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Differential diagnoses in 83 dogs with icterus[1]

Maria C. Andrade[2], Letícia B. Oliveira[2], Ágna F. Santos[2], Matheus V.L. Moreira[2], Felipe Pierezan[3] and Roselene Ecco[3]*

ABSTRACT.- Andrade M.C., Oliveira L.B., Santos A.F., Moreira M.V.L., Pierezan F. & Ecco R. 2020. **Differential diagnoses in 83 dogs with icterus.** *Pesquisa Veterinária Brasileira 40(6):451-465.* Setor de Patologia, Departamento de Clínica e Cirurgia, Escola e Veterinária, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil. E-mail: ecco@vet.ufmg.br

Icterus (jaundice) is a yellowish pigmentation resulting from the depositing of bilirubin in tissues due to its high plasmatic concentration. The pathogenesis of icterus includes metabolic changes or obstructed bilirubin excretion and it is classified as pre-hepatic, hepatic and post-hepatic. This study aimed to evaluate and classify different causes of icterus in dogs during *post mortem* examination. These dogs were examined from 2014 to 2017, using macroscopic and histologic exams as well as ancillary tests. Eighty-three dogs were examined macroscopically and microscopically. They were separated into groups of icterus types: 24 (28.9%) dogs had pre-hepatic icterus, 45 (54.2%) had hepatic, 13 (15.7%) pre-hepatic and hepatic and one (1.2%) had post-hepatic icterus. Many factors were identified as a cause of icterus, including infectious agents (51/83), neoplasms (13/83), hepatic degeneration (11/83), chronic hepatic diseases (6/83), and obstructive causes (1/87). Among the infectious causes, leptospirosis, ehrlichiosis and disorders suggestive of septicemia were diagnosed. Neoplasms associated with icterus were cholangiocarcinoma, hemangiosarcoma and lymphoma. Other causes of icterus included degenerative diseases, such as lipidosis and glycogen degeneration. Hepatic fibrosis (cirrhosis) as a chronic disease and cholelithiasis also produced icterus. PCR was performed to confirm leptospirosis and ehrlichiosis. Samples of total DNA were used to amplify a fragment of a gene from *Leptospira interrogans* and *Ehrlichia canis*. In some dogs, co-infection of these agents was detected. The classification and identification of icterus etiologies in dogs is very important due to the number of diseases with this alteration, where *ante mortem* diagnosis is not always easily performed when some of these conditions are present.

INDEX TERMS: Diagnosis, dogs, icterus, jaundice, bilirubin, histopathology, PCR, serology, leptospirosis, ehrlichiosis, neoplasm, septicemia.

RESUMO.- [**Diagnósticos diferenciais em 83 cães com icterícia.**] Icterícia é a pigmentação amarelada decorrente da deposição de bilirrubina em tecidos devido à elevada concentração plasmática. A patogênese da icterícia inclui alterações no metabolismo ou na excreção de bilirrubina, sendo classificada em pré-hepática, hepática ou pós-hepática. O objetivo desse estudo foi identificar, avaliar e classificar as causas de icterícia em cães necropsiados de 2014 a 2017, associando as lesões macroscópicas, histológicas e exames complementares. Foram avaliados macro- e microscopicamente 83 cães com diferentes intensidades de icterícia. Os cães foram separados em grupos de acordo com o tipo de icterícia: 24 (28,9%) cães com icterícia pré-hepática, 45 (54,2%) cães com icterícia hepática, 13 (15,7%) com icterícia pré-hepática e hepática e um (1,2%) com icterícia pós-hepática. Foram identificadas várias etiologias associadas à icterícia, dentre elas pode-se destacar, agentes infecciosos (51/83), neoplasmas (13/83), processos degenerativos (11/83), crônicos (6/83) e obstrutivos (1/83). Dentre as causas infecciosas, destacam-se a leptospirose, a erliquiose e as lesões sugestivas de septicemia. Entre os neoplasmas associados com icterícia destacaram-se

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[2] Graduate Program in Animal Science with emphasis in Veterinary Pathology, Departamento de Clínica e Cirurgia, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil.

[3] Setor de Patologia, Departamento de Clínica e Cirurgia, Escola e Veterinária, Universidade Federal de Minas Gerais (UFMG), Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil. *Corresponding author: ecco@vet.ufmg.br

o colangiocarcinoma, hemangiossarcoma e linfoma. Outras causas de icterícia incluiriam os processos degenerativos como as degenerações gordurosa e glicogênica. Fibrose hepática (cirrose) e colelitíase foram também diagnosticados como causa de icterícia. A PCR foi utilizada para o diagnóstico confirmatório de leptospirose e erliquiose. Amostras de DNA total foram utilizadas para amplificar um fragmento dos genes de *Leptospira interrogans* e de *Ehrlichia canis.* Em alguns cães foi detectada co-infecção por estes agentes. A classificação e a identificação das causas de icterícia em cães são relevantes devido ao grande número de doenças que apresentam essa alteração, muitas vezes sem diagnóstico *ante mortem*.

TERMOS DE INDEXAÇÃO: Diagnóstico diferenciais, cães, icterícia, bilirrubina, caninos, histopatologia, PCR, sorologia, leptospirose, erliquiose, neoplasia, septicemia.

INTRODUCTION

Icterus refers to the yellow pigmentation of tissue caused by accumulated deposits of bilirubin. Bilirubin deposition is more evident in the mucous membranes and in tissues with highest proportion of elastin, such as the aorta and sclera. Icterus can be classified into pre-hepatic, hepatic and post-hepatic (Murdoch 1976, Beckingham & Ryder 2001).

Hyperbilirubinemia is characterized as unconjugated (indirect) or conjugated (direct) (Murdoch 1976). Unconjugated bilirubin (bilirubin bound to albumin) is formed during the metabolic degradation of hemoglobin as hemolysis. Unconjugated bilirubin is also created when there is insufficient bilirubin uptake or insufficient conjugation of bilirubin with glucuronic acid due to hepatocyte degeneration or necrosis (Thompson 1970, Beckingham & Ryder 2001). Conjugated bilirubin (bilirubin bound to glucuronic acid) can also be associated with liver diseases that decrease bilirubin uptake (e.g., hepatitis) or associated with reduced biliary tract drainage due to obstruction and/or compression of the bile canaliculi (Thompson 1970).

Prehepatic icterus is caused by increased bilirubin production due to hemolysis and may be caused by infectious agents such as *Babesia* spp., *Leptospira* sp., and *Rangelia vitalii* (Krauspenhar et al. 2003, Loretti & Barros 2005, Fighera et al. 2010) or immune-mediated diseases (Murdoch 1976). This excess of bilirubin overloads the liver's capacity to remove bilirubin from the plasma and secrete it into bile. Severe hemolysis may also result in hypoxia and consequently promote hepatic lesions, resulting in pre-hepatic and hepatic icterus (Cullen & Brown 2013). Hepatic icterus occurs due to insufficient bilirubin uptake as well as insufficient bilirubin conjugation and excretion. The etiology is related to acute or chronic severe hepatic disease, such as toxic liver diseases, neoplasms, liver fibrosis (cirrhosis) or infectious agents such as *Leptospira* sp. Post-hepatic icterus occurs in cases of obstruction and/or compression of bile ducts, resulting in reduced bile drainage. This leads to the accumulation of bilirubin in the blood (Murdoch 1976).

Icterus is a clinical finding common to several diseases. Therefore, the aim of this study is to classify the types of icterus and associate with the etiology, clinical, pathological and laboratory findings in 83 dogs.

MATERIALS AND METHODS

Samples. A prospective study was performed from June 2014 to June 2017 in the "Setor de Patologia" of the "Escola de Veterinária" of the "Universidade Federal de Minas Gerais" (UFMG). Eighty-three dogs with mild to marked icterus were examined. The data collected from these dogs, including medical history and clinical pathology exams, were obtained from the integrated veterinary hospital archive of UFMG. The reference values for the hemograms (45/83) and serum biochemical tests (43/83) performed in this study were interpreted according to Kaneko et al. (1997).

Serology anti-*Leptospira*. The microscopic agglutination test (MAT) was standardized and utilized according to Fields et al. (1965) for the following serovars: Icterohaemorragiae, Wolfi, Hardjo, Bratislava, Autumnalis, Canicola, Grippothyphosa, Pomona, Ballum, Bataviae, Copenhageni and Tarassovi. This test was performed on 13 out of 40 dogs suspected of leptospirosis. The results were interpreted according to WHO (2003) and Azócar-Aedo et al. (2017).

Gross and histopathology. The *post mortem* examinations were performed on all 83 dogs. The procedures in this study were performed in accordance with the recommendations by the Ethics Committee of the UFMG (Protocol 61/2017). Samples of the liver, kidneys, lymph nodes, spleen, bone marrow, lungs, heart and other organs that were found to have changes during necropsy were collected. The samples were sectioned, fixed in 10% neutral buffered formalin for 48 to 52 hours for histological evaluation. The tissues were dehydrated in increasing ethanol series, diaphanized in xylene and paraffin embedded to obtain sections of 4.0µm, stained by hematoxylin and eosin (HE) (Luna 1968) and examined using white light microscope.

Cytopathology was only performed with samples from dogs with clinical suspicion and/or macroscopic lesions suggestive of leishmaniasis or to rule out babesiosis. The smears were performed using bone marrow and spleen samples. A thin layer of tissue was spread on the glass microscopy slide, air-dried and stained with a quick stain (Diff Quick) for subsequent microscopic examination.

Polymerase chain reaction. For all dogs, samples of the liver, kidneys, lymph nodes, spleen and bone marrow were collected and stored at -20°C until PCR was performed to detect *Ehrlichia canis* and *Leptospira interrogans* DNA. PCR was only performed with samples from dogs with histologic lesions suggestive of these diseases. The total DNA extraction method was based on the use of sodium iodide and silica according to the method described by Vogelstein & Gillespie (1979). To amplify a fragment of the gene named p28 from *E. canis,* a set of primers: ECp28-F 5'-ATGAATTGCAAAAAAATTCTTATA-3' and ECp28-R 5'-TTAGAAGTTAAATCTTCCTCC-3' were used. These primers generated a product of 843 base pairs (bp) (Nakaghi et al. 2010). For positive control, DNA was extracted from a cell culture infected with *Ehrlichia* sp., provided by the parasitology laboratory of UFMG. To detect DNA of *L. interrogans,* the primer sets utilized were: LipL32-F 5'-CGCTTGTGGTGCTTTCGGTG-3' and LipL32-R 5'-GCGCTTGTCCTGGCTTTACG-3'. This reaction generated a product of 152 bp, according to the method described by Coutinho et al. (2014) with modifications. PCR conditions were 95°C for 5 min, followed by 30 cycles at 95°C for 30 seconds, annealing for 1 min at 52°C, extension at 72°C for 2 min and final extension at 72°C for 5 min. An inactivated bacterial sample of *L. interrogans* serovar Icterohaemoragiae were provided by the "Laboratório Nacional Agropecuário" (Lanagro-MG) as a positive control. As a negative control, the primers were used in conjunction with PCR Master Mix and 2µl of ultrapure water without DNA. To assess viability and quality of extracted DNA, all negative samples were subjected to PCR

to detect *β-actin* (endogenous control gene), using the previously described primers (Turchetti et al. 2015) with modifications. The following PCR conditions were used: 95°C for 10 min, 35 cycles at 95°C for 30 seconds, annealing temperature for 30 seconds at 60°C, extension at 72°C for 30 seconds and a final elongation at 72°C for 5 min. PCRs were performed using a final volume of 25µl (PCR Master Mix Promega, Madison/WI, USA) and 200-300ng of DNA template, in a thermocycler (Veriti® Thermal Cycler, Applied Biosystems, Inc., Foster City/CA, USA). The final product of each reaction was subjected to electrophoresis in 1.5% agarose gel stained with ethidium bromide along with a molecular weight marker of 100 bp (LowRanger100bp DNA Ladder Norgen®). This final product was visualized using an UV trans-illuminator.

RESULTS

From 2014 to 2017, 83 dogs that presented with icterus were necropsied. Forty-five dogs (54.2%) were male with an average age of eight years and two months. Their ages varied between seven months and 20 years. Thirty-eight dogs were female (45.8%), with an average age of seven years and eight months. Their ages varied between two months and 14 years. The most common breeds were mixed-breed (43.4%) followed by Poodles (7.2%) and Golden Retrievers (7.2%), Yorkshire Terriers (6.0%), German shepherds (4.8%) and various others (31.4%).

The type of icterus in the dogs of this study was evaluated and classified according to the diagnoses (Table 1). Twenty-four dogs had pre-hepatic icterus (28.9%), 45 (54.2%) had hepatic, 13 (15.7%) had pre-hepatic and hepatic and one (1.2%) had post-hepatic icterus. The classification of the types of icterus was based on macroscopic and histologic lesions. Dogs classified with pre-hepatic icterus had multicentric hemorrhages and/or hemoglobinuria. Dogs classified with hepatic icterus had degenerative, necrotic or compressive lesions in the hepatic parenchyma. Dogs with multiple hemorrhages and hepatocytes lesions were classified with pre-hepatic and hepatic icterus. Dogs with obstructed and/or compressed extrahepatic bile ducts were classified with post-hepatic icterus. Different diagnoses were obtained, including infectious etiologies (51/83), neoplastic diseases (13/83), degenerative diseases (11/83), chronic conditions (6/83), neoplastic associated with degenerative diseases (1/83) and biliary tract obstruction (1/83). Regarding the infectious diseases, the most frequent were leptospirosis (35/51), ehrlichiosis (7/51) or coinfectious by both agents (5/51). Septicemia was diagnosed in three dogs (3/51). One of these dogs had bacterial pancreatitis and hepatitis associated with ehrlichiosis. The neoplastic disease diagnoses were cholangiocarcinoma (5/13), hemangiosarcoma (4/13), pancreatic carcinoma (2/13) and lymphoma (3/13). The degenerative hepatic diseases included lipidosis (8/11) and glycogenic degeneration (3/11). One case of lipidosis accompanied with multicentric lymphoma was also diagnosed. Hepatic fibrosis was diagnosed in six dogs (6/6). Finally, one dog (1/83) was found with obstructive cholelithiasis. Cytopathological smear examinations from liver, spleen and bone marrow for *Babesia* sp. infection, performed during *post mortem* examination, were negative in these dogs. Similarly, no histological lesions indicative of *Rangelia vitalli* were found.

Hematology tests were performed on 45 dogs (Table 2). The majority of animals with icterus also had anemia. Most of these anemia cases were regenerative as they were associated with an increased number of reticulocytes.

Biochemical tests were performed on 43 dogs (Table 3). The values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and

Table 1. Classification of icterus intensity and histopathologic diagnostic

Icterus % (n/total)	Histopathologic diagnosis	N	(%)
Pre-hepatic 30.1% (25/83)	Leptospirosis	11	45.8
	Ehrlichiosis	7	29.1
	Hemangiosarcoma	3	12.5
	Septicemia	2	8.3
	Septicemia and suggestive of ehrlichiosis	1	4.16
Total		24	100
Hepatic 53.0% (44/83)	Leptospirosis	15	33.34
	Lipidosis	8	17.78
	Hepatic fibrosis (cirrhosis)	6	13.34
	Cholangiocarcinoma	5	11.11
	Glycogenic degeneration	3	6.67
	Leptospirosis and ehrlichiosis	2	4.44
	Metastatic pancreatic carcinoma	2	4.44
	Lymphoma with hepatic metastases	2	4.44
	Lipidosis and multicentric lymphoma	1	2.22
	Bacterial hepatitis and pancreatitis	1	2.22
Total		45	100
Pre-hepatic and hepatic 15.7% (13/83)	Leptospirosis	9	69.2
	Leptospirosis and ehrlichiosis	3	23.1
	Hemangiosarcoma	1	7.7
Total		13	100
Post-hepatic 1.2% (1/83)	Cholelitiasis	1	100

gamaglutamiltransferase (GGT) were generally high when the icterus was classified as hepatic. In the majority of pre-hepatic icterus cases the ALP values were generally high. For animals with pre-hepatic and hepatic icterus, the AST and ALP values were generally increased. When the total protein values were low, this was mostly due to decreased albumin values. Creatinine and urea sera values were usually above the reference values, independent of icterus classification. Biochemical exams were not performed on the dog with post-hepatic icterus.

Leptospirosis

Laboratory diagnosis of leptospirosis was performed in 40 out of 83 dogs using histopathology in conjunction with serology and/or PCR. Macroscopically, mild to marked levels of icterus were found in the mucous membranes (Fig.1A), skin, subcutaneous and visceral adipose tissue, intimal layer vessels, serosae and joints. In some dogs, numerous multifocal petechial hemorrhages in the mucous membranes, subcutaneous and/or serosae were also present. Hemorrhages in the lungs (Fig.1B) were also seen in some dogs. The livers were mildly to moderately enlarged and red-yellow to red-green (Fig.1C). The kidneys were yellow-green to red-yellow with evident alterations especially in the medullar region (Fig.1D).

The histological lesions found were mild to marked multifocal or diffuse dissociation of hepatocytes (15/40), mild to marked randomly multifocal necrosis of hepatocytes (21/40) with mild to marked biliary stasis (40/40) (Fig.2A). There was also mild to moderate multifocal cholemic nephrosis (19/40), mild to moderate multifocal hemoglobinuric nephrosis (4/40) (Fig.2B and 2C). In two dogs with hemoglobinuric nephrosis, Prussian blue stains were positive, revealing iron inside the cytoplasm of the epithelium and the lumen of the tubules (Fig.2D). Mild to marked multifocal membranous glomerulonephropathy (27/40), mild multifocal glomerulosclerosis (6/40), and moderate multifocal proteinuria (8/40) were also seen. In the spleen and lymph nodes there was lymphoid depletion with moderate to marked increase differentiation of plasm cells (plasmacytosis) (12/40) and mild to moderate multifocal erythrophagocytosis (15/40). In addition, there were macrophages infected with *Leishmania* spp. amastigotes in the bone marrow (11/40).

From 40 dogs diagnosed with leptospirosis, serology to detect antibodies anti-*Leptospira* spp. were performed in 13 dogs. Ten were reagents and three were negative. Sorovars reagents were the following: Icterohaemorrhagiae (6/10), followed by Bratislava (3/10), Autumnalis (3/10), Canicola (3/10), Grippothyphosa (2/10), Pomona (1/10), Andamana (1/10), Ballum (1/10), Bataviae (1/10), Copenhageni (1/10), Pyrogenes (1/10) and Tarassovi (1/10). There were five (5/13) dogs with multiple sorovars. The antibody titers for these serovars ranged from 1:100 to 1:800. The highest titer (1:800)

Table 2. Hematological results from 45 dogs with icterus

Hematological exams		Pre-hepatic (n=11)	Hepatic (n=29)	Pre-hepatic and hepatic (n=5)
Anemia		81.8% (9/11)	79.3% (23/29)	60.0% (3/5)
Increased	Leucocytes	54.5% (6/11)	72.4% (21/29)	80.0% (4/5)
	Bastonets	36.4% (4/11)	53.6% (15/28)	75.0% (3/4)
	Segmented cells	54.5% (6/11)	75.8% (22/29)	80.0% (4/5)
	Monocytes	36.4% (4/11)	75.9% (17/29)	40.0% (2/5)
	Platelets	0	6.9% (2/29)	0
Decreased	Leucocytes	18.2% (2/11)	3.4% (1/29)	0
	Segmented cells	18.2% (2/11)	0	20.0% (1/5)
	Lymphocytes	72.2% (8/11)	48.3% (14/29)	80.0% (4/5)
	Monocytes	18.2% (2/11)	3.4% (1/29)	40.0% (2/5)
	Platelets	63.6% (7/11)	75.9% (22/29)	100.0% (5/5)

Table 3. Serum biochemical results from 43 dogs with icterus

Biochemical profile		Pre-hepatic (n=13)	Hepatic (n=25)	Pre-hepatic and hepatic (n=5)
Increased	Urea	61.5% (8/13)	76.0% (19/25)	100.0% (5/5)
	Creatinine	53.8% (7/13)	63.6% (14/22)	40.0% (2/5)
	ALT	30.8% (4/13)	60.0% (15/25)	40.0% (2/5)
	AST	23.1% (3/13)	72.0% (18/25)	60.0% (3/5)
	ALP	75.0% (9/12)	84.0% (21/25)	60.0% (3/5)
	GGT	23.1% (3/13)	56.0 (14/25)	25.0% (1/4)
	Amilase	23.1% (3/13)	44.0% (11/25)	50.0% (2/4)
	Total protein	7.7 (1/13)	4.2% (1/24)	40.0% (2/5)
	Globulin	46.2% (6/13)	20.8% (5/24)	40.0% (2/5)
Decreased	Amilase	7.7% (1/13)	8.0% (2/25)	0
	Total protein	46.2% (6/13)	41.7% (10/24)	40.0% (2/5)
	Albumin	92.3% (12/13)	79.2% (19/24)	100.0% (5/5)
	Globulin	0	25.0% (6/24)	20.0% (1/5)

ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamaaglutamyltransferase.

was detected for serovars Icterohaemorrhagiae, Bratislava, Autumnalis, Bataviae and Pomona. Considering the clinical history (vaccinated or not vaccinated dogs), titers above 1:200 were considered positive results (Azócar-Aedo et al. 2017).

PCR for *Leptospira interrogans* resulted positive in 37/40 dogs, for one or more organs. In dogs that tested positive, DNA amplification was performed on samples from the liver (24/40), kidney (16/39) and spleen (20/39). One of the dogs that had a negative PCR result for *L. interrogans* (1/40), had a positive serological test for Bratislava, Ballum and Pyrogenes serovars. In five of these dogs there were lesions suggestive of coinfection by *Ehrlichia* sp., with four testing positive for *E. canis* using PCR.

In the hematological tests (21/40), anemia was diagnosed in 17 dogs (17/21). The types of anemia were normocytic normochromic (11/17), macrocytic normochromic (5/17) and normocytic hypochromic (1/17). There was also moderate to marked leukocytosis (16/21) by neutrophilia (17/21) with swift to the left (12/21) and monocytosis (12/21). Lymphopenia (11/21) and mild to severe thrombocytopenia (17/21) were also detected. In the biochemical exams, the most relevant changes were a mild to accentuated increase in the sera values of urea (15/18), creatinine (12/17), ALT (7/18), AST (9/18), ALP (14/17) and GGT (6/18). A mild to accentuated increase in the sera values of amylase was detected in nine (9/18) dogs. In addition, the total protein values were found to have decreased (6/18) more often than increased (3/18).

Ehrlichiosis

A diagnostic of ehrlichiosis, using histological lesions and/or PCR, was performed on 12 out of 83 animals. Dogs diagnosed with ehrlichiosis were characterized macroscopically by anemia and mild to moderate icterus (Fig.3A). There were also multifocal petechial hemorrhages in the subcutaneous tissue, serosae (Fig.3B) and mucous membrane. The lymph nodes were dark-red to brown (Fig.3C) and the kidneys light-red to yellow (Fig.3D). Histologically, the spleen and lymph nodes had diffuse lymphoid depletion (Fig.4A), marked multifocal plasmacytosis, several Mott cells and erythrophagocytosis. Hemossiderosis was also found in the lymph nodes in six out of 13 dogs. There was also mild multifocal perivascular (2/12) random (2/12) or centrilobular (1/12) lymphoplasmacytic hepatitis with mild multifocal bile stasis (3/12). In addition, there was mild multifocal hepatocytic necrosis (2/12). In the kidneys, there was multifocal moderate to marked membranous

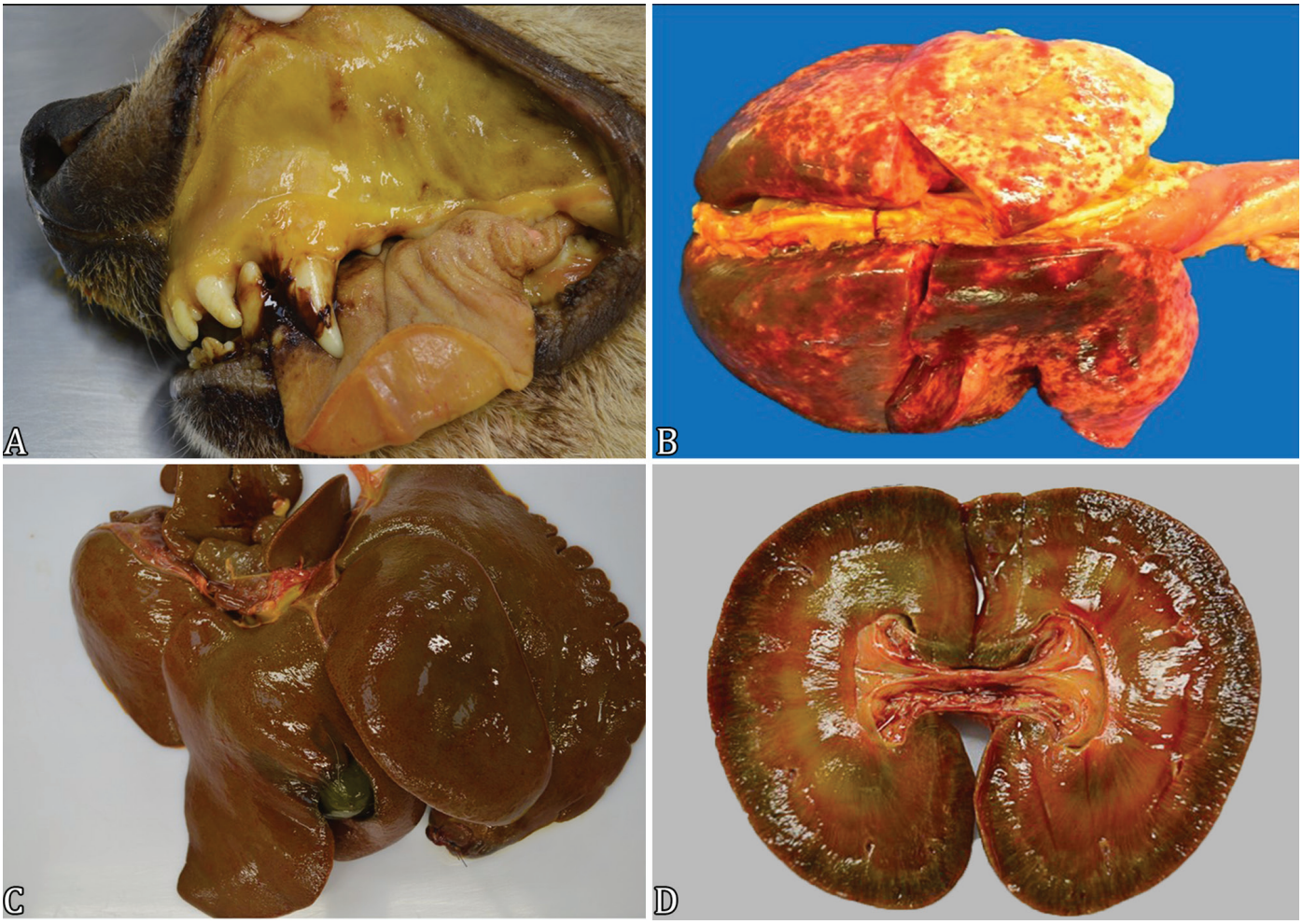

Fig.1. Adult male German Shepherd dog, diagnosed with leptospirosis. (**A**) Markedly yellow oral mucous membrane with ecchymoses. (**B**) Lung of a dog with numerous hemorrhagic areas in the visceral pleura. (**C**) Liver diffusely yellow-red and slightly green. (**D**) Kidney of dog from Figure 1A with diffusely red-brown cortical and medullar with yellow and green areas.

glomerulonephropaty (5/12), multifocal moderate fibrosis (5/12) and nephrosis (5/12), majority bilirubinuric (3/12), multifocal mild to moderate proteinuria (2/12) and moderate multifocal, interstitial nephritis, predominantly by plasm cells (4/12). In the bone marrow, varying degrees of hypocellularity (Fig.4B) and plasmacytosis were seen. In three dogs (3/12), macrophages with intracytoplasmic amastigotes compatible with *Leishmania* spp. were also found.

In the PCR for *E. canis,* five out of 12 dogs had one or more positive organ: bone marrow (2/12), lymph node (2/12) and spleen (2/12). In seven dogs (7/12), *E. canis* DNA was not amplified.

Three dogs (3/12) diagnosed with ehrlichiosis had hematologic tests. One dog presented macrocytic and normocytic anemia and another dog had normocytic and normochromic anemia. In addition, these dogs had severe leukopenia by neutropenia, lymphopenia, monocytopenia and thrombocytopenia. The biochemical exams (4/12) detected a mild increase in serum values of urea in one dog (1/4) and a marked increase in urea and creatinine values in another dog (1/4). For AST (2/4), ALT (1/4) and GGT (1/4) there were mild increases and a moderate increase for ALP (4/4).

Septicemia

Three dogs (3/83) were diagnosed with septicemia in accordance with their macroscopic and histopathological findings. Macroscopically, these dogs had mild to moderate icterus. The probable cause of septicemia in each of these cases were: fibrinous pericarditis; pyelonephritis and prostatitis; epididymitis and hepatitis (this dog also had ehrlichiosis). Multiple hemorrhagic areas were present in all three dogs (3/3).

Liver histopathology examinations revealed random multifocal histiocytic and lymphoplasmacytic hepatitis (3/3), moderate multifocal hyperemia (1/3) and mild multifocal biliary stasis (3/3). In the kidneys, there was moderate

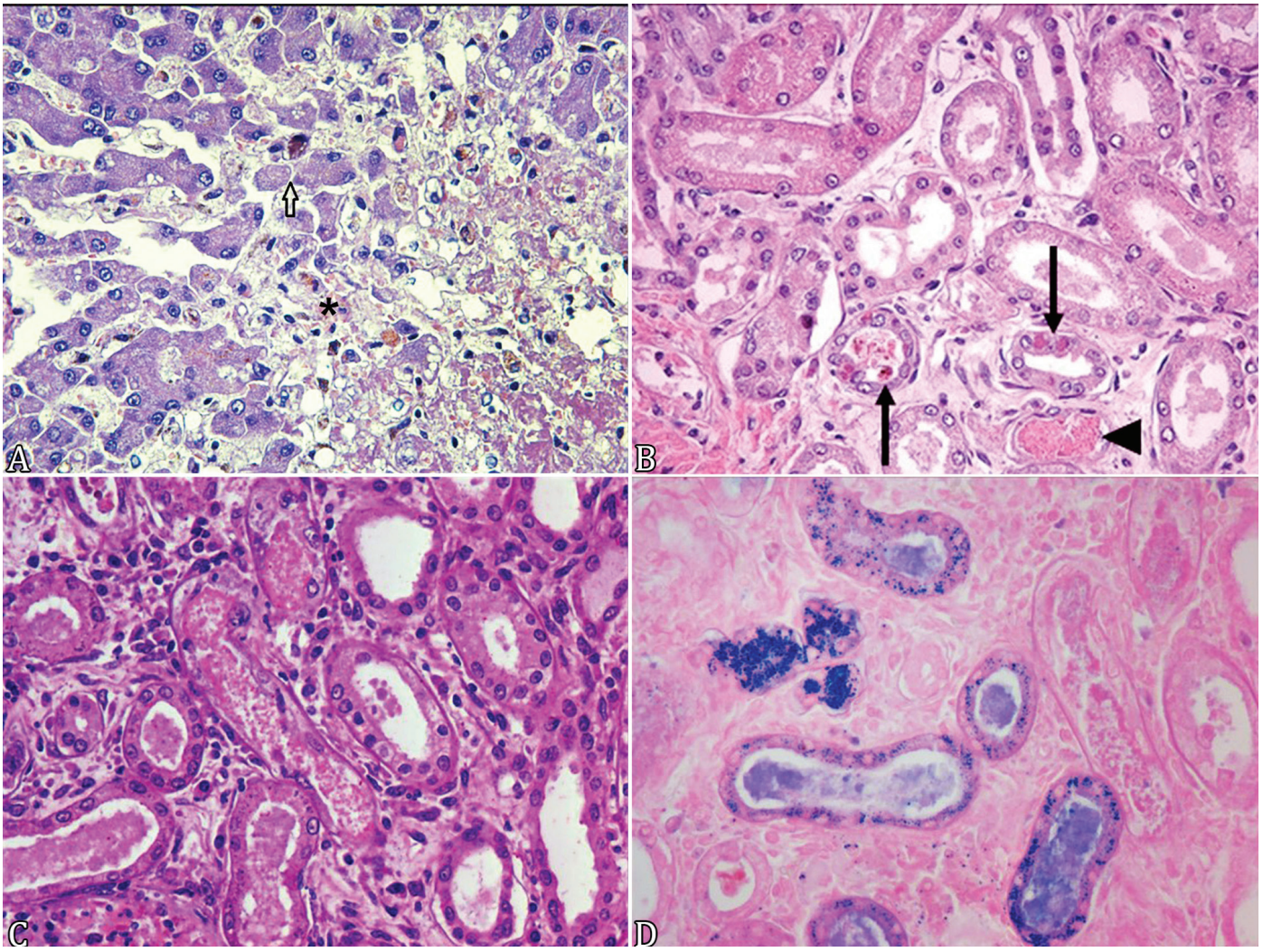

Fig.2. Histologic lesions in dogs with leptospirosis. (**A**) Adult male White Swiss Shepherd dog. Liver. Individual coagulative necrosis (*) and dissociation of hepatocytes (arrow). HE, obj.40x. (**B**) Kidney of dog from Figure 2A. Tubular lumen (arrow head) and cytoplasm of epithelial cells (arrow) containing yellow-brown pigment compatible with bilirubin (bilirubinuric nephrosis). HE, obj.40x. (**C**) Female mixed-breed dog. Kidney. Tubular lumen filled with eosinophilic to yellow casts and drops compatible with bilirubin (bilirubinuria) and brown granules within the cytoplasm of the epithelial cells compatible with hemosiderin. Necrosis of tubular epithelium can also be seen. HE, obj.40x. (**D**) Kidney from Figure 2C. Blue casts and granules indicating hemosiderin. Prussian Blue stain, obj.40x.

Fig.3. (**A**) Nine-year-old female German shepherd dog, diagnosed with ehrlichiosis caused by *Ehrlichia canis*. Moderately yellow oral mucous membrane. (**B**) Parietal pleura with numerous multifocal to coalescing petechial and ecchymoses hemorrhages. (**C**) Cervical and axillary lymph nodes, diffusely dark-red (drainage hemorrhages). (**D**) Kidneys diffusely pale-red in the cortical region and yellow in the medullar and pelvic adipose tissue.

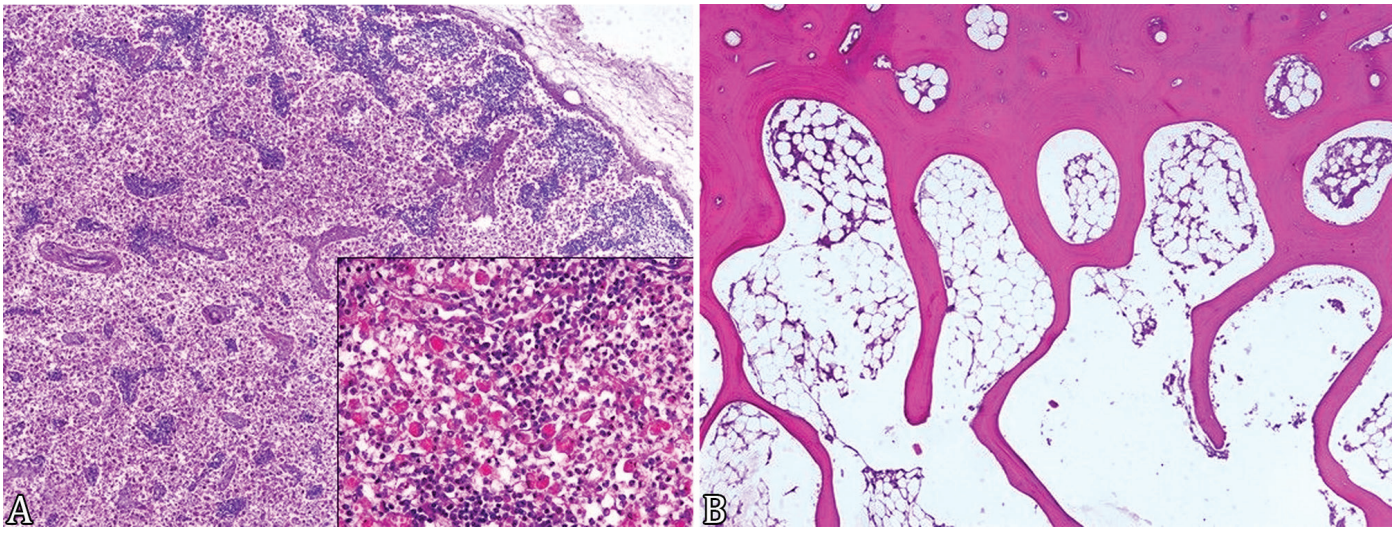

Fig.4. Histopathology of an adult male German shepherd dog with icterus diagnosed with chronic ehrlichiosis caused by *Ehrlichia canis.* (**A**) Lymph node. Marked decrease of lymphocyte density in the cortical region, and loss of follicular organization. HE, obj.50x. The inset shows the medullar area of the lymph node with marked erythrophagocytosis. HE, obj.20x. (**B**) Bone marrow. Diffuse decrease of cellularity characterizing marked hypoplasia. HE, obj.50x.

multifocal *membranous glomerulonephropathy* (1/3), mild multifocal to moderate interstitial lymphoplasmacytic nephritis (3/3), moderate multifocal tubular necrosis (2/3) and mild multifocal bilirubinuric nephrosis (3/3). There were also moderate multifocal hemorrhages (1/3), hyaline cylinders (1/3) and mild multifocal neutrophilic and lymphoplasmacytic pyelonephritis (1/3).

In the spleen and lymph nodes, there was mild to moderate lymphoid depletion (3/3). In addition, there were mild to moderate hemosiderosis (3/3), mild to moderate erythrophagocytosis (2/3) and hyperemia (1/3). The dog with concomitant ehrlichiosis also had plasmacytosis with some Mott cells. In the bone marrow and lymph nodes, there were some macrophages with intracytoplasmic amastigotes compatible with *Leishmania* spp.

Hematological tests in two dogs (2/2) revealed moderate to marked normocytic normochromic anemia (2/2), a mild to accentuated neutrophilic left shift (1/2) and thrombocytopenia (1/2). In two dogs the biochemical tests detected a mild to moderate increase in the plasma concentrations of urea and creatinine (1/2). A mild increase for AST and ALP (1/2) were also found.

Bacterial pancreatitis and hepatitis

One dog (1/83) was diagnosed with bacterial pancreatitis and hepatitis based on macroscopic and histopathological findings. Macroscopically the icterus was mild. The liver had accentuated lobular pattern and was mildly yellowish with several multifocal whitish areas (0.5cm). At histopathology, there were necrotizing and lymphoplasmacytic hepatitis and pancreatitis. In addition, thrombosis was found in the kidneys and lungs.

Hematological tests detected moderate normocytic normochromic anemia with marked neutrophilia, left shift neutrophils and monocytosis. Biochemical tests revealed increase in the sera values for AST, GGT, ALP and marked hypoproteinemia by hypoalbuminemia and hypoglobulinemia.

Cholangiocarcinoma

Five dogs (5/83) were diagnosed with primary hepatic neoplasia compatible with cholangiocarcinoma based on the macroscopic and histopatological findings. Macroscopically, these dogs had mild to moderate icterus. In the liver there were multiple well-demarcated, yellow-whitish nodules. In some prominent nodules, umbilication was present (5/5). In one dog, similar nodules were also present in the kidneys (metastases). At histopathology, neoplastic cells were arranged in tubular forms interspersed by abundant connective tissue stroma. These areas widely replaced the hepatic parenchyma (5/5). In addition, there were random multifocal necrotic areas and moderate multifocal biliary stasis (5/5). Some of these dogs had metastatic cholangiocarcinoma in the lymph nodes (2/5), pancreas and kidneys (1) and bone marrow (1/5).

Hematological and biochemical tests were performed on two dogs (2/5). The hematological tests revealed neutrophilic left shift (1/2), neutrophilia (1/2), lymphopenia (2/2) and mild to marked thrombocytopenia (2/2). The biochemical tests revealed a moderate to marked increase in the sera values of urea (2/2), a marked increase of creatinine (1/2), a marked increase of ALT and ALP (1/2), a moderate increase of GGT (2/2) and a mild to marked increase of AST (2/2).

Hemangiosarcoma

In four dogs (4/83), vascular endothelial neoplasia consistent with metastatic hemangiosarcoma was diagnosed. Macroscopically, they had mild to moderate icterus. Well-demarcated dark-red nodules were found in the liver (2/4), lungs (2/4), spleen (2/4), lymph nodes (1/4), brain (1/4) and heart (1/4).

Liver histopathology showed neoplastic mesenchymal proliferation, forming vascular spaces filled with erythrocytes and aligned with one or more poorly differentiated endothelial cells layer (2/4). The parenchyma adjacent to the metastatic areas was compressed and necrotic (1/4). In all dogs there was moderate multifocal biliary stasis. In the kidneys there were mild to marked multifocal bilirubinuric nephrosis (4/4), tubular hemosiderosis (1/4) mild to moderate multifocal *membranous glomerulonephropathy* (3/4), glomeruloesclerosis (2/4) and moderate multifocal interstitial lymphoplasmacytic nephritis (2/4). Mild lymphoid depletion (3/4), erythrophagocytosis (3/4) and hemosiderosis (4/4) were found in the lymph nodes. Hemosiderosis in the renal tubules was confirmed by Prussian blue stain method.

Hematological tests were performed in two dogs (2/4). One dog had moderate normocytic normochromic anemia with neutrophilia, left shift neutrophils and monocytosis. Another dog had neutropenia and monocytopenia. Both had anemia and thrombocytopenia. Biochemical tests (2/4) revealed a marked increase in the plasma concentrations of urea and creatinine and mild hyperglobulinemia.

Lymphoma

Three dogs (3/83) were diagnosed with lymphoma. Macroscopically, they had moderate icterus. In two dogs all lymph nodes were markedly enlarged with replacement of normal parenchyma with neoplastic cells. Microscopically, the neoplastic cells had morphologic characteristics of lymphoma. These dogs had metastatic multicentric lymphoma with extensive liver invasion and multiple neoplastic nodules in the lungs, spleen and kidneys. One dog was also diagnosed with alimentary lymphoma. The jejunum and ileum had transmural infiltration by this neoplasia with focal luminal stenosis. Metastases in the lungs, liver and kidneys were found.

Hematological and biochemical tests were performed on all three dogs. Results revealed macrocytic normochromic (1/3) or normocytic normochromic (2/3) anemia with neutrophilic left shift (3/3), lymphopenia (2/3) and thrombocytopenia (2/3). Plasma concentrations were mildly increased for urea and creatinine (1/3), GGT (1/3) had a marked increased and AST and ALP (2/3) had moderate increases. Two dogs had hypoalbuminemia.

Pancreatic carcinoma

Two dogs (2/83) were diagnosed with pancreatic carcinoma based on pathological findings. Macroscopically, there was mild to moderate icterus. One dog had a solid and single yellow mass and another dog had multiple white to yellow nodules in the pancreas. Metastases in the liver, lungs, pancreatic lymph node, kidneys and bone marrow were found in one dog. Microscopically, neoplastic cells had morphology compatible with acinar pancreatic cells. Hematological tests detected normocytic normochromic anemia (1/2), neutrophilic left shift (1/2), monocytosis (2/2) and thrombocytopenia (2/2).

These dogs also had moderate to marked increases in the plasma concentration of urea and creatinine (2/2), ALT, AST, ALP and GGT (1/2). One dog had hypoalbuminemia.

Lipidosis

Nine dogs (9/83) were diagnosed with lipidosis with moderate to marked icterus (Fig.5A). The liver was enlarged, diffusely yellow (Fig.5B) and friable. Microscopically, hepatocytes were seen markedly enlarged with well-demarcated vacuoles within the cytoplasm, displacing the nucleus. Moderate to marked biliary stasis was present in all dogs. In the kidneys, there was mild to moderate multifocal bilirubinic nephrosis. Mild or marked *membranous glomerulonephropathy* (7/9), glomeruloesclerosis (2/9), moderate multifocal interstitial lymphoplasmacytic nephritis (4/9) and mild proteinuria (4/9) were also found. The lymph nodes had moderate multifocal hemossiderosis (3/9).

The hematological tests detected normocytic normochromic (3/5) and macrocytic normochromic (2/5) anemia, neutrophilia, left shift neutrophils (4/5) and monocytosis (3/5). In four dogs (4/5) mild to moderate lymphopenia and mild to marked thrombocytopenia were detected. In five dogs (5/9), their urinary and hepatic biochemical profiles showed mild to marked plasma concentration increases in urea (5/5), creatinine (3/5), ALT (3/5), ALP (5/5) and GGT (3/5). Total protein values were mildly to moderately decreased (3/5).

Glycogenic degeneration

Three dogs (3/83) were diagnosed with marked glycogenic degeneration in the liver. Macroscopically, these dogs had mild to marked icterus. In all dogs the liver was moderately enlarged and diffusely red-orange with *accentuation* of the normal *lobular pattern. The bile was* thick and *grumous.* Histopathologically, the hepatocytes were markedly enlarged with poorly demarcated vacuoles within the cytoplasm and the nucleus was generally centrally located. There was also a moderate multifocal biliary stasis. In the kidneys there was mild multifocal bilirubinic nephrosis (2/3) and moderate glomerulonephropathy (2/3). In the spleen and lymph nodes there were mild to moderate lymphoid depletion (3/3) and hemosiderosis (2/3).

Hematological tests in three dogs (3/3) detected normocytic normochromic (2/3) and macrocytic normochromic (1/3) anemia, moderate neutrophilia (2/3), monocytosis (1/3) and thrombocytopenia (1/3). The urinary and hepatic biochemical profiles showed moderate plasma concentration increases in urea and creatinine (2/3), AST (1/3), and mild to marked increases in ALT, ALP and GGT (3/3).

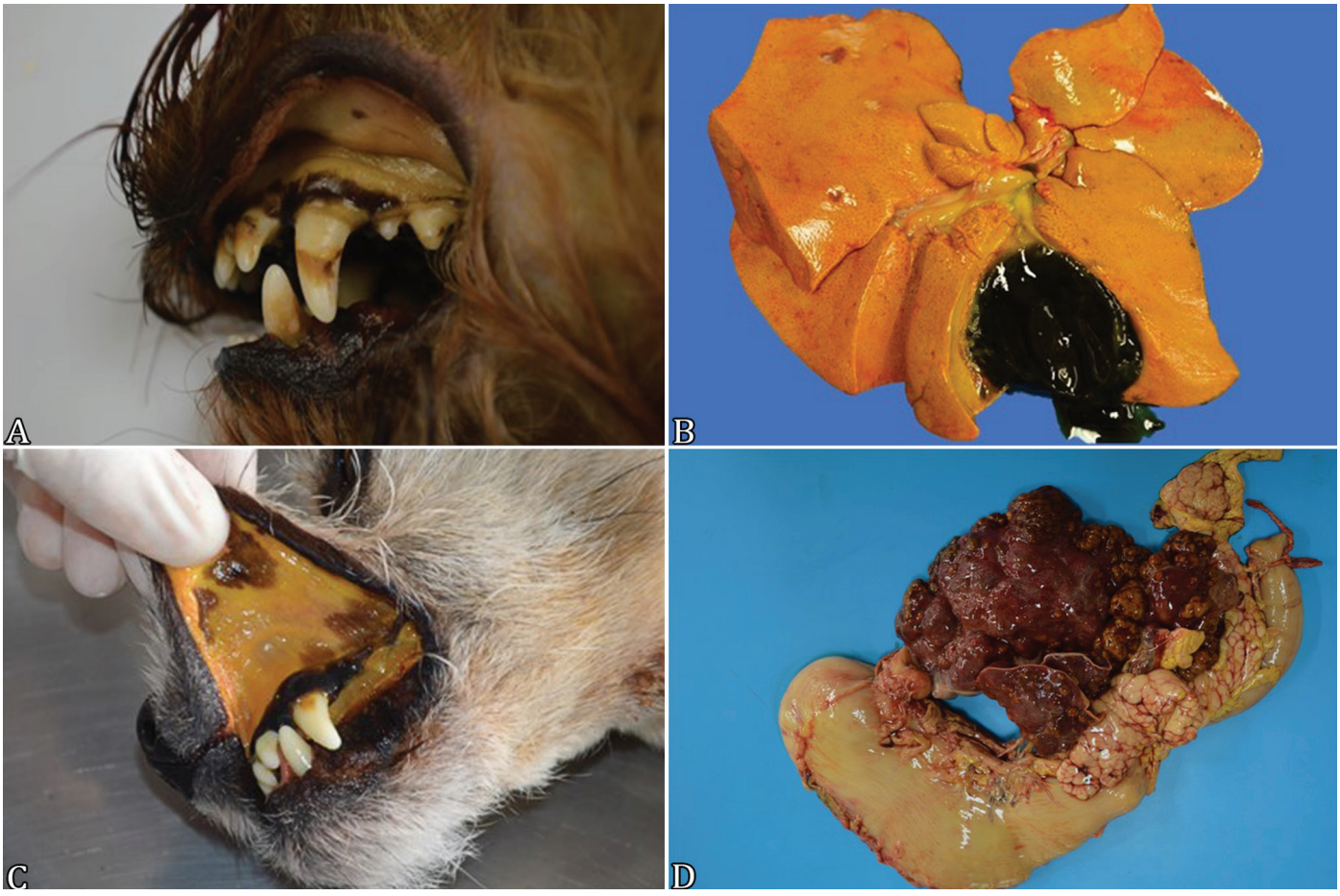

Fig.5. Liver with degenerative lesions. (**A**) Oral mucous membrane with moderate icterus. (**B**) Liver of the dog from Figure A with marked diffuse yellowish discoloration (lipidosis). (**C**) Terminal hepatic fibrosis in an adult male mixed-breed dog. (**D**) Liver of the dog from Figure C. Liver markedly decreased in size with irregular surface and yellow-greenish nodules (cirrhosis).

Hepatic fibrosis (cirrhosis)

Six dogs (6/83) had advanced hepatic fibrosis. Macroscopically, all dogs had moderate to marked icterus (Fig.5C). The liver of these dogs was moderately to markedly smaller than expected, with an irregular surface and with multiple yellow nodules (Fig.5D).

Histopathologically, there was a loss of normal hepatic architecture and replacement with portal-portal to portal-central bridging fibrosis, biliary duct proliferation and nodular areas of regenerating hepatocytes. In addition, multifocal necrosis was associated with moderate lymphoplasmacytic and neutrophilic infiltration (6/6) and biliary stasis. In the kidneys there was multifocal bilirubinic nephrosis (6/6) and a mild to moderate multifocal membranous glomerulonephropathy (3/6). In the spleen and lymph nodes there was mild lymphoid depletion (2/6) and moderate hemosiderosis (6/6). In the bone marrow of one dog (1/6) there was a mild number of macrophages with amastigotes compatible with *Leishmania* spp. within the cytoplasm.

Hematological tests were performed in three dogs. Normocytic normochromic anemia was diagnosed in three dogs (3/3). In addition, there were moderate neutrophilia, left shift neutrophils and monocytosis in two dogs. All dogs had moderate to marked thrombocytopenia. In three dogs (3/6), the biochemical profile showed a mild increase in the sera values of GGT (1/3), a moderate ALT increase (2/3), a mild to moderate AST increase (3/3) and a marked ALP increase (2/3). Two dogs had hypoalbuminemia.

Biliary duct obstruction

Complete extrahepatic biliary duct obstruction caused by cholelithiasis was diagnosed in one dog (1/83). Clinically, the animal presented anorexia and recurrent vomiting. Macroscopically, there was marked icterus (Fig.6A) and the gallbladder was markedly enlarged and distended. The lumen was filled with yellow-green thick grumous bile. Gallstones were found blocking the ductal lumen (Fig.6B). Microscopically, the gallbladder mucous membrane had moderate neutrophilic infiltration and multifocal hemorrhages. Hematological and biochemical tests were not performed.

DISCUSSION

Pre-hepatic and hepatic icterus were the most frequent types of icterus in this study. Only one was classified as post-hepatic icterus. Leptospirosis stood out as the main cause of pre-hepatic or hepatic icterus in the dogs of this study.

The pre-hepatic form can occur as a result of erythrocytes destruction inside blood vessels or erythrophagocytosis in the lymphoid organs (Valli et al. 2016). The hepatic icterus can be related with several diseases, such as *Leptospira interrogans* infections; degenerative liver diseases (lipidosis and glycogenic degeneration); primary hepatic neoplasia (cholangiocarcinoma) or metastases and hepatic fibrosis with loss of hepatocytes. This type of icterus occurs as a result of lesions in the hepatocytes and a decrease in or unconjugation of bilirubin (Murdoch 1976, Barros 2011). Toxins, medicaments and viruses can also cause hepatic icterus (Barros 2011). Post-hepatic icterus was diagnosed in a dog with biliary stones in this study. The occurrence of this condition in dogs is not common (Cipriano et al. 2016). Generally, biliary stones associated with cholecystitis can cause severe biliary obstruction of the main duct with marked icterus and subsequent rupture of the gallbladder (Cullen & Stalke 2015). Other reported causes related to duct obstruction include extrahepatic biliary tumors (Eulenberg & Lidbury 2018).

One important etiology of icterus is *Leptospira interrogans* (Murdoch 1976, Bernheimer & Bey 1986). In this study, leptospirosis mainly occurred in mixed-breed adult dogs older than one year old, similar to other studies (Batista et al. 2005). The fact that adult dogs are more affected by *Leptospira* spp., could be related to increased exposure and contact with contaminated external environments than puppies. Puppies normally receive more care from their owners (Greene et al. 2006). Another factor could be leptospirosis vaccination failure or an inadequate vaccination schedule (Batista et al. 2005, Adler & de la Pena-Moctezuma 2010).

The most frequent serovars in our study were Icterohaemorrhagiae and Bratislava, followed by Canicola and Autumnalis. Other researchers in Brazil have reported similar results, whilst others have found different results. One study detected the serovars Autumnalis, Copenhageni and Canicola as the most frequent (Batista et al. 2005). Another study revealed that predominant reactive serogroups were Icterohaemorrhagiae, Australis, Pomona, Butembo, and Castellonis (Miotto et al. 2018). A study from the United States detected antibodies against serovars Autumnalis, Grippotyphosa, Pomona, and Bratislava as the most common in dogs (Gautam

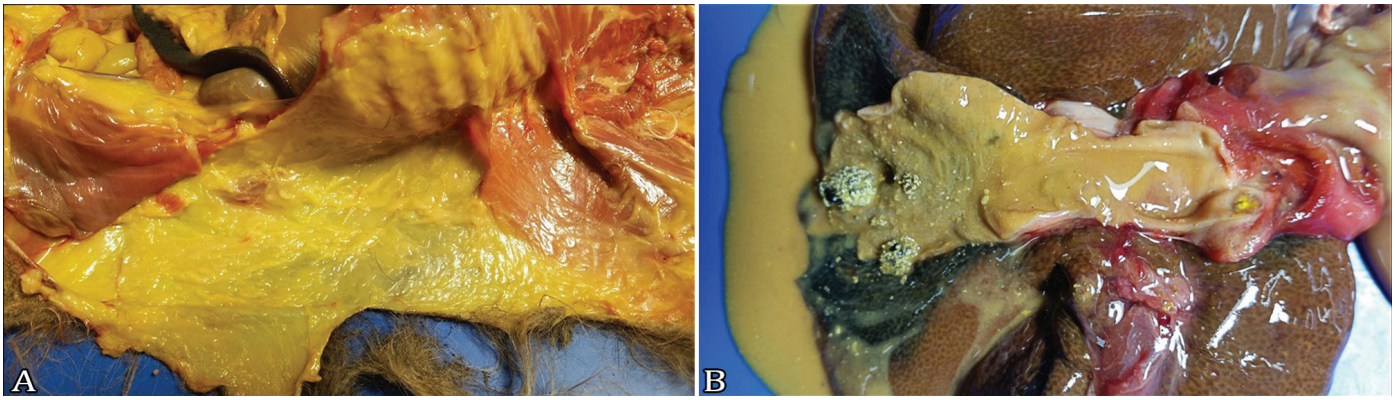

Fig.6. Five-year-old male Yorkshire Terrier dog. (**A**) Subcutaneous adipose tissue and abdominal serosa characterized by marked icterus. (**B**) Liver. Complete obstruction of biliary duct by gallstones accompanied by a viscous yellow exudate.

et al. 2010). Cross- reaction between different serovars can occur. In this case, the serovar with the highest titer (>1:800) should be interpreted as the infecting serovar (Brown et al. 1996, Adler & de la Pena-Moctezuma 2010). The serovars with the highest titers (1:800) detected in the dogs from this study were Icterohaemorrhagiae, Bratislava, Autumnalis, Pomona and Bataviae. Autumnalis and Bratislava could possibly be the serovars responsible for clinical cases, because they are not included in the vaccines against leptospirosis in Brazil.

Hemoglobinuria was not common in dogs diagnosed with leptospirosis in this study. The mechanism for hemolysis production in the leptospirosis is not completely elucidated. Some studies related the hemolysis with production of sphingomyelinases or phospholipases activity by pathogenic serovars Icterohaemorrhagiae and Pomona (Kasãrov 1970). These enzymes produce pores in the cellular membrane of erythrocytes and subsequently cell death (Kasãrov 1970, Trowbridge et al. 1981, Picardeau et al. 2008, Adler & de la Pena-Moctezuma 2010). The main types of phospholipids in the erythrocyte membrane were found to be susceptible to the action of specific leptospiral enzymes. However, the type of these phospholipids differs among animal species as well as the different types of haemolytic enzyme produced by each serovar (Kasãrov 1970). This could be related to differences in the susceptibility of an animal species to each specific serovar. Hemolytic anemia associated with leptospirosis infection has been previously reported in some animal species including lambs (Smith & Armstrong 1975) cattle (Bernheimer & Bey 1986) and horses (Delph et al. 2018). An experimental study in hamsters demonstrated hemolytic activity of the serovar Pomona (Sobroza et al. 2014). For canine leptospirosis, different results were found in some studies, which described the diseases with hemorrhagic, renal and hepatic lesions without hemolysis. The icterus manifestation in these studies was related to hepatic lesions and hyperbilirubinemia (Tochetto et al. 2012), as diagnosed in several dogs in our study. Similar clinical manifestations were reported for serovars Australis and Bratislava infections in Europe. Dogs from these reports were routinely vaccinated against serovars Canicola and Icterohaemorrhagiae (Major et al. 2014). *L. interrogans* serovars Icterohaemorrhagiae was described by Murdoch (1976) as causing icterus in dogs because haemolysis and concomitant hepato-cellular lesions. Icterus is a common manifestation of acute leptospirosis and may be the result of hemolysis or hepatocellular lesions (Cianciolo & Mohr 2016). A dog examined in the present study diagnosed with hemoglobinuria had MAT titers for serovar Icterohaemorrhagiae, another dog for Bataviae and Bratislava and another for serovar Bratislava only. The hemoglobinuria in these dogs was interpreted as chronic because of iron positive stain in the renal tubules using Prussian blue stain.

The serovar Icterohaemorragiae is especially implicated with hepatic lesions, and the serovar Canicola mainly with renal lesions. Other serovars could affect both organs, although the lesions can be less marked than both the serovars aforementioned (Freire et al. 2008, Adler & de la Pena-Moctezuma 2010). Hepatic and renal lesions found in the dogs of the present study varied among serovars and no correlation was found with a specific serovar. Hematological results from several positive leptospirosis dogs in our study included regenerative anemia, neutrophilic left deviation and thrombocytopenia, corroborating with other studies (Sykes et al. 2011, Maele et al. 2008).

L. interrogans detection could be performed by PCR using DNA extracted from blood, urine, kidney or liver (Coutinho et al. 2014, Maele et al. 2008). In the present study, this test produced confident results in approximately 90% of dogs using samples from the liver, kidney and spleen. Based on these results, a limitation detection may have occurred. *Leptospira* spp. are readily eliminated by antibiotics, particularly using doxycycline (Truccolo et al. 2002) or doxycycline and rifampicin, decreasing the PCR sensitivity (Kim & Byun 2008). In some dogs from this study, with compatible pathological diagnosis of leptospirosis and PCR negative results, we speculated about the antibiotic therapy influence.

The biochemical profile of the dogs with leptospirosis examined in this study revealed increased plasma concentrations of urea and creatinine in most of the dogs. These results are similar to other leptospirosis studies, which identified that sera values of these renal catabolic were increased but can vary with the clinical evolution of the disease (Sykes et al. 2011, Maele et al. 2008).

Increased activities of enzymes indicating hepatic dysfunction was also detected in dogs from this study. ALT, AST and ALP, which are almost always associated with azotemia, are usually described in dogs with leptospirosis (Azócar-Aedo et al. 2017). Increased sera concentrations of ALP usually occur in association with biliary tree lesions (Valli et al. 2016). Increased sera concentrations of ALT and AST can also be found in other hepatic conditions (Murdoch 1976), as described in the present study.

Chronic glomerular lesions present in several dogs from this study and not related with leptospirosis could have contributed to renal biochemical changes. These lesions were frequent and suggestive of chronic visceral leishmaniasis, which was confirmed in some dogs from this study with chronic glomerular lesions. The frequency of this infection is high in the animals from the region studied (Lima et al. 2004, Rigo et al. 2013). Glomerular alterations are commonly described in dogs with leishmaniasis and associated with functional changes (Costa el al. 2003).

Co infection with *Ehrlichia canis* and *Leptospira* sp. was found in some dogs from the present study, similar to another report (Morais et al. 2011), confirming that concurrent infections could occur for these etiologic agents. The PCR was an important ancillary test to confirm *E. canis* infections, in addition to the histopathology exam helping to confirm the disease.

Icterus associated with ehrlichiosis is a clinical manifestation (Rungsipipat et al. 2009) possibly related to hemorrhages caused by thrombocytopenia, hemorrhagic drainage by the lymph nodes and erythrophagocytosis with the consequent production of bilirubin. In this study, two dogs with icterus caused by ehrlichiosis (single infection), had hematological changes similar to those reported in other studies (Moreira et al. 2003, Greene 2006, Harrus 2015). There are some hypotheses about the pathogenesis of hematological changes of ehrlichiosis. Thrombocytopenia detected in dogs diagnosed with ehrlichiosis, could be caused by immune-mediated mechanisms (Waner et al. 2000, Rungsipipat et al. 2009, Harrus 2015). Thrombocytopenia, anemia and lymphopenia were also related to the action of *Ehrlichia* in the bone marrow

and lymphoid tissues (Mylonakis et al. 2004, Greene 2006). Recent studies about iron metabolism in animals infected with *Ehrlichia canis*, reported a marked decreased in circulating iron, suggesting that the host may be induced by the bacteria to transport iron to infected tissue for bacterial multiplication, contributing to anemia (Bottari et al. 2016). This may explain the numerous hemosiderin granules commonly found in the bone marrow, liver, lymph nodes and spleen from animals with ehrlichiosis in this study. Also, in our dogs, the histopathologic findings included plasmacytosis in the lymph nodes and spleen. In addition, lymphocytic and plasmacytic perivascular cuffing were found in numerous organs, including the liver, kidneys and bone marrow. In these dogs, bone marrow was also marked hypoplastic. These findings are similar to those described by Waner & Harrus (2013) in dogs with chronic ehrlichiosis.

Septicemia associated with icterus was also found in dogs from the present study. Some mechanisms underlying this process have been researched. Systemic bacterial endotoxins can activate inflammatory mediators, such as TNF-α and IL-6 cytokines. This could reduce biliary excretion by the canaliculi (Chand & Sanyal 2007). Biliary excretion is dependent on ATP. If septicemia is present, a decrease in cell energy is possible (ATP), as a result of hypoxia, toxins or cytokines effects. This interfers with the transport proteins of bile and its components causing bile stasis. This results in an increase in unconjugated bilirubin and consequently icterus (Nesseler et al. 2012). In addition, high amount of bacterial toxins and cytokines in the spleen could produce necrosis and inflammation (Ackermann 2013). Endothelial lesions can cause hemorrhages, erythrophagocytosis and bilirubin production, contributing to icterus (Goyette et al. 2004). A marked increase in leucocytes, especially neutrophils, could suggest the presence of septicemia (Klosterhalfen et al. 1996). This increase was detected in the dogs in this study diagnosed with septicemia. A dog with lesions compatible with septicemia had pancytopenia, with marked neutropenia. These hematological changes could occur due exhaustive production of progenitor cells (Aird 2003).

Lipidosis was the second most frequent cause of hepatic icterus in the present study, followed by primary hepatic neoplasia and advanced hepatic fibrosis. Lipidosis in dogs mainly occurs due to excessive ingestion of carbohydrates, hypoxia, abnormal hepatocyte function, decreased apoprotein synthesis, hormonal diseases and medication intoxication. The degeneration of the hepatocytes could reduce bilirubin uptake and their increased size compresses the bile canaliculi, resulting in reduced biliary tract drainage (Cullen & Brown 2013). Lipidosis was common in the dogs from the present study but the etiology was not well determined. Nevertheless, high caloric diets were inferred to be a possible cause based on the clinical history and absence of others etiologies.

Glycogenic degeneration was associated with icterus in this study. This condition is caused by endogenous or exogenous steroids. Endogenous steroids are found in high concentrations in cases of hyperadrenocorticism (functional cortical adrenal neoplasia) or functional tumors in the adeno-hypophysis (inducing cortical adrenal hyperplasia). High steroid plasma concentrations occur due to excessive or prolonged doses of exogenous glucocorticoids. The overload of glycogen in the liver could induce diffuse and marked degeneration of the hepatocytes (Cullen & Stalke 2015), as seen in the animals from the present study. Most dogs had a clinical history of therapeutic corticoid use. Its likely these lesions were caused by this medication.

Dogs with advanced hepatic fibrosis could also present icterus (Murdoch 1976, Elhiblu et al. 2015) due to loss of hepatocytes and canaliculi (Elhiblu et al. 2015). Hepatic fibrosis can be induced by lipidosis, glycogen degeneration, hepatitis and toxic hepatopathies (Cullen & Stalke 2015). In the resolution phase, depending upon the distribution, intensity and persistent of injury caused to the hepatocytes, and basal membrane destruction, fibrosis and nodular regeneration can occur (Cullen & Stalke 2015, Eulenberg & Lidbury 2018). In the present study, the lesions were markedly chronic and confirmation of the etiology responsible for the pre-fibrotic lesions was not possible.

Cholangiocarcinoma originates from hepatic biliary ducts, usually composed of small to wide nodule proliferations, replacing the normal liver parenchyma and causing icterus (Aloia et al. 2012). Regarding primary hepatic neoplasms, cholangiocarcinoma was the most frequent hepatic neoplasm is this study, similar to another study in Brazil (Aloia et al. 2012). The dogs studied here had macroscopic and microscopic changes interpreted as primary intrahepatic ductal origin and no other changes indicating other cause for the icterus were found.

Dogs with hemangiosarcoma usually have frequent hematological changes including microangiopathic hemolysis, disseminated intravascular coagulation (DIC) and hemorrhages (Hammer et al. 1991, Valli et al. 2016). These changes are mainly characterized by thrombocytopenia, an increase of fibrin degradation products and fragmented erythrocytes (Hammer et al. 1991), which are removed by splenic macrophages (Valli et al. 2016). DIC in dogs with hemangiosarcoma are frequent (Maruyama et al. 2004). Besides this, in the neoplastic vessels with turbulent blood flow, mechanical trauma can produce physical lysis to the erythrocytes and consequently icterus (Valli et al. 2016). In the present study, dogs with disseminated hemangiosarcoma had anemia, mild icterus, thrombocytopenia and histologic lesion of hemoglobinuria in absence of hepatic lesions, characterizing intravascular hemolysis and pre-hepatic icterus. Also, a marked hemosiderin deposition was found in the renal tubular cells of one dog with hemangiosarcoma of this study. Intravascular hemolysis generally is characterized by hemoglobinuria, and hemosiderosis are seen in the renal tubular cells consequent of reabsorbed filtered hemoglobin (Valli et al. 2016).

Metastatic neoplasms in the liver from the dogs of this study, such as lymphoma, were also responsible for producing icterus. In dogs, the most common metastatic hematopoietic, mesenchymal, and epithelial neoplasms are lymphoma, hemangiosarcoma, and pancreatic carcinoma, respectively. These neoplasms can cause icterus either by extrahepatic bile duct obstruction or by intrahepatic cholestasis, or both (Cullen & Stalke 2015), as diagnosed in our study.

CONCLUSIONS

Hepatic and pre-hepatic icterus were the most frequent type of icterus diagnosed in the 83 dogs examined during the 36 months of this study.

The most frequent infectious etiologies were *Leptospira interrogans* and *Ehrlichia canis*.

Degenerative hepatic diseases, end-stage hepatic fibrosis as well as primary and metastatic neoplastic diseases were also diagnosed and considered important differential etiologies of the infectious causes.

Clinical pathology tests, serology, molecular and other complementary tests, such as a liver biopsy, are important auxiliary tools for clinical presumptive diagnosis.

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