancy were able to induce a tumor, similar to Wilm’s tumor in humans [9]. Due to a single animal in the present report and limited etiological reasons in the development of the tumor, we agree with those of the authors suggesting that tumor develops in neonatal stages [14]. Tumors can be huge and cause abdominal distention. The nephromas are firm, pale, spherical masses having nodular or lobulated appearance in cut and external surfaces [14,18]. The tumors are usually distinctive and can weigh up to 34 kg (sow) with different dimensions [18]. A distinct fibrous capsule usually surrounds the tumor, most often unilaterally and sometimes bilaterally [12]. The tumor is characteristically myxomatose, soft and sponge-like with wide hemorrhage necroses, and grayish white in color on cross sections [10]. Nephroblastosas are usually bilateral in animals [2,11], and humans, [1,23] and cause common external metastases, and thus are considered malignant [1,2,9]. Metastatic organs usually include regional, sublumbar, mesenterial lymph nodes, lungs, liver, spleen, and the other kidney. Epithelial and mesenchymal components can be found in metastatic organs [18]. More than half of the tumors in dogs are reported to have metastases of the lungs and liver [18,20]. Metastases in pigs, calves, and poultry are rare [12,15]. Nephroblastosas in rabbits are usually benign with no metastases [2,6,18]. In the present case, occurrence of lung metastases are different from those reported in the literature [2,16,22,26] which may indicate that the tumor causes metastases in a way similar to those in humans and dogs.

Histologically, the tumors contain primitive glomerulus, undeveloped tubules, and loss and spindle-type cells of stroma. Capillary vascularization, common hemorrhage, and necrotic areas in smooth or striated muscle fibers, cartilage, bone, and fat tissue are also found in the stroma of tumors. The presence of bone and cartilage tissues in the stroma indicates the metaplastic characteristic of the tumor [18]. The tumors generally consist of mesenchymal and epithelial elements. It was reported that epithelial elements are more common in pigs [12] and sheep, [21] whereas mesenchymal elements are more common in cattle, calves [18], and in dogs [5,20]. Mesenchyme tissue can be more than epithelial tissue in ruminants, and fibro sarcomas characterized by wide areas of fibrous proliferations can be encountered [9]. Differentiations of tubules and glomeruluses indicate a good prognosis, while anaplasia and sarcomatous stroma as an indication of malignant tumors indicate a good prognosis, while anaplasia and sarcomatous stroma can be more than epithelial tissue in ruminants, and fibro sarcoma as an indication of malignant tumors indicate a good prognosis, while anaplasia and sarcomatous stroma are reported in [2,14,18], which were similar to that in our case. Mitotic activity was noticeable in the tubular epithelium and blastomatous areas. Although not reported in previous studies, [2,16,17,22,26] the observation of sarcomatous stroma, atypical tumor cells, and lung metastases in the present case suggests that nephroblastoma could be characteristically malignant in rabbits.

In an immunohistochemical staining of nephroblastoma it was showed positive immunoreactivity with Avidin-Biotin-Peroxidase staining in primitive tubular cytokeratin, and positive with vimentin and desmin staining in metanephric blastem and spindle cells [24]. In another immunohistochemical staining in dogs, immunoreactivity for cytokeratin antigen in tubules, and vimentin antigen in blastemal areas were observed [27]. It was observed in horse that strong positive reaction with cytokeratin in well-differentiated tubular epithelium and squamous epithelium and vimentin positive immunoreactivity in stromal cells and foci of tubular epithelium interpreted as primitive collecting ducts [13] while a weakly positive reaction with vimentin in blastema undergoing tubular differentiation. In the present case, vimentin-cytokeratin primer antibodies gave positive immunoreactivity with blastema, stromal, and epithelial elements, and these results were consistent with the reported results [22,27].

Results of the present instance indicated that in an Angora rabbit triphasic nephroblastoma (tumor) originated from embryonal metanephric blastema, which was diagnosed using histopathological and immunohistochemical examinations.

References


