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A clinical case of a mixed testicular tumour with cryptorchidism in a dog of the Yorkshire Terrier breed

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Abstract. Histological examination of testicular tumours in cryptorchid males allows determining the nature of neoplasia, prognosis of long-term consequences in the form of metastases, and defining the optimal method of treatment of the animal, which determines the relevance of research in this area. The aim of the

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work is to determine the pathohistological structure of neoplasia of the left, cryptorchid, and morphology of the contralateral testes in a Yorkshire Terrier male with unilateral cryptorchidism. General clinical, ultrasonographic, radiological and histological methods were used in the examination of the animal. The article presents data on a mixed tumour of the intraperitoneal left testis of a 6-year-old Yorkshire Terrier, which is an atypical clinical case. It was found that the characteristic ultrasonographic features of the cryptorchid left testis tumour were numerous isoechoic foci of different diameters (from 4 to 26 mm), hyperechoic septa, and tuberos contours with a hyperechoic capsule. At laparotomy, the altered testis had an uneven surface saturated with blood vessels. The section showed milky-white neoplastic areas separated by fibrous bands that did not correspond to the normal structure of the organ. Histological examination of the parenchyma revealed areas of similar rounded cells in the form of a group of shapeless masses separated by a fibrous barrier. Neoplastic tubules had a developed fibrovascular stroma with an eosinophilic necrotic area. The protein membrane also had numerous fibrous formations. The layers of multifaceted cells were located perpendicular to the basal lamina, often with central necrosis, and the testicular mass contained multifocal haemorrhages. Such signs are characteristic of the lesion of Sertoli cells and interstitial Leydig cells, which indicated a mixed type of tumour of the left extraperitoneal testis of the Yorkshire Terrier. The contralateral right testis was unchanged and corresponded to the natural morphological and histological structure. The prostate was normal on macroscopic and histological examination, which has not been previously reported. Six-month follow-up of the dog showed no signs of metastatic process. The practical significance of the work is to obtain new scientific knowledge about the development of mixed tumours of the testis in cryptorchid males and the prospects for the treatment of diseased animals

Keywords: neoplasia of the testis; extraperitoneal testis; Sertoli cell tumour; interstitial Leydig cells; fibrovascular stroma

INTRODUCTION

Neoplasms of the canine gonads are playing an increasingly important role in small animal veterinary medicine due to the improvement of clinical diagnosis and treatment regimens. Cryptorchidism is a common abnormality of male reproductive development. With an increase in temperature (namely, body temperature), the germ cells of the testis are depleted, inflammatory reactions develop that negatively affect somatic cells (Sertoli, Leydig), and can provoke neoplasia. The strategy for treating males with cryptorchidism should be based on an understanding of the characteristics of neoplastic processes in the testes. Depending on the histological characteristics of the altered cryptorchid testis, the feasibility of using either surgical treatment alone or supplementing surgical intervention with chemotherapy is determined. The morphological characteristics of the tumour determine the choice of chemotherapeutic drugs, the scheme of their use, the list of methods for diagnosing distant metastatic processes in the body, as well as the prognosis for life, so the topic of research is relevant.

H. Aupperle-Lellbach *et al.* (2022) note that an important part of diagnosing patients with neoplasia is the histopathological examination of neoplasia, taking into account changes in the behaviour of the sick animal. According to the World Health Organization (n.d.), testicular tumours are quite common and are classified into interstitial cell tumours (Leydig cell tumours), seminomas and sustentacular cell tumours

(Sertoli tumours). Other neoplastic tumours are less common and are divided into mixed germ cell, stromal, and spermatocytic cord tumours. In dogs, testicular tumours are the most commonly reported genital tumours (16.8%), of which 56.3% are cryptorchid and 44.7% are scrotal (WHO, n.d.). Based on the results of studies of the prevalence, clinical manifestation, macroscopic and histological aspects of testicular neoplasms in males, Nascimento *et al.* (2020) noted the development of gonadal neoplasia in animals over 8 years of age.

The Fédération Internationale Cynologique Internationale has officially recognized about 350 dog breeds as being prone to diseases, including neoplasms. A group of researchers (Baioni *et al.*, 2017) noted that in the development of tumours in dogs of the German Shepherd, English Setter, Italian Hound, Maremma Shepherd breeds, the proportion of affected animals depends on the popularity of the breed in different time periods of human life. The work of C. Gradil & R. McCarthy (2023) shows that one of the risk factors for testicular neoplasia in males is cryptorchidism, which causes a 26-fold increase in the risk of Sertoli cell tumours and a 15-fold increase in seminoma. Dog breeds with more frequent occurrence of cryptorchidism than others include Chihuahua, Zwergschnauzer, Pomeranian Spitz, German Shepherd, Siberian Husky, Poodle, and Yorkshire Terrier. It has not been possible to establish the exact cause of cryptorchidism in

animals, but it is believed that this pathology is associated with a defect in the auto-recessive sex trait, which is often recorded in these breeds of dogs. The diagnosis of cryptorchidism (intra-abdominal or inguinal) is difficult, as such testes cannot be palpated, so it is advisable to use radiographic and ultrasonographic studies, which also help in the diagnosis of testicular neoplasia (Othman *et al.*, 2022).

N.I. Mykhalenko & D.V. Voitsekhovych (2017) found that interstitial cell tumours (Leydig cell tumour), seminomas and Sertoli cells are the most commonly reported tumours in dogs. Most of the publications on these testicular neoplasms are case reports of dogs of different breeds. There are only a few cohort studies that report on a population-based tumour registry in dogs (Kudo *et al.*, 2019; Manuali *et al.*, 2020), but they do not describe the clinical characteristics of testicular neoplasia. E. Chiti *et al.* (2022) report the need for curative orchiectomy for bilateral mixed testicular tumour, which has a positive result.

The analysis of the literature shows that cryptorchidism is a risk factor for testicular neoplasms in males; testicular tumours in males are common; histological examination of testicular tumours in cryptorchid males is important for establishing the diagnosis; orchiectomy gives positive treatment results; clinical and histological characteristics of cryptorchid testes with neoplasia are described sporadically and are not described in the national scientific literature. Thus, the study of a case of a mixed testicular tumour with cryptorchidism in a dog is useful for establishing the prevalence of testicular neoplasia in animals, characterizing the factors associated with the occurrence of this tumour, and describing original histological features that may be diagnostic in understanding their pathogenesis. This study aimed to histologically investigate and describe a clinical case of a testicular neoplasm removed from the abdominal cavity of a left-sided Yorkshire Terrier, which was first documented in the literature.

MATERIALS AND METHODS

The study was conducted at the Veterinary Medicine Clinic in Poltava in 2022-2023. The owners of a 6-year-old male Cryptorchid Yorkshire Terrier complained of decreased appetite and anxiety, especially at night. The dog is vaccinated against plague, parvovirus, leptospirosis, infectious hepatitis, parainfluenza, rabies. The dog is treated twice a year (in early spring and autumn) for ectoparasites. The animal consumed high quality food in accordance with all feeding standards. Water was freely available.

The dog was examined according to the following scheme: collection of anamnestic data, examination of

habitus, palpation, auscultation, thermometry, ultrasonographic examination of the abdominal cavity. Ultrasonographic examinations were performed with an ultrasound machine Sono Scape A6 (China), sectoral transducer (frequency 6.0 MHz). After the ultrasonographic detection of the neoplasm, an examination radiography of the animal was performed to exclude metastatic tumours. After that, a decision was made to surgically remove the tumour. The laparotomy was performed along the white line of the abdominal wall, according to the generally accepted method. The right testicle was removed in an open way, with ligation of the spermatic cord.

After extirpation of the testicular neoplasm, histological examinations were performed on the selected organs, which were numbered and fixed with a 10% aqueous solution of neutral formalin. The organ tissue pieces were washed under running water, dehydrated with aqueous solutions of ethyl alcohol in increasing concentration, and then embedded in paraffin. The paraffin blocks were cut on a sled microtome (type MPS-2, China) to obtain a histological section (7-10 µm thick), stained with Karatzi's iron haematoxylin and eosin. The obtained histological sections were examined under a light microscope MICROmed XS-5520 (objective ×4, ×10, ×40; eyepiece ×10, ×20). A 3 Mpix MICROmed digital camera (China) was used to take digital photos of the histological material.

The histological characteristics were assessed according to the World Health Organization (WHO) histological classification of genital tract tumours in domestic animals. All studies were conducted in accordance with modern requirements, standards, and methods (requirements of DSTU ISO/IEC 17025:2006 (2006)), Law of Ukraine No. 27 (2006), Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 (2012), European convention for the protection of vertebrate animals used for research and other scientific purposes (1986).

RESULTS AND DISCUSSION

After evaluating the results of the anamnestic data, the animal was diagnosed with sleep disorders. Abdominal wall palpation revealed a dense mass in the abdominal cavity. Ultrasonographic examination revealed an 11×13 cm mass of heterogeneous echogenic structure. The structure of the neoplasm was represented by numerous isoechoic foci, ranging in diameter from 4 to 26 mm, with hyperechoic septa of various thicknesses, uneven contours (Fig. 1). The lesion was well vascularized and corresponded to a degenerated parenchymal organ, its contours were tuberos and had a hyperechoic capsule. In general, it is impossible to determine the type of neoplasm by ultrasonography.



Figure 1. Ultrasound examination of a dense mass in the abdominal cavity

Based on anamnestic data, a diagnostic and therapeutic laparotomy was performed. An abdominal examination showed that the neoplasm was a modified left testis, which was removed. The right testis was unchanged and corresponded to the anatomical characteristics of clinically healthy dogs, with its own vaginal membrane formed by serous tissue, extending to the epididymis and mesentery. The connective tissue capsule of the testis was dense, closely fused with the

serous membrane, and from the head end of the organ it was immersed in its thickness and formed the mediastinum of the testis. Numerous partitions extended from the mediastinum, dividing the testis into separate chambers. The right testis was small, 22×16 mm in size, round-ellipsoidal in shape.

Histological examination of the contralateral testis showed that the protein membrane was a dense collagenous tissue with elastic fibres and formed partitions that divided the testis into lobes. The basis of the lobules was loose connective tissue permeated with tortuous seminiferous tubules, which represented the parenchyma. The wall of the seminiferous tubules was formed by its own membrane, which had basal, myoid and fibrous layers. Thus, the histological structure of the contralateral testis was normal.

Macroscopically, the retroperitoneal cryptorchid testis was characterized by the presence of an uneven, hyperaemic surface with dilated vessels (Fig. 2A). Loss of normal architecture was observed on the surface of the testis section. Multifocal cavitation haemorrhages were recorded over the entire area of the incision and on the surface of the testis (Fig. 2B). The prostate gland and mediastinal lymph nodes were severely enlarged. Homogeneous white parenchymal masses separated by dense connective tissue septa were found on the section.

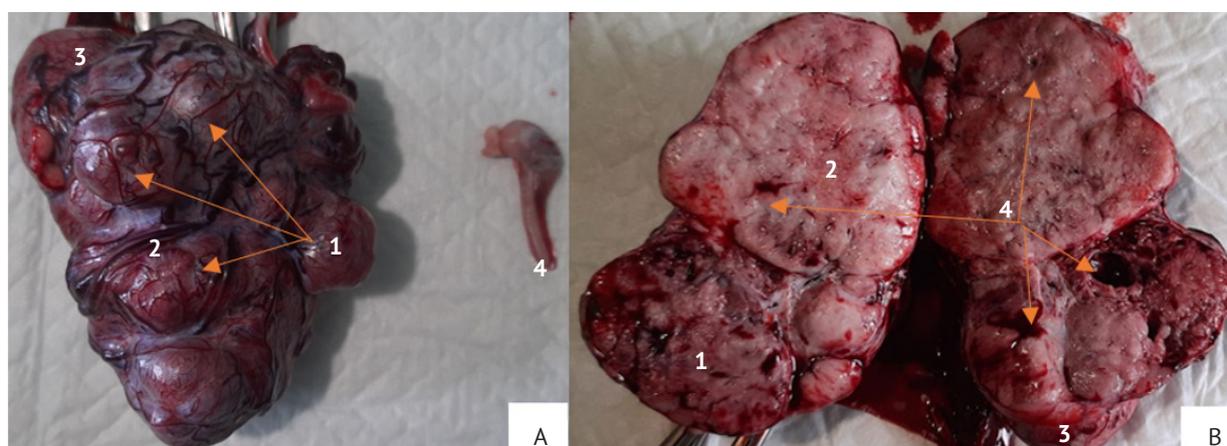


Figure 2. Macroscopic changes of the testis during unilateral cryptorchidism

Note: A – Semin (cryptorch) with the development of a seminoma: 1 – neoplasm; 2 – venous stasis; 3 – epididymal appendage; 4 – right testicle, without pathology (removed by castration). B – macroscopic changes in the testicle (sagittal section) with unilateral cryptorchidism: 1 – neoplasm; 2 – testicle parenchyma; 3 – accessory edge of the testicle; 4 – multifocal cavitation haemorrhages

Pathologically, the retroperitoneal cryptorchid testis was diagnosed as a mixed sertolioma (a tumour of Sertoli and Leydig cells of non-germ cell aetiology). Histological examination of the neoplasms revealed areas of parenchyma with similar rounded cells (Fig. 3A) with clear contours, light cytoplasm and large nuclei that formed conglomerates of shapeless masses (Fig. 3G). Such groups of cells were separated by fibrous partitions (Fig. 3B). In some areas, the growth

of germ cell epithelium was observed, which filled the cavity with the convoluted seminiferous tubules (Fig. 3B, 3C), which led to the damage to Sertoli cells, and thus made further gametogenesis impossible. Similar research results have been reported by other researchers, in particular, the authors pointed to the coexistence of a Sertoli cell tumour and proliferation of interstitial Leydig cells in a male that was not a cryptorchid (Chiti *et al.*, 2022).

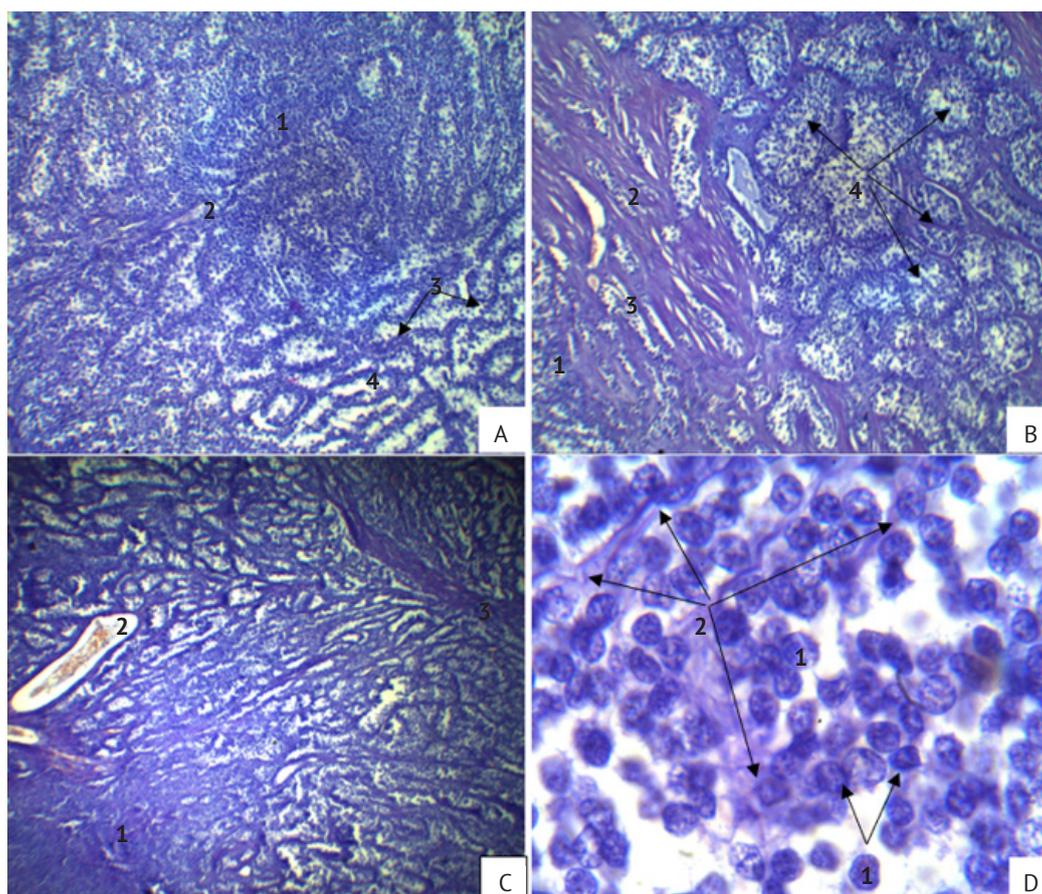


Figure 3. Histological changes of the testis in male seminoma

Notes: Haematoxylin and eosin staining. Magnification Ax64, Bx64, Cx160, Dx640. A: 1 – growth of germinogenic epithelium; 2 – capillary blood filling; 3 – walls of tortuous seminiferous tubules; 4 – lumen of tortuous seminiferous tubules; B: 1 – growth of germinogenic epithelium; 2 – connective tissue elements forming testicular septa; 3 – leukocyte infiltration; 4 – tortuous seminiferous tubules; C: 1 – growth of germinogenic epithelium; 2 – capillary blood filling; 3 – connective tissue elements forming testicular septa; D: 1 – germinogenic epithelium in the lumen of the seminiferous tubule behind seminomas; 2 – fibrous partitions;

It was found that the protein membrane of the retroperitoneal cryptic testis was thickened, with numerous fibrous formations. Densely packed neoplastic tubules of irregular size were separated, with a well-developed fibrovascular stroma. The convoluted seminiferous tubules consisted of many layers of moderately pleomorphic spindle-shaped cells or multifaceted cells arranged perpendicular to the basement membrane, with or without central necrosis. These tumour cells had a rounded or elongated nucleus and/or dense eosinophilic or vacuolated cytoplasmic lesions with an indistinct cell border and pattern. Some large neoplastic tubules contained an eosinophilic central necrotic area. Many small immature tubules penetrated the thickened protein membrane. Severe multifocal haemorrhages and necrotic foci were also observed in the testicular mass. Morphologically, the tumour cells resembled Sertoli cells in the testis.

Unilateral cryptorchidism provokes the development of testicular parenchymal atrophy, causing dystrophy, necrosis, and neoplasia, which subsequently

grows into metastatic tumours (Crescio *et al.*, 2022). According to the study of the clinical condition of the dog, after removal of the neoplastic cryptorchid testis, no metastases were detected within six months of development. The data obtained on the clinical signs of this pathology only partially correspond to similar cases of cryptorchidism, in particular, in bilateral cryptorchidism in an 11-year-old poodle, clinical signs were characterized by bilateral symmetrical alopecia swelling of the prepuce, gynaecomastia, seborrhoeic dermatitis with scales (DeForge, 2020), in a 4-year-old Lhasa Apso Cryptorchid – hyporexia and tremors (tremors), blood tests in case of cryptorchid neoplasia in dogs were characterized by anaemia and thrombocytopenia. There have also been reports of lameness and tenderness on scrotal palpation (Padaliya *et al.*, 2023). Considering the results of studies by other authors, it can be noted that the dog reported in this article had only sleep disturbance as clinical symptoms. Palpation revealed the presence of a tumour in the hypogastrium,

but the animal did not demonstrate pain. The appetite was slightly reduced.

Most veterinarians are of the opinion that the clinical signs of cryptorchid testicular neoplasia are not specific, so a combination of imaging methods is used for the final diagnosis – abdominal ultrasound, colour Doppler, and plain radiography (Tannouz *et al.*, 2019). To confirm the diagnosis, an ultrasound examination of the abdominal cavity was used, which revealed the formation of a heterogeneous echogenic structure with a hyperechoic capsule of uneven contours. Similar changes in the studies of dogs with testicular tumours, including cryptorchid tumours, were noted by Italian researchers R. Orlandi *et al.* (2022), including complex structural formations surrounded by a hyperechoic capsule. Along with testicular neoplasia, an enlargement of the prostate gland is reported, its parenchyma becomes heterogeneous and alternates with polycystic formations (Sun *et al.*, 2017), which was not observed in the case described in this article. The prostate gland ultrasonographically, macroscopically and histologically did not differ from that of clinically healthy animals. Probably, the condition of the prostate was maintained by the endocrine function of the non-cryptic testis. Obviously, in bilateral cryptorchidism, the hormonal status changes more clearly than in unilateral cryptorchidism.

One of the imaging methods for neoplasia is radiography, which can detect metastases to bone tissue, parenchymal and other organs. Radiological diagnostics in separate studies by M. Russo *et al.* (2021) demonstrated bone proliferation and lysis of the humerus, lesions of the left tenth rib and lumen of both proximal humerus metaphysis. At the same time, the described cases of radiography do not indicate a clear localization of the testis into the hypogastrium, which is associated with a variety of breed characteristics and pathophysiological conditions. According to other researchers, in particular E. Park *et al.* (2017), in the case of a combined tumour (Sertoli cells and interstitial Leydig cells), no metastasis was detected on plain radiography. This indicates the feasibility of surgical treatment. In order to assess the degree of the pathological process, it is proposed to determine the concentration of testosterone and estradiol in the blood, the level of which increases in the case of cryptorchidism.

Intraperitoneal testes (cryptorchid) have a high risk of developing neoplasia compared to intrascrotal testes. Changes in the cryptorchid testis are macroscopically characterized by dense parenchyma and the presence of rounded lesions in the periphery. Leydig cell hyperplasia is more common in older dogs, while Sertoli cell tumours, seminomas and leydigomas are reported in the testes with approximately equal frequency, but a significant percentage of neoplasia in retroperitoneal cryptic testes is diagnosed as seminoma. Histopathologically, there is abundant eosinophilic cytoplasm with round (oval) nuclei and punctate nucleoli (Pecile *et al.*, 2021).

In the case of malignant seminoma, metastases penetrate the lymph nodes and bones, with possible foci in the scrotum, vaginal membrane, and, after a while, the skin and perioesophageal structures.

In order to treat metastatic seminoma in dogs, multimodal therapy consisting of surgery and chemotherapy is used (Kisani *et al.*, 2017). In the case described in this article, both the retroperitoneal cryptorchid testis and the contralateral unaltered testis were surgically removed from the dog. Since the tests performed on the animal did not reveal a metastatic process, chemotherapy was not used. Further monitoring of the dog's condition, as already reported, showed the appropriateness of this decision. According to V.G.S. Tannouz *et al.* (2019), unilateral cryptorchidism has a right-sided localization, which is due to the fact that the right testis is formed more cranially than the left, and therefore the path to the scrotum is longer. An unusual case of unilateral cryptorchidism of the left testis with neoplasia was recorded in the dog under study, which has not been reported in the literature.

CONCLUSIONS

Clinical examination of a 6-year-old male Yorkshire Terrier revealed a dense 11×13 cm mass in the abdomen. Ultrasound examination of the mass was characterized as a neoplasia with numerous isoechoic foci with hyperechoic trabeculae of various shapes and contours, with advanced vascularization. Given the history of unilateral cryptorchidism, the tumour was considered as a neoplasia of the extraperitoneal testis. Given the potential malignancy of the neoplastic process described in the literature, an additional plain radiography was performed, which did not reveal metastases in the thoracic cavity and skeleton. The extraperitoneal testis was surgically removed, as well as the contralateral testis.

Histological examinations showed that the parenchyma consisted of conglomerates of shapeless masses formed mainly by uniform round cells, light cytoplasm and large nuclei. In addition, there was an overgrowth of germ cell epithelium, filling the cavity with the seminiferous tubules. Thus, structurally, the tumour is a combination of Sertoli cells and interstitial Leydig cells. At the same time, the contralateral testis had a natural histological structure. The prostate gland looked normal macroscopically and histologically, which was not previously described. Postoperative six-month follow-up showed no evidence of metastatic disease. This study emphasizes the importance of early diagnosis and treatment of neoplastic tumours in animals, especially those with certain risk factors such as cryptorchidism, and shows that histological analysis is an important tool in determining the structure of the tumour and choosing further treatment tactics. Additional monitoring and statistical data may help to develop a better understanding of the frequency and nature of neoplastic processes in cryptorchid males, as

well as their impact on the health of these animals in the future. The results of the study also highlight the importance of an interdisciplinary approach to medical diagnosis and treatment of animals, including the use of ultrasound, histology and other methods to obtain a complete clinical image of the tumour. The data also indicate the possibility of developing more individualized treatment methods, taking into account the histological characteristics of each specific case of neoplasia in an animal. This approach can improve treatment outcomes and increase the chances of recovery.

The prospect of further research is the accumulation of our own statistical data on the frequency of neoplasia in cryptorchid males, their histological structure and the impact on metabolic processes in the body of these animals.

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CONFLICT OF INTEREST

None.

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Клінічний випадок змішаної пухлини сім'яника з крипторхізмом у собаки породи йоркширський тер'єр

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Анотація. Гістологічне дослідження пухлин тестикул у кобелів-крипторхів дозволяє визначити характер неоплазії, прогноз розвитку віддалених наслідків у вигляді метастаз, визначити оптимальний метод лікування тварини, що визначає актуальність досліджень у цій сфері. Метою роботи є визначення патогістологічної будови неоплазії лівого, крипторхічного, та морфології контрлатерального сім'яників у кобеля породи йоркширський тер'єр за одностороннього крипторхізму. У обстеженні тварини використано загальноклінічні,

ультрасонографічні, рентгенологічні та гістологічні методи дослідження. У статті наведено дані щодо змішаної пухлини внутрішньоочеревинного лівого сім'яника йоркширського тер'єра віком 6 років, що є нетиповим клінічним випадком. Встановлено, що характерними ультрасонографічними ознаками новоутворення крипторхового лівого сім'яника були численні ізоехогенні осередки різного діаметру (від 4 до 26 мм), гіперехогенні перегородки, горбисті контури з гіперехогенною капсулою. За лапаротомії змінений сім'яник мав нерівну просочену кровонаповненими судинами поверхню. На розрізі реєстрували молочно-білі неопластичні ділянки, розділені волокнистими смугами, які не відповідали нормальній будові органу. За гістологічного дослідження в паренхімі реєстрували ділянки однотипних округлих клітин у вигляді групи безформної маси, що були розділені фіброзним бар'єром. Неопластичні канальці мали розвинену фіброваскулярну строму, з еозинофільною некротичною ділянкою. Білкова оболонка також мала численні фіброзні утворення. Шари багатогранних клітин розташовувались перпендикулярно до базальної пластинки, часто з центральним некрозом, тестикулярна маса містила мультифокальні крововиливи. Такі ознаки характерні для ураження клітин Сертолі та інтерстиціальних клітин Лейдіга, що вказувало на змішаний тип пухлини лівого позаочеревинного сім'яника йоркширського тер'єра. Контрлатеральний правий сім'яник був без змін і відповідав природній морфогістологічній будові. Простата за макроскопічного та гістологічного дослідження відповідала нормі, що раніше не повідомлялось. Шестимісячне спостереження за собакою наявність ознак метастатичного процесу не виявило. Практичне значення роботи полягає у отриманні нових наукових знань щодо розвитку змішаних пухлин сім'яника у кобелів-крипторхів та перспектив лікування хворих тварин

Ключові слова: неоплазія сім'яника; позаочеревинний сім'яник; пухлина клітин Сертолі; інтерстиціальні клітини Лейдіга; фіброваскулярна строма
