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Retrospective Study of Hepatic Neoplasms in Dogs (1999-2012)

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Abstract

Primary hepatic neoplasms (PHN) account for 0.6 to 1.5% of canine tumours and constitute a major portion of chronic liver disease. The canine hepatic neoplasms can be classified as hepatocellular adenoma/carcinoma (HA/HCC), cholangiocellular adenoma/carcinoma (CCA/CCC), neuroendocrine carcinoma of tumors of mesenchymal origin, vascular or hematopoietic. Thus, because of its importance in veterinary oncology and hepatology, we performed a retrospective study of primary canine liver cancer in the Department of Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo, between 1999 and 2012. Was evaluated 6886 histopathological analysis, 106 (1.5%) were related to canine liver diseases, in which the tumors accounted for 27 cases (25.47%). Liver neoplasms, were classified as HA (2 cases -7.4%); CCA (5 cases - 18.5%); HCC (5 cases - 18.5%); CCC (6 cases - 22.2%) and 9 tumors of mesenchymal, hematopoietic or vascular (33.3%). Epidemiological data showed similarities to previously reported data, predominantly CCC, HCC and CCA in older animals and females.

Key Words: primary hepatic neoplasms, epidemiology, veterinary oncology, veterinary hepatology, vimentin.

Introduction

With advances in diagnostic and therapeutic methods in veterinary medicine, dogs have shown a higher survival rate and therefore, more likely the development of neoplastic lesions. Primary hepatic malignancies are an important portion of chronic liver diseases in dogs and represent 0.6 to 1.5% of all canine tumors (4,6,23). The etiology of these tumors has not yet been precisely established, but potential causes such as aflatoxins, nitrosamines, liver parasites (Clonorchis spp and Platynossomun concirum) and radioactive components (strontium 90 and cesium 144) have been reported in experimental studies and spontaneous findings (19). Contrary to reports in human medicine, there is no association between the incidence of liver tumors and viral infections in dogs and cirrhotic livers are not likely to develop hepatocellular carcinoma (16).

Primary liver proliferative diseases in dogs can be classified as nodular hyperplasia, hepatocellular adenoma/carcinoma (HA/HCC), cholangiocellular adenoma/carcinoma (CCA/CCC), neuroendocrine carcinoma (NC) and hepatoblastoma. Tumors of mesenchymal origin, vascular or hematopoietic have also been described in dogs, for example, leiomyosarcomas, lymphomas and hemangiosarcoma (3,22,23). Metastatic tumors are about three times more common than primary and stem from a variety of cells (2,3,7,10,14,23,24). The distinction between primary and metastatic cancer is essential to lead to the most appropriate therapeutic procedures, as well as to determining the prognosis of the disease (21). Often, the reported hepatic metastases are from malignancies of the mammary gland, spleen, adrenal glands, pancreas, bones and lungs (14).

Clinical signs of dogs with liver tumors are nonspecific, usually indistinguishable from other liver diseases and are generally recognized only in advanced

stages or when metastases are already present (13,22). Among the clinical signs, Johnson (11) highlights the presence of anorexia, lethargy, weight loss, polyuria, polydipsia, vomiting and abdominal distension. Disruption of the tumor and liver bleeding are more frequently observed in cases of HA/HCC and hemangiosarcoma. Approximately 10% to 40% of the dogs have icterus (3,16).

Considering the growing importance of veterinary oncology and hepatology, the study of the incidence of primary liver tumors in dogs is of great importance in veterinary medicine. Even the study of epidemiological data allows a better understanding of these diseases, allowing the adoption of more appropriate treatment protocols and, consequently, improved quality of life and prognosis. Thus, this study evaluated the qualitative and quantitative distribution of hepatic neoplasms in dogs examined at the Veterinary Hospital of the Faculty of Veterinary Medicine, USP, and forwarded to the Department of Pathology from January 1999 to October 2012.

Material and methods

We reviewed 6886 reports obtained at the Department of Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Brazil, from January 1999 to October 2012. From the reports concerning dogs, we selected only those animals with primary hepatic neoplasms (PHN), nodular hyperplasia, hepatocellular adenoma/carcinoma (HA/HCC), cholangiocellular adenoma/carcinoma (CCA/CCC), neuroendocrine carcinoma (NC) and hepatoblastoma. Glass slides from all cases , stained with hematoxylin and eosin (HE) were reviewed by two veterinary pathologists (BC and MLZD) and classified according to histopathological criteria established for the diagnosis of liver diseases in dogs and cats by the World Association of Veterinarians for Small Animals (18). After this classification, we calculated the prevalence of each condition relative to the total PHN diagnosed. We also recorded the PHN more frequent epidemiological data as age and sex of the animals. The most common clinical symptoms were also reported.

Immunostaining was performed on deparaffinized sections using an immunoperoxidase method with the avidin-biotin amplification system. The specimens were deparaffinized in xylene and rehydrated. Endogenous peroxidase was blocked with 3% H_20_2 in methanol for 5 minutes. The blocking of nonspecific reactions was performed by Protein Block (*Dako*) for 30 minutes. The primary antibodies (Anti-Vimentin, *Dako* - 1:100) were incubated overnight at +4°C and were sequentially followed by a complex of avidin and biotinylated horseradish peroxidase (*Dako*) for 30 min. Careful rinses with several changes of phosphate-buffered saline were performed between each stage of the procedure. The reaction was developed with a solution of diaminobenzidine in the dark. For vimentin positive cell counts were used to score low, moderate and strong.

Results and discussion

From the histopathological analysis of 6886 lesions recorded at the Department of Pathology, University of Sao Paulo, 106 reports (1.5%) were related to chronic liver disease in dogs. Of these, 27 cases (25.47%) were primary hepatic malignancies and were distributed as follows: two cases of hepatocellular adenoma (7.4%), 5 cases of cholangiocellular adenoma (18.5%), five cases of hepatocellular carcinoma (18.5%) six cases of cholangiocellular carcinoma 6 (22.2%) and 9 tumors of mesenchymal origin, vascular or hematopoietic (33.3%) (Figure 1).

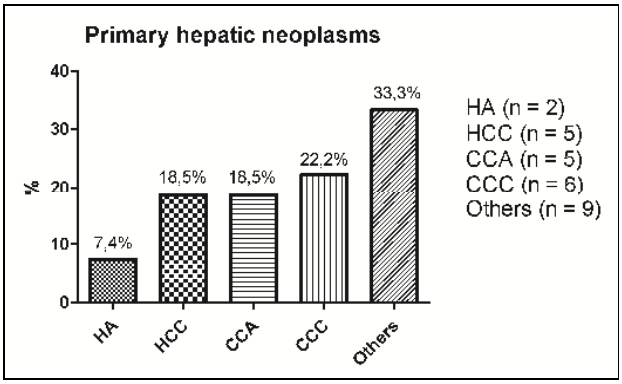

Figure 1. Distribution of the main primary hepatic neoplasms in dogs identified in the Department of Pathology, Faculty of Veterinary Medicine and Animal Science - USP, from 1999 to 2012.

Vimentin (intermediate filament) is a marker for cells of mesenchymal origin, widely used in the determination of tumor histogenesis. Typically, vimentin is not expressed in epithelial cells. However, tumor cells may express it, which usually is associated with a poor prognosis. Thus, we evaluated the expression of vimentin (Figure 2) in samples from dogs with HCC (n = 3) and CCC (n = 4) obtained from the Department of Pathology, FMVZ-USP. Sections were incubated with monoclonal anti-Vimentin (1:50 - Dako) and revealed by DAB (Dako). The cytoplasmic markers were classified according to the severity: mild, moderate and severe.

The results demonstrated the presence of strong positive cells of mesenchymal origin in cases of HCC and CCC, such as sinusoidal cells and smooth muscle cells. However, 50% of HCC and 100% of CCC showed mild to moderate cytoplasmic reactivity in neoplastic epithelial cells. Some authors have demonstrated co-expression of cytokeratin and vimentin in a focus on HCC pale canine cells and found that these cells were derived from the differentiation of hepatic progenitor cells. The hepatocyte and ductular oval cells also express vimentin, and may be involved in the histogenesis of HCC in dogs.

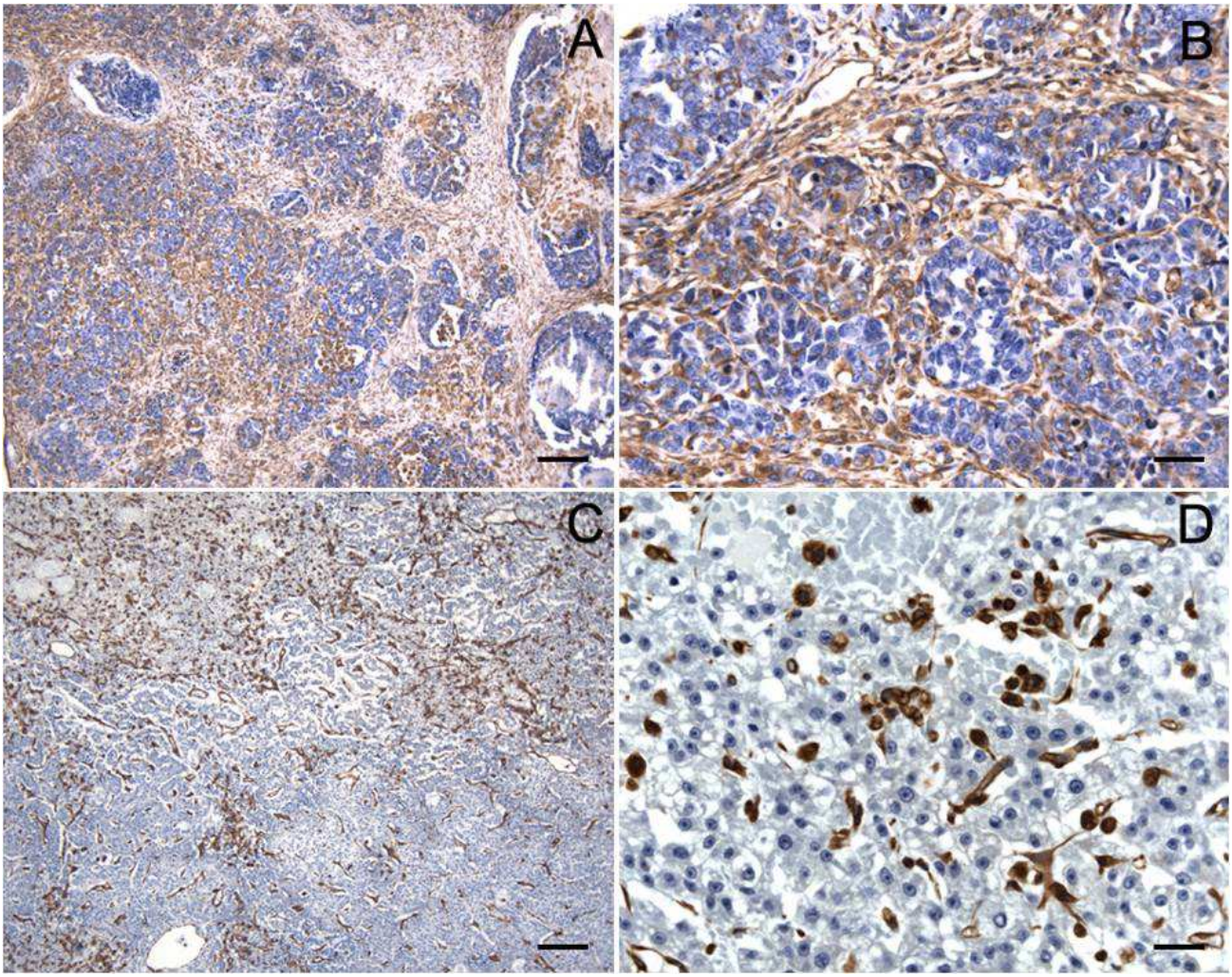

Figure 2. Immunohistochemical staining for vimentin. Intense positive reaction in cells of mesenchymal origin, such as endothelial and stellate cells. (A-B) Cholangiocarcinoma. (C-D) Hepatocellular carcinoma. (B and D) Presence of neoplastic epithelial cells vimentin+. Scale bars: (A/C) 20 µm; (B) 5 µm, (D) 3 µm.

Hepatocellular carcinoma affects 6% of all cancers in humans and is the fifth most common malignant tumor found in the world (10), whereas this tumor represents less than 1% of all tumors dogs (3). The frequency of Canine cholangiocellular carcinoma is uncertain, because retrospective studies conducted in the U.S. claim that the HCCs are more frequent than the CCCs. In contrast, studies conducted in Scandinavia, South Africa (3), Brazil (5) and the University of Kansas (21) indicate a higher prevalence of CCCs in dogs. Some authors claim that the CCC is 22% to 41% of malignant liver tumors observed in dogs (16,23). Our data indicate percentage of incidence of CCC similar to the latter authors, but there was no significant difference in distribution between the CCC and HCC tumors in the examined dogs.

Cholangiocarcinomas were the most frequent hepatic neoplasms in this study, all animals were female and the mean redorded age was 10.8±2.2 years. In cases of cholangiocellular adenoma, all animals were male and the mean age was 11±2.3 years. Among animals with hepatocellular carcinoma, the male: female ratio was 1:5, and the mean age 10.6±2 years.

Liver tumors are observed in dogs over the age of 10 years, and males have a higher incidence of HCC, while concerning cholangiocarcinoma spayed females are more frequent affected (3,7,10,14). Other studies only report a higher frequency of CCC in females, not determining whether they are spayed or not (7,16,26). Regarding the CCC, our results are consistent with previously reported data and demonstrate a higher incidence in females with a mean age of 10.8±2.2 years. However, unlike the literature, this study showed a greater incidence of HCC in females with the mean age as expected (10.6±2 years).

In humans, dogs and cats there is an association between parasitism by Clonorquis sinensis and cholangiocarcinoma, but the mechanism by which this parasite promotes tumor development is still unknown (2,3,12,21). Changes in the biliary tract associated with

this parasitism are adenomatous hyperplasia and proliferation of the biliary epithelium, with minimal inflammatory reaction (12). Ancylostoma spp. and Trichuris vulpis are also associated with increased incidence of cholangiocarcinoma, but is not yet clear whether these parasites act as the direct cause for tumor development or whether they act by facilitating the exposure to other agents (4,7).

Burton et al. (1) reported the presence of ectopic HCC first in dogs, which has also been reported in humans (8, 13,20). SHIGA et al. (2001) reported the presence of combined hepatocellular carcinoma and cholangiocarcinoma (HCC-CCC) within the same tumor tissue, and as in humans, this type of tumor is extremely rare and not found in our retrospective study. Likewise, the neuroendocrine carcinoma is also of rare type of cancer. Hepatoblastoma, a tumor reported in children, has not been described in dogs (20) and, similarly, was not demonstrated in this series. Metastases occur in 60-88% of cases of cholangiocarcinoma, and the preferred sites include lymph nodes, lungs and the peritoneal cavity via the lymphatic system, blood and implantation, respectively (2,3). Other sites include kidney, pancreas, spleen, heart and bone (2,15). The results of this study indicate the presence of metastasis in 50% of CCC, located in the lungs and kidneys.

The most frequent clinical symptoms observed in animals in this series are represented by anorexia, abdominal swelling, vomiting, malaise and jaundice. The clinical signs of canine HCC are nonspecific, usually indistinguishable from other liver diseases, while the signs of dysfunction usually only occur when the tumor is at an advanced stage (16,22). The animals in this study had tiredness and impaired liver function. Likewise, clinical signs in dogs that occur with CCC are nonspecific, however jaundice is observed in 10% to 40% of the dogs (3,16). The clinical symptoms most frequently found in dogs with CCC were anorexia, abdominal swelling, vomiting and prostration. Jaundice was observed in 33% of the animals and is consistent with previously reported data.

In conclusion, epidemiological data herein included showed similarities with the data described in the literature, predominantly CCC, CCA and HCC in older animals and females. The presence of neoplastic epithelial cells may indicate a vimentin positive precursor cell with active participation in the histogenetic process of hepatic tumorsin dogs. The results represented in this series provide an important tool in the identification of primary liver cancer in dogs confirmed in epidemiological and clinical data.

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