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The clinical, imaging and microscopic evaluation of hepatic tumoral processes in dogs

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Abstract Veterinary oncology studies have stated the scarcity of hepatic primary tumors in dogs, as well as the role of the liver as main target organ for the metastases of many tumors developed in other organs and tissues. This study is structured in two parts including clinical and imaging data, as well as paraclinical and microscopic data. The importance of clinical examinations as keys to the identification of hepatic general pathology is emphasized; they must be complemented by the imaging exams in order to confirm the presence of lesions in this organ. The described exams have been performed in 25 dogs in order to diagnose the presence of hepatic neoplasias. The results of blood tests and microscopic examination have a pivotal role in establishing the etiology of the hepatic tumoral pathology. The study highlights the foundations of the examination strategy that is to be assumed when diagnosing hepatic tumoral pathologies in dogs.

Keywords Dog, liver, tumor, exam.

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Introduction

Of the entirety of tumoral pathologies encountered in dogs, primary hepatic tumors are assigned a percentage between 0.6 and 1.3% (Patnaik et al [33]). Arrais Aloia et al. [1] mention the following main types of proliferative diseases of the liver in dogs: nodular hyperplasia, hepatocellular carcinoma/adenoma, cholangiocellular carcinoma/adenoma and hepatoblastoma. It is assumed that neoplasms of the hepatocellular type are more frequent in dogs, those of the cholangiocellular type occurring more frequently in cats (Stalker and Hayes [38]); nevertheless, this assumption has not been unanimously confirmed by the studies on this topic (Cullen and Popp [13]). According to Scherk and Center [36], the peak incidence of hepatobiliary neoplasms in dogs occurs at 10 to 12 years of age.

Generally, hepatic neoplasms in dogs are classified as adenomas, carcinomas, cholangiocarcinomas, mixed forms of carcinomas and cholangiocarcinomas and carcinoid tumors (Cullen and Popp [13], Stalker and Hayes [38]). Moreover, it is known that the patients (dogs) are brought to consultations when in advanced stages of tumoral pathologies, due also to the extraordinary capacity of the liver for function recovery (Wypij et al [44]). Investigations of the liver under suspicion of a tumoral pathology comprise the clinical examination, the imaging examination, blood tests and macroscopic and microscopic examination of anatomic pathology specimens.

Clinical examination

Owners usually bring dogs to be examined in subacute to chronic stages, since the evolution of hepatic tumors is an insidious one; only processes that include bile ducts and the bladder evolve with signs of pain (Wypij et al [44]). The main symptoms consist of digestive disorders (vomiting and the modified aspect of the feces) and the visible weakening of the organism; on the other hand the abdomen is enlarged, either due to hepatomegaly or due to ascitis. Sometimes jaundice may also be present, manifesting itself through the yellow color of the mucosal surfaces and skin.

Deep palpation, performed of a penetrating manner under the right costal margin, highlights the moderate pain sensitivity over the hepatic projection area. In case of ascitis, abdominal palpation shall be performed bimanually and bilaterally, assessing the increased tension in the abdominal walls and if possible the wave-like sensation caused by the movement of the fluid.

Radiological exam

The radiological examination provides good diagnosis directions to practitioners.

The radiological examination of the liver implies using the following positions (patient positioning): lateral left and right and ventro-dorsal. The radiological assessment of the liver is limited, but it may orientate a large amount of cases to other imaging exams, such as ultrasound (Nyland and Park 1983, Cullen and Popp [13], Vulpe and Meomartino 2014).

In the right lateral position, the hepatic silhouette appears slightly larger and its caudoventral edge appears superimposed on the spleen to a higher extent than in the case of the left lateral position. Saritaş and col. (2014) found caudal enlargement in the neoplastic liver in a ventro-dorsal radiograph. Hepatomegaly, or liver enlargement, may be detected radiologically, although minor size modifications cannot be assessed precisely. Classical radiological signs in generalized hepatomegaly consist of the rounding or "blunting" of the caudo-ventral edges of the liver, accompanied by the extension beyond the right costal margin. Liver enlargement leads to the compression and change of topography in other adjacent organs (stomach, spleen, intestines); sometimes, analyzing the image, diffuse radioopacity may be found over the entire abdominal region and the decrease of the visibility of the serous surfaces – this signifies the presence of ascitis (Figure 1, Figure 2).

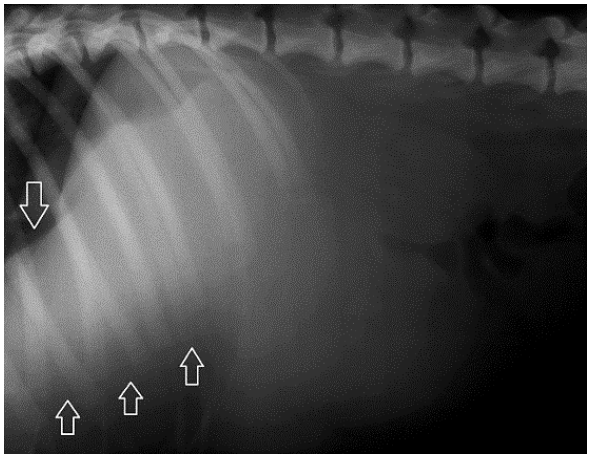

Figure 1. Dog, 9 y.o. Abdominal radiography, lateral positioning. Radioopacity extended over the liver projection area with displacement (and enlargement) of the spleen and kidney – the arrows are pointing to the initial, topographically normal position of the edge of the liver (original photograph).

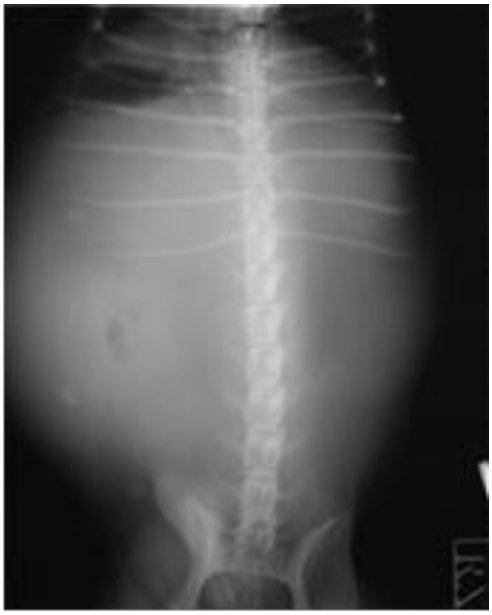

Figure 2. Dog, 5 y.o. Abdominal radiography, VD positioning. Radioopacity extended over the entire abdominal region due to ascitis (original photograph).

Radiological examination is frequently used for identifying pulmonary metastases of hepatic origin (Figure 3).

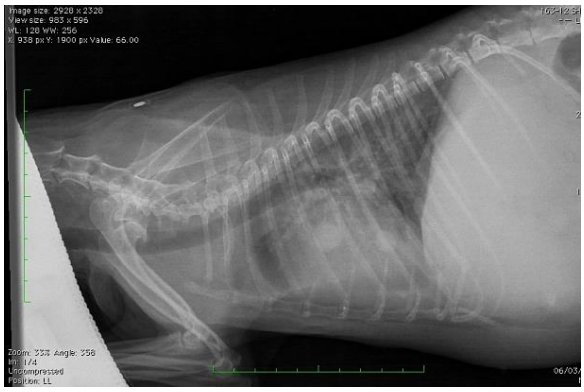

Figure 3. Bitch, cross-breed, 13 y.o. Lateral thorax radiograph; Mixed pulmonary pattern with the presence of two well delimited formations on the cardiac area. Pulmonary metastases (original photograph).

Ultrasound

This is a widely-used method in liver examination, providing data on the organ echostructure (Crabtree et al [11]). Conventional biopsy is preferred for histological confirmation of diffuse benign hepatic diseases; most ultrasound-guided fine needle biopsy studies provide a diagnosis in 77% up to 94% of the cases. Lately, punction has become ultrasound-guided using the Doppler system – the technique is used especially in patients harboring focal hepatic lesions (Begon [4]).

There are several ultrasound-guided fine needle biopsy techniques. Transducers with biopsy needle canals are used or the needle is inserted through an attachment on the lateral side of the transducer. The thinner the punction needle (punction cytology using 22 and 21 gauge needles), the lower the technique sensibility (the hepatic tumor cytology examination requires a highly competent cytologist), but the risks are also much lower compared to those of a punction using a 18 or 19 gauge needle for the histologic exam (Cole et al. [9], De Rycke et al [14]).

The normal liver has a homogeneous echogenicity, its edges being sharp and flat. The hepatic vein and portal vein appear as anechogenous structures. The gall bladder is anechogenous, round and pear-shaped. Many diffuse hepatic diseases cause detectable ultrasonographic modifications only in advanced stages, when an increase or decrease in echogenicity may be found. Hyperechogenicity implies the increase of the echogenicity as compared to that of the renal cortex or of the spleen. Focal hepatic disease is much easier to differentiate from normal regions of the liver (Figure 4, Figure 5). Hyperplastic nodules appear as hypoechoic or isoechoic structures. In neoplastic processes, the liver may appear as an inhomogeneous structure with areas of varying echogenicity (Lodi et al [28], Nyland and Park 1983, Kealy and McAllister 2005). Jain et al (2016) found using ultrasonography multiple hypoechogenic nodules with irregular borders in a case of hepatocellular carcinoma in a German Shepherd.

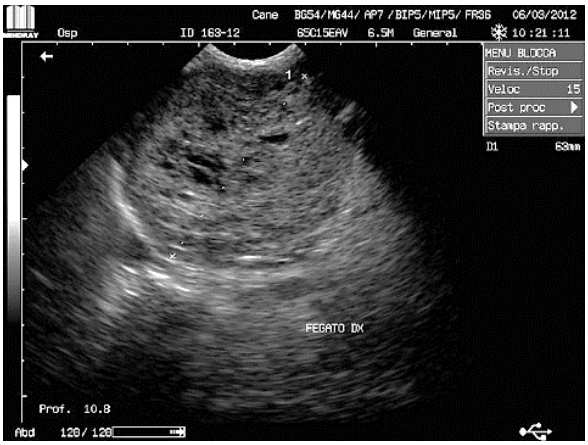

Figure 4. Bitch, cross-breed, 13 y.o. Liver ultrasound; well delimited area, with nonhomogeneous echostructure and small areas of anechogeneous character. Metastatic neoplasia of a mammary adenocarcinoma (original photograph).

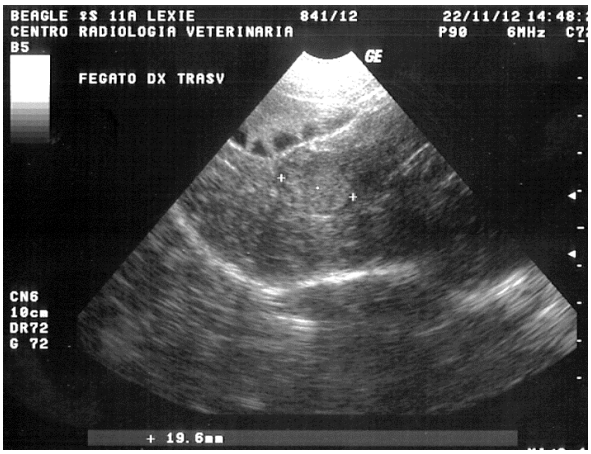

Figure 5. Bitch, Beagle, 11 y.o. Liver ultrasound; diffuse hyperechogeneous aspect, well delimited central area. Metastasis of a renal adenocarcinoma (original photograph).

Computed tomography is an ever more frequently used imaging technique in veterinary medicine. Computed tomography performed without contrast substance detects lesions with different densities from that of the normal liver. Contrast medium is then injected into an intravenous bolus, followed by intravenous perfusion of the contrast medium and the execution of step II of the examination. The vascular canals become opaque during the contrast infusion and can be compared to the low density canals representing the hepatic and portal veins during the first step (Schwarz and Saunders 2011).

The detection of focal or diffuse hepatic anomalies depends mainly on the difference in contrast from the normal liver. By performing sections after the iodinated substance injection, one may find vascular hepatic lesions that were of the same density in the primary phase of the examination. In the case of vascular lesions of the hemangioma type, they are quickly injected, initially presenting lower density than the rest of the tissue. The increased density compared to the rest of the liver appears in the hemangioma at 5-10 minutes from injection, and this aspect, combined with the hypodense aspect from the initial step, constitute signs typical of hemangioma. An early and persistent increase of the density after injection is achieved in hepatic lymphoma, the excessive accumulation probably a result of tumoral "capture" (Fukuda et al. [17]).

Comparing classical computed tomography to ultrasound, most studies conclude that the former is of higher value (Irausquin et al [19], Fukushima et al [18], Fukuda et al [17]). This is due to the need for finding images of different density from that of the hepatic parenchyma. The advantage of computed tomography consists of the use of contrast medium, which increases the density of initially hypodense hemangiomas. Moreover, some initially „invisible" tumors are rendered visible by applying contrast medium because their density does not change; another advantage consists of the increasingly frequent application of computed tomography-guided punction or biopsy (Cerci et al [7], Fukushima et al [18]).

Magnetic resonance imaging (MRI) allows the visualization of the studied volume as direct sections obtained not only in the transverse plane (as in CT), but also in the frontal and sagittal plane. Fatty tissue, found adjacently to the aponevroses in the abdominal wall, as well as in the sub and retroperitoneal space, provides a high-resonance signal. Blood vessels that are found in parenchymatous organs (liver, kidney, spleen) or in the peritoneal or retroperitoneal space emit a very weak or no signal. The liver appears as a structure with a homogeneous signal, surrounded by perivisceral fat that provides good contrast. The liver is crossed by vascular structures that are more visible in images. Thus, it is possible for numerous lesions with a cystic structure and liquid content to be distinguished as finely as solid lesions (Dennis 2003, Clifford et al 2004).

With its multiple section planes, MRI has the advantage of locating any tumors, especially in the posterior hepatic quadrant, where the transverse images obtained by CT make it hard to differentiate between adrenal, renal and hepatic tumors. The evaluation of the MRI sensitivity in the diagnosis of hepatic tumors also depends on their dimensions; the imaging aspect of hepatocarcinomas varies depending on the extent of steatosis and fibrosis. Ebara and col. (1986) describe various aspects related to hypo-, isointensity, hyperintensity and inhomogeneous appearance in tumoral processes.

Imaging examination of the gallbladder

The simple radiological exam of the gallbladder does not yield important details on gallbladder pathology. Cholecistography is nevertheless infrequently used, since obtained data is limited to the diagnosis of biliary stasis.

Ultrasound is the most frequently used, as an easy diagnosis tool for inflammations, but also for tumoral processes at this level (Bennet et al 2001). Suspicions of cholangiocarcinoma (Figure 6) may arise when using color Doppler ultrasound (Komuta et al 2008).

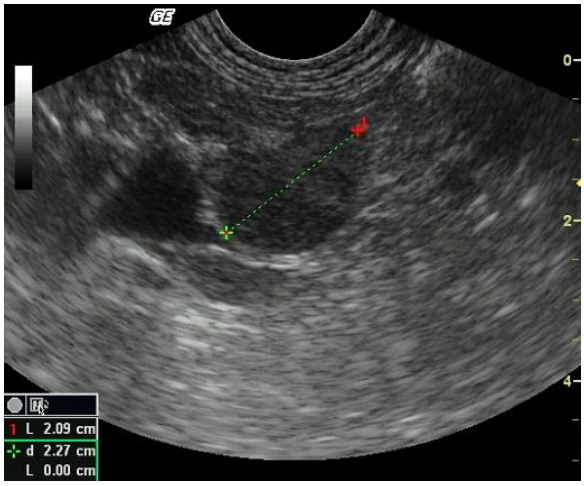

Figure 6. Bitch, Teckel, 8 y.o. Liver ultrasound, formation attached to the common hepatic duct. Suspicion of cholangiocarcinoma (original photograph).

Examination of blood parameters

The measurement of biochemical blood parameters alone does not suffice for a diagnosis, but in association with other types of examinations (clinical examination, abdominal imaging examination comprising radiology, ultrasound and CT, cytological examination) it increases the possibility of distinguishing between the involved pathologies as well as neoplasia types.

In the clinics for hepatic disease of a suspected tumoral nature, white blood cell composition and blood biochemistry analyses are used. Leukocytosis, thrombocytopenia (found in approx. 50% of the dogs with hepatocellular carcinoma) and moderate nonregenerative anemia are found in dogs with hepatic tumors.

Serum enzymes measured by biochemical blood examination include alkaline phosphatase (ALP), alanin aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamiltransferase (GGT). In a study performed on 48 dogs with hepatocellular carcinoma (Liptak et al [26]), the following upper limits were proposed for these enzymes: 141 U/l for ALP, 120 U/l for ALT, 40 U/l for AST and 6 U/l for GGT. A case study published by Teshima and col. (2013) proposes the following reference ranges: 14-44 U/l for AST, 14-68 U/l for ALT, 47-254 U/l for ALP and 2-15 U/l for GGT.

According to Liptak et al. [25], alkaline phosphatase and alanin transferase present increased values usually in dogs with primary hepatic tumors, while in the case of tumors with hepatic metastases, increased values of the bilirubinemia and AST may be found. Increased alkaline phosphatase values may reflect the presence of a neoplastic process located in the bile duct, causing its obstruction; thus, the increase of this parameter is greater in neoplasias than in degenerative processes. Some studies show that this comparison also stands when considering bilirubinemia (Neumann 2004). Pronounced increases in ALT and AST values indicate a grave prognosis, signifying a fast-paced development of the hepatic tumor associated with a considerable size and high malignity (Center [6], Liptak [27]). Wypij et al [44] also mention that in the case of dogs undergoing surgical intervention for cholangiocarcinoma extirpation, increased creatininemia frequently suggests a grave prognosis.

Other biochemical modifications frequent in dogs with hepatic tumors consist of hypoalbuminemia, hypoglobulinemia, increase in bile acids, as well as altered synthesis of coagulation factors (Balkman [3]). Exceptionally, a study published by Cooper et al [10] reported a single case of hyperalbuminemia consequent to the development of hepatocellular carcinoma, advancing a hypothesis of increased albumin synthesis by malignant hepatocytes as well as one of diminished negative feedback due to altered osmocellular receptivity. Hypoglycemia constitutes a less frequently encountered consequence of hepatic adenocarcinoma in dogs, resulting from the synthesis of a substance similar to insulin (Cullen and Popp [13]).

According to a study published by Yamada et al [45], the determination of serum alpha fetoprotein, a parameter also used in human medicine, is of great aid in differentiating types of primary hepatic tumors, as well as differentiating primary tumors from hepatic metastases: in dogs with hepatocellular carcinoma, the average value reaches 2860 ng/ml, unlike in cholangiocarcinoma cases, with the average value of serum AFP of 75 ng/ml, or cases with non-tumoral pathologies, with an average value of 310 ng/ml, relatively close to that from cases with metastatic tumors (366 ng/ml). The presence of increased AFP values in dogs with hepatocellular carcinoma was confirmed by Kitao et al [23] after performing preoperatory biochemical examination in 4 dogs.

Lodi et al [28] state that cytological diagnosis represents an efficient and quick diagnostic tool, and total proteinemia does not undergo significant changes in hepatocellular carcinoma cases.

In the cases that we studied, working techniques were based on blood samples for hematological and serum biochemistry exams obtained by punction of the saphene, cephalic or jugular vein. Blood was collected in vacutainer tubes with anticoagulation agent (EDTA) for hematological exams and clot–activator tubes for serological exams. During the current clinic activity, blood samples were collected when the general state of the patient had to be

evaluated quickly (severely modified) or when imaging exams did not suffice.

Of the 23 cases of hepatic carcinoma, 17 were subjected to hematological analyses. All these cases presented anemia, with a decrease in red blood cell count by up to 2,1 mil/mm^3 below the accepted lower limit (5,7 mil/mm^3) and by up to 5,3 g/dL below the accepted lower limit (12,9 g/dl) for hemoglobin. Thrombocytopenia was found in 13 of the 17 analyzed cases, with a decrease reaching 55 000/mm^3. Leukocytosis was found in 10 cases, the white blood cell increase reaching 4500/mm^3.

The anemia syndrome (normocytic, non-regenerative) accompanying the hepatic carcinoma was consequent to the chronicization of the tumoral process and hepatic function alteration, as well as iron deficiency. Leukocytosis is encountered in over half of the hepatic carcinoma cases, since the gradual, but continuous compression by the tumor on the (normal) marginal hepatic tissue is accompanied by inflammation, necrosis and vascular disorders (Morris and Dobson [30]; Liptak et al [25]).

All 17 cases presented hypoproteinemia (decrease up to 2 g/dL) and hypoglycemia (decrease up to 24 mg/dL); moreover, an increase was found in ALT by up to 350 u/dL (accepted higher value of 107). In 11 cases, the ALP enzyme increased by up to 55 u/dL (accepted higher value of 150). In eight cases, an increase in GGT by up to 57 u/dL (accepted higher value of 14) was found. In nine cases, an increase in AST by up to 67 U/dl (accepted higher value of 55) was found; six cases presented an increase in total bilirubin by up to 2 mg/dl (accepted higher value of 3).

Our results are coherent with those found in the literature. The increase of the transaminase values and especially the maintenance of the AST/ALT ratio below 1 indicate the presence of complex hepatic tumors, with the possibility of occurring metastases. In addition to these one can also mention the increase in alkaline phosphatase, which may suggest the presence of hepatic primary tumoral processes. If bilirubin is also increased, this signifies that the tumor may have also reached the intrahepatic biliary system or even the gallbladder (as in the case of the cholangiocarcinoma found by us) or that the hepatic tumor is a metastasis. Hypoglycemia and hypoproteinemia provide details about the organ function, the diminishment of the gluconeogenesis function etc. (Liptak et al [25]).

Microscopic examination

The microscopic examination is performed on samples collected by fine needle punction (cytological examination) or by live biopsy or on samples collected at necropsy. Frequently, for an accurate diagnosis, excision biopsy or cup forceps biopsy is used, since samples of adequate dimensions can be taken easily and safely from multiple hepatic lobes, providing a realistic representation of the disease (Wang et al [43]). Samples collected antemortem require analyses for the verification of the quality of hemostasis in the patient: physical exam, blood smear, routine coagulation tests, activity in high risk breeds and bleeding time of the oral mucosa.

Anatomic pathology exams were performed on liver samples from the Necropsy diagnosis service of the Faculty of Veterinary Medicine of Iaşi. Samples were fixed in 10% buffered formalin solution and paraffin embedded with a LEICA TP1020 (LEICA MICROSYSTEMS GmbH, Germany) tissue processor. 5µm thick sections were made with a SLEE CUT 6062 (SLEE MEDICAL GmbH, Germany) microtome, then deparaffinized and HE (hematoxylin – eosin – methylene blue)-stained. Photographs were made using an attached LEICA ICC50 HD digital camera with LEICA APPLICATION SUIT SOFTWARE (LAS) version 4.2.

The results led to an anatomopathological picture specific to hepatic tumoral disease diagnosed in dogs from the north-eastern region of Romania (2012 – 2014): hepatic carcinoma (11 cases), cholangiocarcinoma (1 case), adenocarcinoma (8 cases), lymphosarcoma (4 cases), 1 multiple myeloma.

Primary tumors consisted of hepatic carcinoma and cholangiocarcinoma, and their microscopic diagnosis is presented below.

Hepatic carcinoma

It is the malignant tumor of hepatocytes and it was identified in old dogs (6-14 years). Hepatocarcinomas are an important pathological process in both human and veterinary medicine, presenting a vast number of highly similar histopathological aspects. Hepatocarcinomas are the most frequent primary tumor in dogs (Patnaik et al [34]); however, the etiology of spontaneous HCC in dogs remains unclear. The histological aspect of carcinomas varies highly depending on the degree of differentiation and the tumoral hepatocyte disposition. There are three types of proliferation specific to hepatocellular carcinoma, as specified in the literature: the trabecular type, the adenoid or pseudo-glandular type and the compact type (Baba [2], Cullen [12]). There can be one or more types of proliferation in a tumor. Masserdotti and Drigo [29] reported the possibility of aberrant cell arrangements and abortive attempts to form bile canaliculi occurring in adenoid tumors. We identified the compact type of carcinoma (Figure 7) and the trabecular one (Figure 8).

The tumors were multiple (multicentric growth), infiltrated in the liver parenchyma and liver capsule, accompanied by the dilatation of the Kiernan space and afferent vessels (Figure 9). In some cases, the tumors invaded the gall bladder too, but in other cases they were also found in the omentum and diaphragm and as metastases in the portal lymph node and spleen.

Cholangiocarcinoma

It is a malignant tumor of the biliary epithelium. The data in the literature shows that this tumor may present moderate to pronounced cellular and nuclear pleomorphism, possessing high mitotic activity (Van Sprundel et al [40]). Javanbakht et al [21] reported the existence of both tubular and acinar patterns, as well as the cuboidal and columnar aspect of neoplastic cells with nuclei ranging from oval to round in cholangiocarcinoma.

The primary tumor is located in the liver and was found by us in a 12 year old Pinscher dog. From a histological standpoint, differentiated neoplasias are composed of cubic or columnar epithelial cells, with clear or finely granular acidophilic cytoplasm and large, vesicle-like nuclei. Bile ducts have a dilated appearance. The epithelium is composed of cubic or rod-shaped cells with large, round nuclei, disposed in one or more layers, with intratubular papilliferous proliferations. Inside the tumoral nodule there are many smaller canals and wide areas with polymorphous cells, anisokaryosis and numerous mitotic figures (Figure 9). The connective stroma, well represented, infiltrates the tumoral mass, delimiting and grouping neoplastic cells.

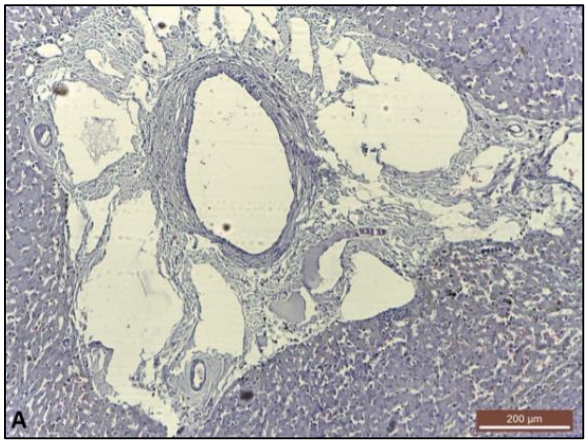

Figure 7. Hepatic carcinoma, dilated venules in the Kiernan space. HE x400 (original photograph).

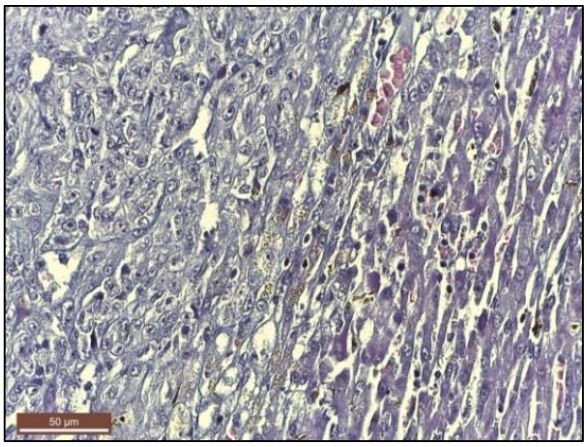

Figure 8. Compact hepatic carcinoma. Round or oval-shaped neoplastic cells; the nuclei exhibit numerous nucleoli. HE x400 (original photograph)

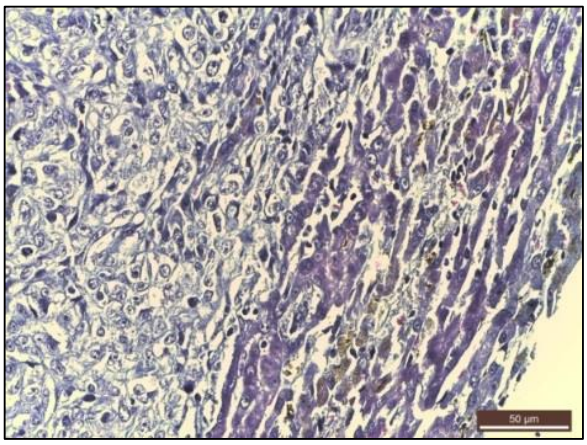

Figure 9. Trabecular hepatic carcinoma; round hepatocytes with nucleolated vesicle-like nuclei. HE x400 (original photograph)

Conclusions

Primary tumors of the liver in dogs are encountered under the form of nodular hyperplasia, hepatocellular adenoma/carcinoma, cholangiocellular carcinoma/adenoma and hepatoblastoma. Metastatic tumors are usually multicentric.

Investigations of the liver in case of suspicion of tumoral disease comprise the clinical examination, imaging examination, blood tests and microscopic examination of the anatomo-pathological specimens. The microscopic diagnosis requires that anatomopathological samples be taken from the live animal by fine needle punction and by intraoperative or live biopsy, or it can be performed on the cadaver during necropsy.

The histological diagnosis indicates the type of hepatic tumor, as well as its benign or malignant character.

The studies performed on dogs from the north-eastern region of Romania led to the finding of the following types of hepatic tumors with the following prevalence: hepatic carcinoma (11 cases), cholangiocarcinoma (1 case), adenocarcinoma (8 cases), lymphosarcoma (4 cases), 1 multiple myeloma.

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