Pathological and immunohistochemical aspects of primary hepatobiliary neoplasms in cats[1]

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ABSTRACT.- Argenta F.F., Mello L.S., Caprioli R.A., Pavarini S.P., Driemeier D. & Sonne L. 2020. **Pathological and immunohistochemical aspects of primary hepatobiliary neoplasms in cats.** *Pesquisa Veterinária Brasileira 40(1)46-54.* Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: lusonne@yahoo.com.br

Primary hepatobiliary neoplasms (PHN) are uncommon in cats, and originate in hepatocytes, intra- and extrahepatic bile ducts, mesenchymal cells, and cells of neuroendocrine origin. The aim of this study was to determine the frequency of PHN in cats diagnosed in the metropolitan region of Porto Alegre (RS), Brazil, for a period of 17 years, determining their epidemiological, anatomopathological and immunohistochemical aspects. Necropsy reports of 2.090 cats were analyzed, 125 were diagnosed with primary hepatobiliary diseases, of which 15 were cases of PHN, representing 12% of the specific hepatobiliary conditions and 0.7% of the necropsies. All PHN were malignant, of which 93.3% had epithelial origin and 6.7% presented mesenchymal origin. Cholangiocarcinoma was the most commonly diagnosed neoplasm, followed by hepatocellular carcinoma and hemangiosarcoma. In general, cats with no defined breed were the most affected. Concerning sex, 60% were females and 40% males. Age ranged from five to 18 years, with a mean age of 10.5 years (median of ten years). Grossly, cholangiocarcinoma and hemangiosarcoma were multinodular and hepatocellular carcinoma was massive. Microscopically, cholangiocarcinomas were arranged in acini and ducts, whereas hepatocellular carcinomas were arranged in solid sheets or trabeculae. On immunohistochemistry, cholangiocarcinomas, hepatocellular carcinomas, and hemangiosarcomas were positive for the antibodies CK 7, Hep Par-1, and vimentin and von Willebrand factor, respectively.

INDEX TERMS: Felines, pathology, immunohistochemistry, hepatobiliary carcinoma, neoplasm, cats, cholangiocarcinoma, hepatic hemangiosarcoma.

RESUMO.- [**Aspectos patológicos e imuno-histoquímicos de neoplasias hepatobiliares primárias em gatos.**] Neoplasias hepatobiliares primárias (NHP) são incomuns em gatos e se originam de hepatócitos, células dos ductos biliares intra e extra-hepáticos, células mesenquimais e ainda células de origem neuroendócrina. O objetivo do trabalho foi determinar a frequência das NHP em gatos diagnosticados na Região Metropolitana de Porto Alegre, no período de 17 anos, abordando seus aspectos epidemiológicos, anatomopatológicos e imuno-histoquímicos (IHQ). Foram analisados os laudos de necropsia de 2.090 gatos sendo que 125 foram diagnosticados com doenças hepatobiliares primárias, destes 15 foram casos de NHP, representando 12% das condições hepatobiliares específicas e 0,7% do total de necropsias. Todos os diagnósticos de NHP eram malignos, destes 93,3% apresentaram origem epitelial e 6,7% mesenquimal. Colangiocarcinoma foi a neoplasia mais diagnosticada, seguido do carcinoma hepatocelular e hemangiossarcoma. De uma maneira geral, os gatos sem raça definida foram os mais acometidos. Em relação ao sexo 60% eram fêmeas e 40% machos. A idade variou de cinco a 18 anos, com a idade média de 10,5 anos (mediana de 10 anos). Macroscopicamente o colangiocarcinoma e hemangiossarcoma eram multinodulares, e o carcinoma hepatocelular, maciço. À histologia, houve predomínio do

arranjo acinar e ductal nos colangiocarcinomas e sólido, no carcinoma hepatocelular. Na IHQ os colangiocarcinomas foram reativos para CK 7, carcinoma hepatocelular para Hep Par-1 e hemangiossarcoma para vimentina e fator de von Willebrand.

TERMOS DE INDEXAÇÃO: Felinos, patologia, imuno-histoquímica, carcinoma hepatobiliar, colangiocarcinoma, hemangiossarcoma hepático.

INTRODUCTION

Primary hepatobiliary neoplasms (PHN) are uncommon in cats (Cullen & Stalker 2016), and originate in hepatocytes, intra- and extrahepatic bile duct cells, mesenchymal cells, and cells of neuroendocrine origin (Head et al. 2003). They usually affect older cats, with no predisposition for breed or sex (Cullen 2017). Anorexia, lethargy, weight loss, hepatomegaly identified on abdominal palpation, and jaundice may be present, but clinical signs are often nonspecific, making clinical diagnosis difficult (Rutgers 1998, Barros 2016). The use of immunohistochemistry assists with the diagnosis of PHN (Patnaik 1992), and treatment and prognosis are determined by gross pattern and histological features, with indication for surgical resection in cases of neoplasms confined to only one hepatic lobe (Stonehewer 2006). The present study aimed to determine the frequency of PHN in cats diagnosed in the metropolitan region of Porto Alegre (RS), Brazil, for 17 years, addressing their epidemiological and pathological aspects.

MATERIALS AND METHODS

PHN cases in the necropsy reports of cats archived at the Department of Veterinary Pathology of the "Universidade Federal do Rio Grande do Sul", from January 1999 to December 2016, were reviewed and selected. Data described in necropsy requests, such as breed, sex, age and gross lesions, were reviewed and compiled. All cases analyzed were from the metropolitan region of Porto Alegre. Of the selected cases, the archived paraffin-embedded blocks were searched for the preparation of 3 μm-thick sections on histological slides for subsequent staining by the hematoxylin and eosin technique and visualization by optical microscopy.

The gross pattern and histological classification of the present study followed the criteria established by World Health Organization (WHO) (Head et al. 2003). For evaluation of the degree of fibrosis and quantification of mucin expression, histological sections were subjected to special Masson's trichrome (MT) and periodic acid-Schiff (PAS) stains. The special staining techniques followed the protocols described in the records of the Armed Forces Institute of Pathology (Gaffney 1992, McElroy 1992).

Neoplasm sections were submitted to immunohistochemistry (IHC) for biliary epithelium cells (cytokeratin 7 - CK 7), hepatocytes (Hepatocyte Paraffin 1 - Hep Par-1), mesenchymal cells (vimentin), and vascular endothelium (von Willebrand factor). The primary antibodies and protocols used are specified in Table 1.

RESULTS

Necropsy reports of 2.090 cats were analyzed, and 125 were diagnosed with primary hepatobiliary diseases, of which 15 were cases of PHN, representing 12% of the specific hepatobiliary conditions and 0.7% of the necropsies. All PHN (100%, 15/15) were malignant; of these, 93.3% (14/15) had epithelial origin and 6.7% (1/15) presented mesenchymal origin. Cholangiocarcinoma was identified in 66.6% (10/15), hepatocellular carcinoma in 26.7% (4/15), and hemangiosarcoma in 6.7% (1/15) of the cats.

In general, cats with no defined breed (NDB) were the most affected, representing 80% (12/15) of the cases, and the Siamese breed represented the remaining 20% (3/15) of the cases. Regarding sex, 60% (9/15) were females and 40% (6/15) males. Age ranged from five to 18 years, with a mean age of 10.5 years (median of ten years).

Cholangiocarcinoma

Cholangiocarcinoma was diagnosed in 66.6% (10/15) of the cases. It affected NDB cats in 80% (8/10) and Siamese cats in 20% (2/10). As for sex, 50% (5/10) were females and 50% (5/10) males. Age ranged from five to 13 years, with a mean age of 10.3 years (median of 11 years). In 80% (8/10) of the cases, cholangiocarcinoma originated in intrahepatic bile ducts and in the remaining 20% (2/10) from extrahepatic ducts (13.3%, 2/15). Grossly, the multinodular pattern was identified in 60% (6/10), and was characterized by multifocal to coalescent nodules, firm on palpation, and whitish with red areas (Fig.1A,B), and sometimes presented central depression (umbilicated aspect). The massive pattern was observed in 20% (2/10) of the cases, with the right lateral lobe affected in one case (50%, 1/2) and the left medial lobe affected one other case (50%, 1/2). These were firm on palpation and red interspersed with whitish areas (Fig.1C). The extrahepatic cases involved the cystic duct and were characterized by whitish to yellowish masses, firm on palpation, and with biliary flow obstruction (Fig.1D). Extrahepatic gross findings were mainly characterized by poor body condition and free slightly reddish

Table 1. Antibodies and immunohistochemical protocols used in hepatobiliary neoplasms in cats

| Antibody	Clone/Code	Antigen retrieval	Dilution	Detection method	Chromogen
Monoclonal Anti-Vimentin	V9, Zymed	3 min/125°C, Citrate buffer pH 6.0	1:200	MACH 4, Biocare Medical	DAB, Dako
Monoclonal Anti-Cytokeratin 7 (CK 7)	M7018, Dako	Proteinase K, 10 min	1:40	MACH 4	DAB
Monoclonal Anti-Hep Par-1	M7158, Dako	40 min/96°C, Citrate buffer pH 6.0	1:100	MACH 4	DAB
Polyclonal Anti-von Willebrand factor	A0082, Dako	3 min/125°C, Citrate buffer pH 6.0	1:200	MACH 4	DAB
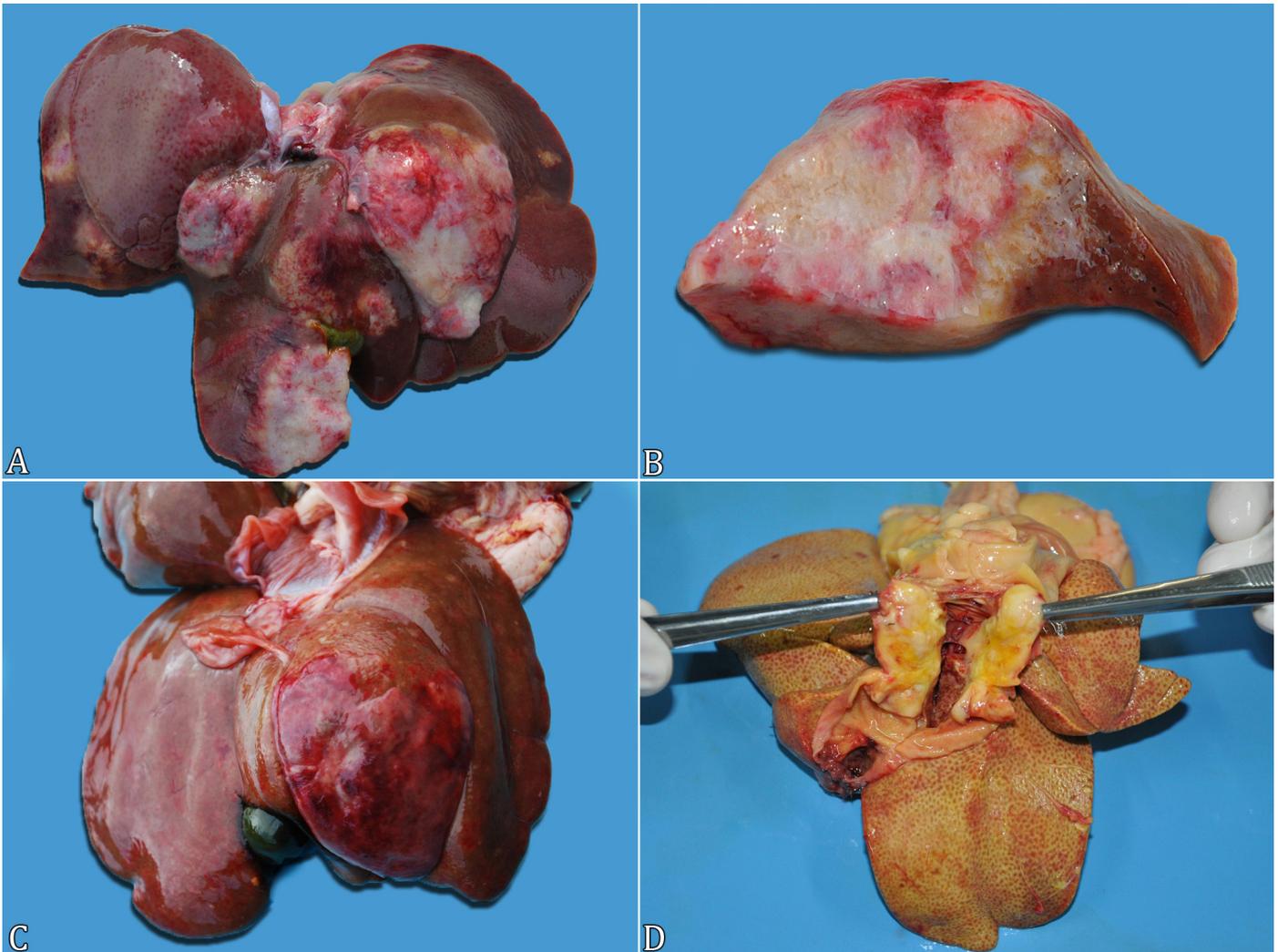
Fig.1. Gross aspects of cholangiocarcinoma in cats. (**A**) Multinodular pattern. Multifocal to coalescent nodules of different sizes, whitish interspersed with red areas. (**B**) Cholangiocarcinoma cutting surface of Figure 1A. (**C**) Massive pattern. Extensive focal mass located in the left medial lobe, red with lighter areas and central depression (umbilicated aspect). (**D**) Extrahepatic cholangiocarcinoma. Yellowish mass located in the cystic duct.

serous fluid in the abdominal cavity (ascites), identified in 50% (5/10) of cats with cholangiocarcinoma. In 40% (4/10) of the cases, nodules with gross appearance similar to those of the liver were identified in various organs, such as regional (hepatic) lymph nodes (75%, 3/4), peritoneum, diaphragm, intestinal and gastric serosa, lungs (50%, 2/4 each), and kidneys (25%, 1/4). Yellow oral and conjunctival mucosa, skin and subcutaneous tissue (jaundice) and yellow slightly diminished liver with lobular pattern accentuation were observed in cats with extrahepatic cholangiocarcinomas.

Histologically, 60% (6/10) of the cases were characterized by replacement of the hepatic parenchyma by proliferation of non-delimited and non-encapsulated epithelial cells, with formation of acini and/or irregular ducts (Fig.2A). In 30% (3/10) of the cases, the solid pattern was predominant. One case (10%, 1/10) was identified as biliary cystadenocarcinoma, characterized by formation of numerous cysts, with papillary projections to their lumen (Fig.2B). Cells were cuboid to rounded, with relatively undefined cytoplasmic borders, moderate and eosinophilic cytoplasm, round nucleus with chromatin,

which ranged from dense to finely stippled, and with one to two conspicuous nucleoli (Fig.2C). Cellular pleomorphism was moderate in 50% (5/10), marked in 30% (3/10), and mild in 20% (2/10) of the cases. The mitotic index per high power field (HPF, 400x) was discrete (1/HPF) in 60% (6/10) of the cases, and moderate (2/HPF) in the remaining 40% (4/10) of the cases. Fibrous connective tissue proliferation, visualized mainly using MT staining, was marked in 50% (5/10) (scirrhous pattern) (Fig.2D), moderate in 30% (3/10), and mild in 20% (2/10) of the cases. Mucin expression was quantified as moderate in 40% (4/10), discrete in 20% (2/10), and marked in 10% (1/10) of the cases. This histological change was not identified in the remaining 30% (3/10) of the cases. Mucin was characterized by amorphous, slightly basophilic material within the acinar and ductal structures, which was intensely stained pink by PAS (Fig.2E). Intratumoral necrosis and hemorrhage were observed in 70% (7/10) and 60% (6/10) of the cases, respectively. Tumor invasion in lymphatic and/or blood vessels was identified in 40% (4/10) of the cases, and extrahepatic metastases were found mainly
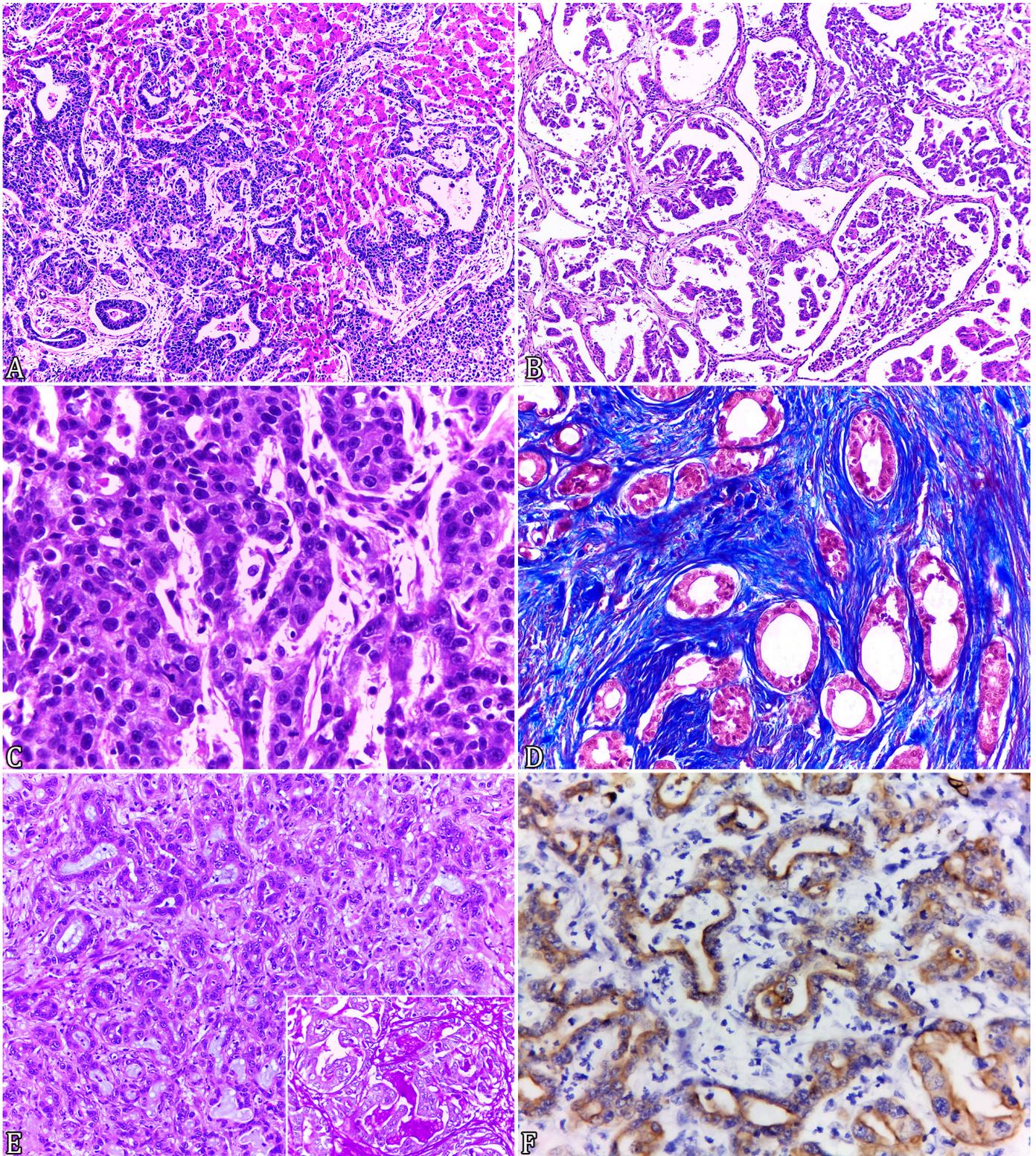
Fig.2. Histological and immunohistochemical aspects of cholangiocarcinoma in cats. (**A**) Replacement of the hepatic parenchyma by neoplastic proliferation of epithelial cells, with formation of acini and irregular ducts, supported by moderate connective stroma. HE, obj.10x. (**B**) Biliary cystadenocarcinoma. Neoplastic proliferation of epithelial cells with formation of cystic structures, sometimes with papillary projections to the lumen. HE, obj.10x. (**C**) Enlargement of Figure 2A showing neoplastic epithelial cells with moderate pleomorphism. HE, obj.40x. (**D**) Proliferation of duct-forming epithelial cells (red), interspersed with marked proliferation of fibrous connective tissue (blue). MT, obj.20x. (**E**) Proliferation of epithelial cells arranged in ductal and acinar pattern, with amorphous slightly basophilic material within these structures (mucin). HE, obj.20x. Inset: evidence of mucin intensely pink stained by PAS. PAS, obj.40x. (**F**) Marked anti-CK 7 staining in the cytoplasm of neoplastic epithelial cells, sometimes more intense near the plasma membranes. IHC, DAB, obj.40x.

in hepatic lymph nodes (4/10), spleen (3/10), lungs (3/10), stomach and intestinal serosa (2/10), peritoneum (1/10), diaphragm (1/10), and kidneys (1/10).

Immunohistochemistry (IHC) showed that intracytoplasmic and membrane staining for CK 7 was marked in 50% (5/10) (Fig.2F), moderate in 40% (4/10), and discrete in 10% (1/10) of the cases. No cases of cholangiocarcinoma showed IHC positivity for Hep Par-1.

Hepatocellular carcinoma

Hepatocellular carcinoma represented 26.7% (4/15) of the diagnoses. All affected cats were NDB (100%, 4/4). Females corresponded to 75% (3/4) and males to 25% (1/4) of the cases. Age ranged from 10 to 18 years, with a mean age of 12.5 years (median of 11 years).

The massive gross pattern was identified in all cats (100%, 4/4), characterized by large masses, predominantly located in the quadrate lobe with extension to the right medial and lateral lobes (75%, 3/4) (Fig.3A). The left medial lobe was also affected in one case (25%, 1/4). The masses were friable, whitish to yellowish, with irregular surface and red multifocal areas. Extrahepatic gross findings were characterized by poor body condition (75%, 3/4), ascites, jaundice in mucosa and subcutaneous tissue, and large amounts of free blood and clots in the abdominal cavity (hemoperitoneum) (25%, 1/4 each).

Histologically, there was disorganization of the organ architecture caused by proliferation of neoplastic hepatocytes with solid pattern (75%, 3/4), which were characterized by dense mantle cells (Fig.3B), sometimes with formation of trabeculae of variable thickness (25%, 1/4). Cells were polygonal, with relatively distinct cytoplasmic borders, abundant and eosinophilic cytoplasm, round nucleus, with finely stippled chromatin and one to two conspicuous nucleoli (Fig.3C). Marked intracytoplasmic vacuolization was identified in 50% (2/4) of the cases. Cell pleomorphism ranged from moderate to severe (50%, 2/4 each), and binucleated cells were identified in 50% (2/4) of the cases.
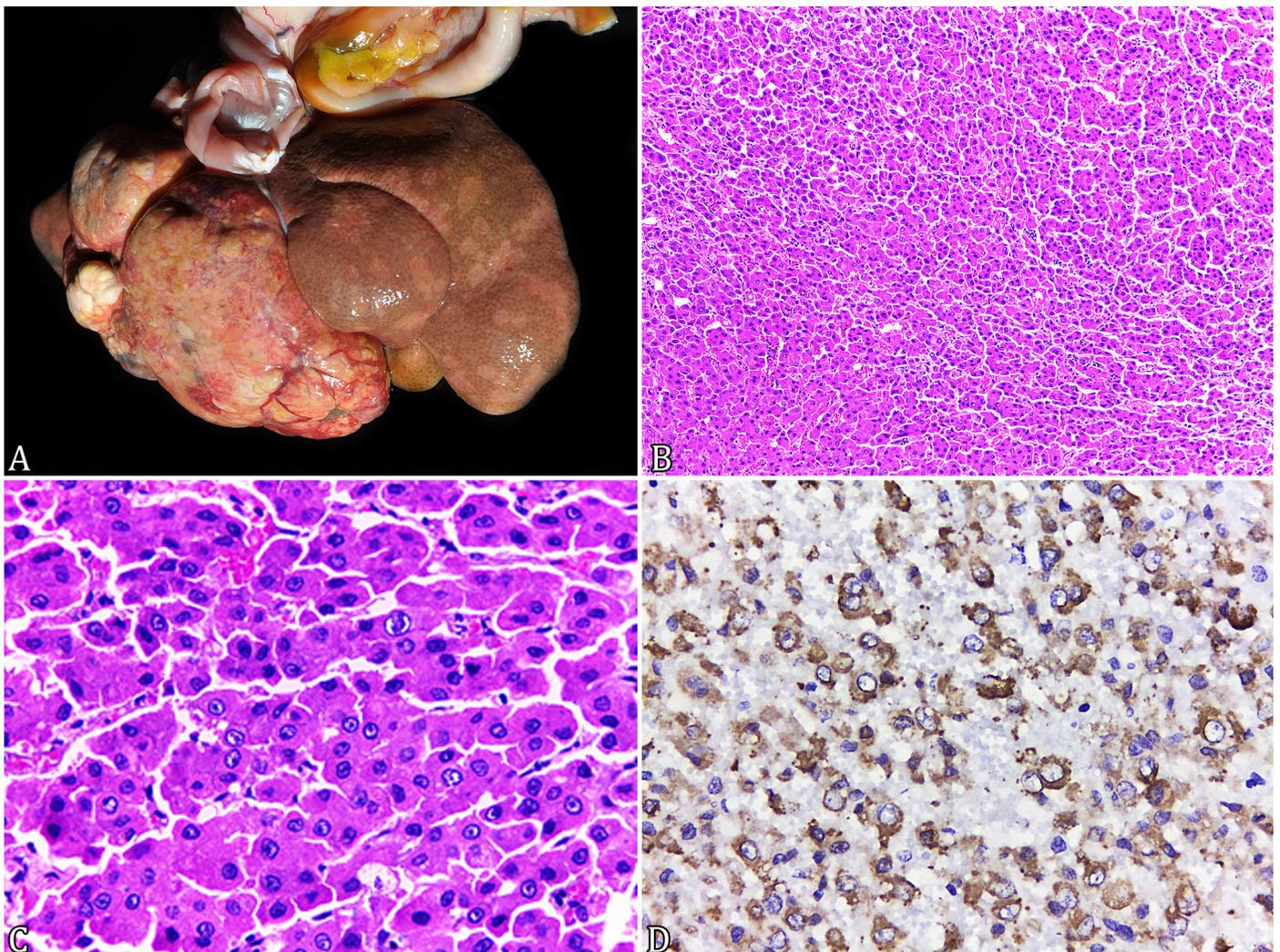

Fig.3. Anatomopathological and immunohistochemical aspects of hepatocellular carcinoma in cats. (**A**) Gross pattern. Large whitish mass with multifocal red and yellowish areas located in the quadrate lobe extending to the right medial and lateral lobes. (**B**) Histological aspect. Neoplastic proliferation of hepatocytes arranged in a solid pattern. HE, obj.10x. (**C**) Enlargement of Figure 3B showing neoplastic hepatocytes with moderate pleomorphism. HE, obj.40x. (**D**) Intense anti-Hep Par-1 staining in the cytoplasm of neoplastic hepatocytes. IHC, DAB, obj.40x.

The mitotic index HPF (400x) was discrete (1/HPF) in all cases (100%, 4/4). Fibrous connective tissue proliferation was classified as mild, and no mucin expression was identified in all cases (100%, 4/4). Intratumoral necrosis and hemorrhage were visualized in 100% (4/4) and 75% (3/4) of the cases, respectively. No tumor invasion in the blood and/or lymphatic vessels or extrahepatic metastases was identified.

All cases (100%, 4/4) showed marked intracytoplasmic, sometimes granular, staining for Hep Par-1 (Fig.3D). No cases of hepatocellular carcinoma presented IHC positivity for CK 7.

Hemangiosarcoma

Only one animal was diagnosed with primary liver hemangiosarcoma, accounting for 6.7% (1/15) of the cases. It was a five-year-old male NDB cat.

At necropsy, the liver showed multiple dark red nodules (Fig.4A) and soft on palpation. On the cutting surface, numerous cystic areas containing blood, interspersed with whitish firm areas were observed. The cat presented good body condition and pale mucous membranes. In abdominal cavity, a large amount of free blood and clots was observed.

Microscopic analysis was characterized by non-delimited and non-encapsulated endothelial cells proliferation, arranged in a solid pattern, often forming vascular structures, and sustained in a discrete conjunctival stroma (Fig.4B). The cells were spindle-shaped, with indistinct cytoplasmic borders, discrete and eosinophilic cytoplasm, oval to elongated nucleus, stippled chromatin, and evident single nucleoli (Fig.4C). Cellular pleomorphism was moderate, and the mitotic index HPF (400x) was discrete (1/HPF). An extensive area of necrosis and intratumoral hemorrhage was observed. No tumor invasion in vessels or extrahepatic metastases was identified.

IHC showed marked multifocal intracytoplasmic staining for vimentin and moderate for von Willebrand factor (Fig.4D).
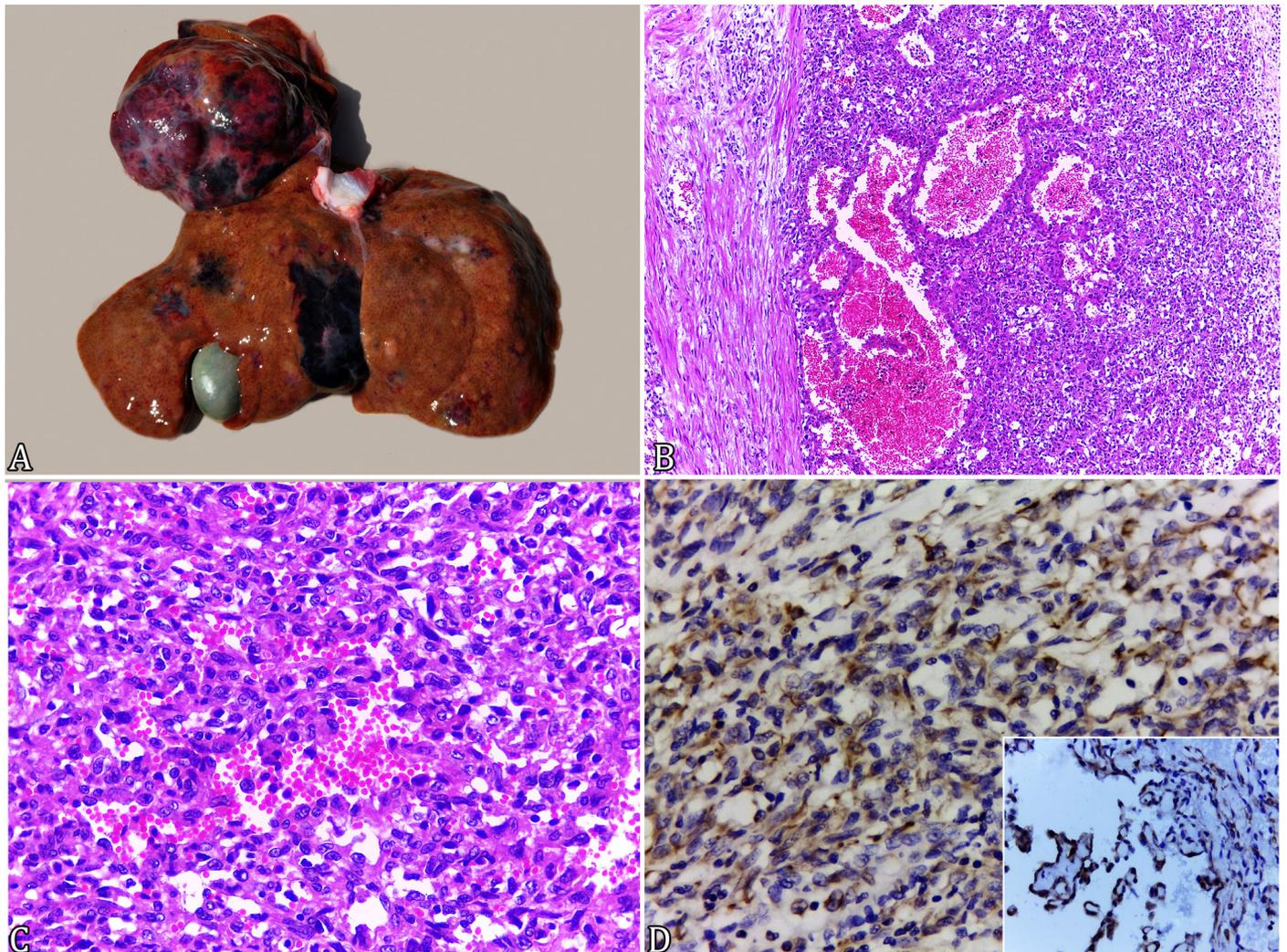

Fig.4. Anatomopathological and immunohistochemical aspects of hepatic hemangiosarcoma. (**A**) Gross pattern characterized by multifocal dark red nodules of varying sizes. (**B**) Histological aspects. Neoplastic proliferation of spindle cells arranged in a solid pattern, sometimes with formation of vascular structures of different sizes and filled with red blood cells. HE, obj.10x. (**C**) Enlargement of Figure 4B showing endothelial neoplastic cells. HE, obj.40x. (**D**) Intracytoplasmic accentuated multifocal staining for vimentin. Inset: intracytoplasmic staining for von Willebrand factor. IHC, DAB, obj.40x.

DISCUSSION

Frequency of PHN in the present study was 0.7% of the necropsies in cats, and when analyzed only the category of hepatobiliary diseases, the frequency was 12%. In similar studies, PHN frequency ranged from 0.7 to 2.3% of the necropsies (Schmidt & Langham 1967, Engle & Brodey 1969, Patnaik et al. 1975, Martins 2016). In the analyses of biopsy samples, these neoplasms corresponded to 9.7 to 11.3% (Gagne et al. 1996, Hirose et al. 2014). Researchers have reported that PHN corresponded to 1.0 to 5.7% of all cat neoplasms (Hammer & Sikkema 1995, Rutgers 1998, Andrade et al. 2012, Van Sprundel et al. 2014). NDB cats were the most affected in this study; however, no breed predisposition to PHN has been described (Balkman 2009). The larger number of NDB cats is probably due to the fact that they are the most assisted in the metropolitan region of Porto Alegre and, consequently, referred to the Department of Veterinary Pathology. There was no apparent sex predisposition, as previously described (Lawrence et al. 1994, Van Sprundel et al. 2014). Elderly cats were the most affected, corroborating the literature (Patnaik 1992, Post & Patnaik 1992, Lawrence et al. 1994, Andrade et al. 2012, Cullen 2017), and this may be associated with their longevity (O'Neill et al. 2015).

In the present study, all PHN were malignant, as described by Patnaik et al. (1975). However, numerous researchers have reported that the benign form is most commonly found (Post & Patnaik 1992, Lawrence et al. 1994). Cholangiocarcinoma was the most commonly diagnosed neoplasm, followed by hepatocellular carcinoma, corresponding to 66.6 and 26.7% of the cases, respectively. In cats, neoplasms originating in bile duct cells occur more frequently than those originating in hepatocytes (Van Sprundel et al. 2014, Otte et al. 2017). Several studies have reported cholangiocarcinoma as the main PHN in cats (Engle & Brodey 1969, Patnaik 1992, Post & Patnaik 1992, Andrade et al. 2012, Martins 2016, Cullen 2017). However, some researchers have described hepatocellular carcinoma (Patnaik et al. 1975) and biliary adenoma (Post & Patnaik 1992, Lawrence et al. 1994) as the most common. Cholangiocarcinomas may develop from intra- or extrahepatic bile ducts (Crawford & Liu 2010), with intrahepatic ducts as the most frequent (Patnaik 1992, Cullen 2009, 2017). In the present study, approximately 13% of the cases originated in extrahepatic ducts, corroborating the literature (Patnaik et al. 1975, Patnaik 1992). Extrahepatic bile duct and gallbladder neoplasms are rare in cats (Feldman et al. 1976, Cullen 2017), but researchers have found a similar frequency between intra- and extrahepatic tumors (Lawrence et al. 1994).

In this study, approximately 6.0% of the cases were of mesenchymal origin. Primary hepatobiliary sarcomas are rare in cats (Balkman 2009). Primary liver hemangiosarcoma varies widely in frequency (Scavelli et al. 1985, Patnaik 1992, Post & Patnaik 1992). This variability can result from two factors: the low incidence of this neoplasm and the difficulty in establishing the primary site when more than one organ is involved (Barros 2016, Cullen 2017). Researchers have reported that hemangiosarcoma is the second most common malignant neoplasm in cats (Post & Patnaik 1992).

Cholangiocarcinoma and hepatocellular carcinoma present different gross appearance (Head et al. 2003, Cullen & Stalker 2016). In the present study, the multinodular pattern was identified in 60% of cholangiocarcinomas, corroborating the literature, which describes this as the most common gross presentation in dogs and cats (Patnaik 1992, Cullen & Stalker 2016). In a survey with dogs, 83% of the cases were multinodular (Flores et al. 2013). In the present study, 20% of cholangiocarcinomas were classified as of massive pattern, and were characterized by large masses that obliterated the entire hepatic lobe (Cullen 2017). In dogs, this presentation has been identified in approximately 17% of bile duct neoplasms (Flores et al. 2013). It is not yet clear whether multiple nodules result from intrahepatic metastases or primary lesions in different *foci* (Barros 2016, Cullen & Stalker 2016). Firm consistency and whitish color are common gross features (Head et al. 2003, Barros 2016, Cullen 2017), and are attributed to the large amount of fibrous stroma present in cholangiocarcinomas (Head et al. 2003, Cullen & Stalker 2016). The umbilicated aspect is probably attributed to intratumor necrosis (Head et al. 2003, Cullen & Stalker 2016, Cullen 2017). Occasionally multiple cystic areas are observed, and when there is predominance of this presentation, the neoplasm is named biliary cystadenocarcinoma (Head et al. 2003, Cullen 2017), as observed in a cat of the present study. Hepatocellular carcinomas exhibited massive gross pattern. This presentation is the most commonly found, and is characterized by large masses involving a single hepatic lobe or extending to adjacent lobes (Barros 2016, Cullen 2017). This neoplasm varies in size and gross appearance, and may be present in massive, nodular or diffuse patterns (Cullen & Stalker 2016). In the present study, quadrate, right medial and lateral lobes were the most affected. According to Patnaik (1992), there is no predilection for hepatic lobe, but the left side is involved in more than two-thirds of hepatocellular carcinomas in dogs (Liptak et al. 2004). Color and consistency vary according to the degrees of hemorrhage and necrosis of the neoplasm and vacuolization of neoplastic cells (Barros 2016, Cullen 2017).

Extrahepatic gross findings were mainly characterized by poor body condition, ascites, and jaundice. These changes are frequent in cases of liver disease (Barros 2016). It has been suggested that ascites occurs as a result of portal hypertension owing to compression caused by neoplasms. Jaundice resulted from bile flow obstruction with consequent intra- and extrahepatic cholestasis (Charles et al. 2006). In the literature, approximately 20% of the cases presented jaundice (Lawrence et al. 1994). Hemoperitoneum as a result of rupture of neoplasms was observed in two cats: one with hepatocellular carcinoma and one with hemangiosarcoma. Generally, when the neoplasm is friable, there is rupture with consequent hemoperitoneum, anemia, and sudden death (Barros 2016, Cullen & Stalker 2016, Cullen 2017). Hemoperitoneum, ascites, and jaundice have been frequently reported in dogs with PHN (Flores et al. 2013).

The histological characteristics of PHN vary according to the degree of differentiation (Head et al. 2003, Cullen 2017). Well-differentiated cholangiocarcinomas are composed of cells that resemble the normal biliary epithelium and present a tubular or acinar arrangement (Barros 2016). As they become undifferentiated, the solid pattern is more prevalent (Head et al. 2003, Cullen 2017). Biliary cystadenocarcinoma is a variation histologically characterized by numerous cysts of varying sizes,

which often contain mucin and present papillary projections to the lumen (Cullen & Stalker 2016). Although the acinar/ductal arrangement was the most frequently found in this study, well-differentiated cholangiocarcinomas were not identified, since their cellular pleomorphism ranged from moderate to severe. Hepatocellular carcinoma usually has three microscopic patterns: trabecular, adenoid and solid (Head et al. 2003, Barros 2016). According to Cullen (2017), the solid pattern is poorly differentiated and characterized by pleomorphic cells (Crawford & Liu 2010, Flores et al. 2013). Vacuolization in the cytoplasm of neoplastic hepatocytes is a frequent finding, and is associated with glycogen or lipid deposition (Cullen 2017). Hemangiosarcomas are composed of neoplastic endothelial cells, often with formation of vascular spaces, but the solid arrangement may be found in some situations (Head et al. 2003, Barros 2016). Hemorrhage, necrosis and thrombus formation are frequent (Cullen 2017), and these findings are associated with the gross presentation, characterized by soft dark red nodules. The mitotic index was discrete in most PHN cases; however, researchers have reported that the mitotic index is more pronounced in cholangiocarcinoma than hepatocellular carcinoma (Head et al. 2003), and can be used to differentiate between them (Barros 2016).

Fibrous connective tissue and mucin are histological features frequent in cholangiocarcinomas and uncommon in hepatocellular carcinomas (Patnaik 1992, Head et al. 2003, Barros 2016, Cullen & Stalker 2016, Cullen 2017). Cholangiocarcinomas may vary in the amount of fibrous connective tissue proliferation, and researchers have reported a scirrhous pattern when there is marked fibrosis (Head et al. 2003). As observed in the present study, researchers have reported that mucin stains strongly with PAS (Barros 2016, Cullen 2017). Multiple *foci* of necrosis and hemorrhage are common in cholangiocarcinomas and hepatocellular carcinomas (Patnaik 1992, Cullen 2017). In the present study, these characteristics were visualized in most of the PHN.

Vascular invasion and extrahepatic metastases were identified in 40% of cholangiocarcinomas. In a similar study, most cholangiocarcinomas showed invasion in lymphatic and/or blood vessels. In dogs, vascular invasion was identified in 64% of these neoplasms (Flores et al. 2013). In hepatocellular carcinomas, invasion in vessels is not a common feature (Flores et al. 2013, Cullen 2017), and this finding corroborates those of the present study. Several authors have described that cholangiocarcinoma is the most commonly found metastatic PHN (Patnaik 1992, Lawrence et al. 1994, Head et al. 2003, Cullen 2017), and that hepatocellular carcinoma metastasis is uncommon in all animal species (Cullen 2009, Cullen & Stalker 2016). Regional lymph nodes are the major sites of metastatic PHN (Cullen & Stalker 2016). Especially in cats, cholangiocarcinomas can invade the Glisson capsule, with implantation of neoplastic cells in the peritoneum and serosa of various organs of the abdominal cavity (Cullen 2017). In a similar research, the frequency of metastatic cholangiocarcinomas ranged from 67 to 80% of the cases (Patnaik 1992, Lawrence et al. 1994). In a study with dogs, metastases were observed in approximately 78% of the cases, with lungs, lymph nodes, and abdominal cavity (omentum, mesentery and parietal peritoneum) as the most frequently affected organs (Flores et al. 2013), corroborating the findings of the present study.

All cholangiocarcinomas expressed CK 7, whereas hepatocellular carcinomas expressed Hep Par-1 in immunohistochemical analysis. In humans, the Hep Par-1 and CK 7 IHC assessments are used to differentiate PHN and in cases of metastatic carcinomas (Lau et al. 2002). Several studies in dogs have reported that CK 7 immunostaining in ductal epithelial cells has good sensitivity and specificity (Ramos-Vara et al. 2001, Flores et al. 2013). Cholangiocarcinoma does not show immunoreaction for Hep Par-1 (Shimonishi et al. 2000, Lau et al. 2002), as observed in the present study. Hep Par-1 is a highly specific and sensitive marker of normal, hyperplastic and/or neoplastic hepatocytes. Therefore, the Hep Par-1 associated with the CK 7 IHC techniques establish the diagnosis of PHN (Ramos-Vara et al. 2001). Hemangiosarcoma showed immunostaining for vimentin and von Willebrand factor, which are mesenchymal and endothelial cell-specific antibodies, respectively (Mello & Alves 1999, Bertazzolo et al. 2005).

In humans, hepatocellular carcinoma is the major PHN, because there are several associated etiological factors, such as viral infection, chronic alcoholism, nonalcoholic steatohepatitis and food contaminants as aflatoxins (Crawford & Liu 2010). In cats, the etiology of PHN is unknown (Lawrence et al. 1994), but *Platynosomum fastosum* infection has been described in the literature as a predisposing factor for the development of cholangiocarcinoma (Santos et al. 1981, Andrade et al. 2012). In the present study, no hepatobiliary trematodes were identified, because occurrence of *Platynosomum* sp. in the metropolitan region of Porto Alegre is infrequent (Michaelsen et al. 2012).

CONCLUSIONS

All primary hepatobiliary neoplasms (PHN) presented characteristics of malignancy and affected mainly elderly cats.

Cholangiocarcinoma was the most commonly diagnosed neoplasm, followed by hepatocellular carcinoma and hemangiosarcoma. Most cases of cholangiocarcinoma originated in intrahepatic bile ducts.

Grossly, cholangiocarcinoma and hemangiosarcoma presented predominance of the multinodular pattern, whereas hepatocellular carcinoma showed predominance of the massive pattern.

Extrahepatic gross findings were mainly characterized by poor body condition, ascites, and jaundice.

Histologically, there was predominance of acinar/ductal arrangement in cholangiocarcinomas and solid arrangement in hepatocellular carcinoma.

PHN showed moderate to severe cellular pleomorphism and mild mitotic index.

Proliferation of fibrous connective tissue and presence of mucin, identified by MT and PAS staining, respectively, were common histological findings in cholangiocarcinoma.

The use of hepatocyte and bile duct epithelial cells specific antibodies, such as Hep Par-1 and CK 7 assisted with the diagnosis of PHN.

Conflict of interest statement.- The authors declare having no conflicts of interest.

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