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CLINICAL AND MOR- PHOLOGICAL STUDY OF HEMANGIOSARCOMA IN CANINES (HSC)

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Abstract: Canine hemangiosarcoma (HSC) is a malignant neoplasm of mesenchymal origin that presents as a multicentric disease. The aim of this study was to review the anatomopathological and immunohistochemical findings and clinical results in canine patients who were referred for necropsy and had a histopathological diagnosis of hemangiosarcoma in the veterinary pathology laboratory of the Universidad de La Salle-Bogotá. We reviewed 794 necropsy histories in the period 2010 to 2019; 17 histories were selected; clinical histories of cases that were attended at the small animal clinic of La Salle University were searched. Ki67, c-kit and SOX2 immunomarkers were used. HSC corresponded to 2.1% of mortality; 82% were males; 65% of the cases occurred in canines over 25 kg; the most affected breed was the Golden retriever (29%); the mean age of presentation was 11 years. Fifty-three percent of the patients were euthanized; 76% presented with CAH of visceral origin; the most affected organ was the lung. The most frequent clinical signs were normocytic normochromic anemia, thrombocytopenia and neutrophilic leukocytosis. Fifty-nine percent of patients were stage III, 35% were grade II and 71% were cavernous. Immunohistochemical labeling was moderate with Ki67 and mild with c-kit and SOX2 in 30%, 60% and 50% of cases, respectively. No patient received chemotherapy treatment. Further research is needed in early diagnosis, metastasis and the search for new therapeutic targets

Keywords: immunohistochemistry, metastasis, neoplasia, pathology, tumor.

INTRODUCTION

Hemangiosarcoma (HSC) is a common, highly malignant tumor in canines, also known as malignant hemangioendothelioma, angiosarcoma (1) or visceral vascular tumor that includes hemangioma and hemangiosarcoma (2). It is characterized by a high mortality rate (3). It represents 12% to 21% of mesenchymal neoplasms in canines (4). It develops from pluripotential cells of endothelial cells in the bone marrow (5,6,7)

The transformation of the progenitor cells of the neoplasm is attributed to genetic events such as the presence of the BIRC3 gene, the mutation of the TP53 gene and of the PTEN genes, the overexpression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (2) and to biological events that occur due to chromosomal translocations and the presence of reactive microenvironments in which IL-8, CXCR4/CXCL12 and sphingosine-1-phosphate receptor 1 (S1P1), among others, participate. Platelet-derived factors, inflammation and hypoxia are probably the main drivers of the disease (6,8).

Although its etiology is unknown, exposure to chemicals including industrial, agricultural and pharmaceutical agents such as arsenicals, vinyl chloride, 2-butoxyethanol, troglitazone, pregabalin, androgens; ionizing radiation including ultraviolet light; hereditary factors; infectious etiologies such as leishmaniasis and increased DNA of *Bartonella* spp. DNA; inflammation; canine hemostasis and the anatomy of the microvasculature (6,7,9,10,11,12).

In canines it commonly presents as a multicentric disease where the most common primary sites are the spleen, heart, skin/subcutaneous tissues, lung, heart and liver (6,13). According to their location they are classified as non-visceral hemangiosarcoma when they affect the skin, subcutaneous tissue and muscle and visceral hemangiosarcoma when they affect the spleen, liver, kidney, heart, lung, oral

cavity, uterus, tongue and retroperitoneum (2). HSC is a highly metastatic neoplasm by hematogenous route or by transabdominal implantation. It is suggested that 25% of canines with splenic HSC may also present the neoplasm in the right atrium. Cutaneous HSC are less aggressive than visceral HSC with a lower metastatic potential and a longer average lifespan; they can be of actinic or sun-independent origin; they are usually well-defined, single or multiple, red, brown to black, superficial masses on the skin of the abdomen when dependent on sunlight (5,8,13,14).

HSC affects animals of average age to geronts between 6 and 17 years. Predisposing breeds are the German Shepherd, Golden retriever and Labrador retriever (1,8,13). In the case of cutaneous hemangiosarcoma, short-haired breeds such as the Greyhound, Whippets and American Pitbull have a higher risk of presentation. There can be predisposition by sex, affecting mainly entire males and sterilized females (1,8).

The clinical signs depend on the affected organ. In the case of splenic HSC it can cause sudden death by hemoabdomen even with the presence of a single mass (15) or pericardial effusion when located in the pericardium or in the myocardium which can lead to sudden death by cardiac tamponade (16). Cutaneous hemangiosarcomas ulcerate and bleed easily, although their metastatic potential is low (5).

Diagnosis includes hematology, serum biochemistry, coagulation test, thoracoabdominal imaging, abdominocentesis, echocardiography, cytology, histopathology, immunohistochemistry, serum biomarkers and serum DNA and microRNA molecular profiles (2). Paraneoplastic signs include regenerative and non-regenerative anemia, with the presence of schistocytes associated with microangiopathic hemolysis and acanthocytes; thrombocytopenia in 75% of cases, neutrophilic leukocytosis and disseminated intravascular coagulation (2,7,13)

The treatment of choice is surgical; however, due to its highly aggressive nature, effective treatment requires local treatment and systemic therapy (26).

The prognosis can be poor in cases of visceral and cardiac HSC, due to the location of the neoplasm and in many cases, due to the late diagnosis. The highly metastatic behavior of visceral HSC results in a short survival of 19 to 86 days in patients treated with surgery and 179 days in patients treated with surgery and chemotherapy (4). Cutaneous neoplasms of actinic origin have a better prognosis than non-actinic subcutaneous masses and their metastatic potential is low (2,7).

The objectives of this work were to describe the clinical and morphologic aspects of CAH related to age, race, sex, clinical stage and clinical signs of patients diagnosed in our geographic region; to establish the importance of paraclinical diagnostic tests in the staging of patients with HSC and their comparison with what is reported in the literature to be taken into account in the early diagnosis of the disease and to evaluate aspects related to clinical stage, histological grade, cellular atypia and response to immunomarkers of cell proliferation (Ki67), tyrosine kinase inhibitors (c-kit) and proliferation, migration and metastasis (SOX2).

MATERIALS AND METHODS

DEMOGRAPHICS

The physical file of necropsy histories of the veterinary pathology laboratory of La Salle University, corresponding to the interval 2010-2019, was reviewed. The necropsy cases of canine patients with an anatomopathological diagnosis of CAH were selected. Data on breed, age, sex, weight, body condition, macro and microscopic description and euthanasia were recorded in an Excel file. Breeds were classified according to weight into small (5-14 kg), medium (15-24 kg) and large (25-50

kg), according to the Fédération Cynologique Internationale (27). Histopathology slides and kerosene blocks were sought immunohistochemical study. The clinical histories of patients referred to pathology and treated at the Veterinary Clinic of the Universidad de La Salle were searched and reviewed.

ANATOMOPATHOLOGICAL STUDY AND STAGING

Necropsy reports were reviewed and data on age, breed, sex, weight, reproductive status, body condition, natural death or euthanasia, color of mucous membranes, presence of pericardial and/or abdominal effusion, and organs affected were recorded in an Excel sheet. The neoplasm was classified according to its location or origin in visceral (splenic, cardiac, hepatic or bladder) and non-visceral (cutaneous, subcutaneous and intramuscular) HSC (2). Cases where visceral organs and skeletal muscle and/or skin were affected were classified as mixed. Body condition was established according to the 1-5 scale of the body condition index, as follows: 1= very thin; 2=thin; 3=optimal; 4=overweight and 5= obesity (28). The ideal value was 2.5-3.

Patients were classified according to their clinical stage at the time of necropsy. The three-level staging system (I, II, III) was used (7). For splenic HSCs, criteria based on the size of the primary tumor and evidence of tumor rupture; the presence of regional lymph node involvement and/or the presence of distant metastases were taken into account (Table 1)

Primary Tumor (T)	
T0	No evidence of tumor
T1	Tumor less than 5 cm in diameter, confined to one organ
T2	Tumor greater than or equal to 5 cm in diameter, ruptured
T3	Tumor with invasion of adjacent structures
Regional lymph nodes (N)	
N0	No regional lymph node involvement
N1	Regional lymph node involvement
N2	Distant lymph node involvement
Distant metastasis (M)	
M0	No evidence of distant metastasis.
M1	Presence of distant metastases
Staging	
I	T0 or T1; N0; M0
II	T2; N0, N1 or N2; M0
III	T1, T2 or T3; N0, N1 or N2; M1

Adapted from Mullin and Clifford (2020) (7)

Table 1. Proposed clinical staging model for visceral HSC (7).

In the case of cutaneous HSC, the depth of the tumor in the dermis, hypodermis and muscle infiltration, lymph node involvement and the presence of distant metastases were taken into account (7,29) (Table 2).

Primary tumor (T)	
T0	No evidence of tumor
T1	Primary tumor confined to the dermis
T2	Primary tumor affecting the hypodermis, with or without concomitant dermal involvement. Without muscle involvement
T3	Any primary tumor with muscle involvement.
Regional lymph nodes (N)	
N0	No regional lymph node involvement
N1	Regional lymph node involvement
N2	Distant lymph node involvement
Distant metastasis (M)	
M0	No evidence of distant metastasis.
M1	Presence of distant metastases
Staging	
I	T0 or T1; N0; M0
II	T2; N0, N1 or N2; M0
III	T1, T2 or T3; N0, N1 or N2; M1

Adapted from Mullin and Clifford (2020)(7) and Ward et al. (1994)(29)

Table 2. Proposed clinical staging model for canine cutaneous SAH (2).

HISTOPATHOLOGY

The growth patterns were classified according to their morphology in three categories; capillary (formation of small vessels), cavernous (formation of dilated vessels) and solid (without vessel formation) (8). A Carl Zeiss Axiostar plus optical light microscope was used to establish the growth pattern and histological grade in each case. The results were agreed by two observers specialized in veterinary pathology.

Histologic grade (I, II and III) was established for each case (18) (Table 3). Each factor was combined to create a final score equivalent to the three-level histologic grade (17,18).

Differentiation	SCORE
Well-differentiated: numerous irregular vascular channels predominate in all fields	1
Moderately differentiated: >50% of the tumor has well-defined vascular channels.	2
Poorly differentiated: solid sheets of spindle cells with few vascular canals	3
Nuclear pleomorphism	SCORE
No difference in nuclear size and shape	0
Minimum variation	1
Moderate variation (2x size difference)-	2
Marked variation (>2x difference in size)	3
Mitotic count (in 10 high-power fields [target x40] in 2.37 mm ²)	SCORE
0 a 10	0
11 a 20	1
21 a 30	2
>30	3
Tumor necrosis	SCORE
No necrosis	0
<25%	1
25% a 50%	2
>50%	3
Histological grade (3 grades)	SCORE
Grade I	≤ 5
Grade II	6 A 9
Grade III	10 A 12

Table 3. Classification scheme of canine splenic hemangiosarcoma by Ogilvie et al. 1996 (18).

Cellular atypia was graded according to 1-3, as follows: (1) mild: spindle-shaped neoplastic endothelial cells with an elongated nucleus (width less than 1 erythrocyte), (2) moderate=spindle-shaped neoplastic cells with an oval nucleus (width of approximately 1-2 erythrocytes). (3) severe=polygonal or cuboid shaped cells with ample cytoplasm and a large round or irregularly shaped nucleus (width of three or more erythrocytes) (19).

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed to observe the expression of the immunomarkers *c-kit*, *ki67* and *SOX-2*. A positive control was included for each case. Positive expression of the immunomarkers was evaluated in tumor cells characterized by brown staining of the cell membrane, cytoplasm or nucleus with diaminobenzamidine (DAB). Expression was graded from 0 to 3 (0 = negative; no brown staining, 1= mild, pale brown membranous staining with minimal cytoplasmic staining/no staining, 2 = moderate, medium/dark brown coarse staining of cytoplasm with or without membranous staining, 3 = high, diffuse dark brown cytoplasm/membranous staining of tumor cells (19).

MEDICAL RECORDS AND PARACLINICAL EXAMINATIONS

The clinical histories of the necropsy cases that were attended at the Veterinary Clinic of the Universidad de la Salle were reviewed. Data on breed, age, sex, weight, type of food (concentrate, homemade and mixed food), behavior at the time of consultation (aggressive, calm, nervous), reason for consultation, clinical signs and results of paraclinical tests performed (blood count, creatinine, total and differential plasma proteins, glucose, alkaline phosphatase) and diagnostic images (radiographs and ultrasound) and concomitant diseases reported in the clinical history were recorded.

STATISTICAL ANALYSIS

The present study corresponds to a retrospective study with a small number of samples. Parametric and nonparametric descriptive statistics were used. The results are presented in tables with percentages and frequencies

RESULTS

DEMOGRAPHICS

A total of 794 pathology histories were reviewed, from which seventeen (17) cases were selected that had as final diagnosis canine hemangiosarcoma (HSC) which corresponded to 2.1% of the casuistry during the period 2010 to 2019

The mean age was 11 years with a range between 3 and 16 years. Eighty-two percent (14/17) were males ranging from 3 to 16 years of age and 18% (3/17) were females ranging from 8 to 12 years of age. The most affected breed was Golden retriever 29% (5/17), followed by Creole 24% (4/17), 47% corresponded to other breeds; in the case of females, 67% (2/3) were Golden retriever and in the case of males, 31% (4/13) were Creole. Fifty-three percent (9/17) weighed more than 25 kg; 23% (4/17) weighed between 15 and 24 kg; 12% (2/17) weighed less than 14 kg and in 12% (2/17) the weight was not reported. The reproductive status of the females was not recorded. Fourteen percent (2/14) of the males were castrated, 79% (11/14) were intact, 7% (1/17) were monorchid (Table 4).

ANATOMOPATHOLOGICAL STUDY AND STAGING

Of the cases that were referred to the veterinary pathology laboratory, 53% (9/17) were euthanized and 47% (8/17) died naturally. Body condition scored optimal in 41.1% (7/17) of the cases; overweight in 41.18% (7/17); lean in 11.76% (2/17) and no body condition was reported in 5.88% (1/17) (Table 5). Subicteric

mucous membranes were observed in 29% (5/17) of cases, pale 23% (4/17), icteric 18% (3/17), cyanotic 18% (3/17), congested 6% (1/17) and pink 6% (1/17). Content was found in the pericardial and abdominal cavities in 47% (8/17) of cases; hemopericardium 50% (4/8) and hemoperitoneum 50% (4/8).

SCORING	BODY CONDITION	CASES	PERCENTAGE
1	Very thin	0	0,00%
2	Delgado	2	11,76%
3	Optimum	7	41,18%
4	Overweight	7	41,18%
5	Obesity	0	0,00%
No information		1	5,88%
		n=17	100,00%

Table 5. Body condition of patients with CAH. Veterinary Pathology Laboratory. La Salle University (2010-2019).

The type of CSH was visceral in 76% (13/17) of the cases, non-visceral in 12% (2/17) and mixed in 12% (2/17). In cases of visceral CSH, several organs were affected in 62% (8/13) of the cases and a single mass was found in 38% (5/13) of the cases, located in the spleen (2/5), heart (1/5), liver (1/5) and lung (1/5). The heart was found to be involved in 38% (5/13) of visceral HSC cases. Cardiac HSC were observed in 29% (5/17) of the cases; 80% (4/5) affected the right heart and of these, only 25% (1/4) had concurrent mass in the spleen; 60%(3/5) occurred in males and 20% (1/5) in females; 80% (4/5) occurred in Golden breed canines; in four (4/5) cases the right heart was affected and involved the lung (3/4) and in one case (1/4) the spleen and lung were found involved. The cases of non-visceral HSC were located in the skin and muscle and the mixed cases involved different internal organs, skin and/or psoas muscle, costal muscles and diaphragm. The affected organs were in order of involvement: lung 58% (10/17), spleen 47% (8/17), heart 29% (5/17), muscle 23% (4/17), liver 18% (3/17), skin 12% (2/17) lymph 12%

SIZE	BREED	WEIGHT	CC	AGE	SEX	REPRODUCTIVE STATUS
SMALL	Poodle	10 kg	4	13 years	Male	Neutered
5-14 KG	Schnauzer	10.6 kg	4	16 years old	Male	Integer
MEDIUM	Basset hound	17 kg	4	11 years	Female	NI
15-24 kg	Springer spaniel	17.5 kg	4	13 years	Male	Integer
	Alaska malamute	21 kg	2	15 years	Male	Integer
	Labrador	20.4 kg	3	16 years old	Male	Integer
BIG	Golden	37kg	4	7 years	Male	Integer
25-50 kg	Golden	29.8 kg	4	NI	Male	Integer
	Golden	25 kg	3	3 years	Male	Monorquid
	Golden	30 kg	3	12 years	Female	NI
	Golden	Ni	3	8 years	Female	NI
	Weimaraner	39.2 kg	3	15 years	Male	Integer
	Great Dane	43 kg	3	9 years	Male	Integer
	Creole	26 kg	NI	14 years	Male	Integer
	Creole	24 kg	4	10 years	Male	Integer
	Creole	NI	3	9 years	Male	Neutered
	Creole	NI	2	6 years	Male	Integer

Table 4. Size according to weight, age, sex and reproductive status of CAH cases. Veterinary Pathology Laboratory. La Salle University. 2010-2019.

*NIF: Does not report

(2/17) and others: bladder, stomach, kidney, mesentery, peritoneum, subcutaneous tissue (Table 6).

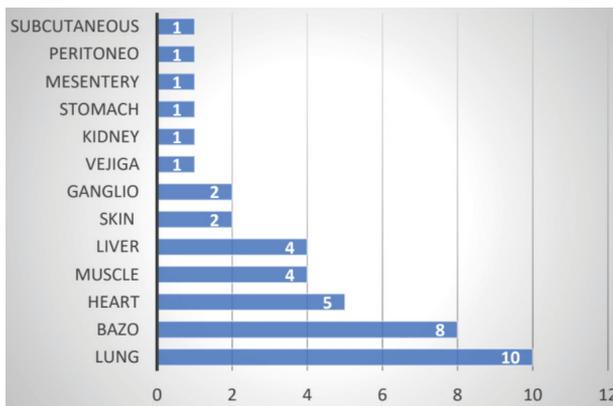


Table 6. Organs affected in patients with CAH. Necropsy cases. Veterinary Pathology Laboratory. La Salle University. 2009-2019.

The anatomopathologic staging of HSC included stage III with 59% (10/17), stage II with 35% (6/17) and stage I with 6% (1/17) of cases (Table 8).

Concomitant diseases such as endocardiosis (4/17), degenerative arthrosis of the spine with evidence of hooks on the ventral surface of the vertebral bodies involving the thoracic, lumbar and sacral region (2/17), prostatic hyperplasia (3/17) and other neoplasms (4/17) were found.

HISTOPATHOLOGY

Histopathologic slides of 12 cases were reviewed, of which 14 organs were evaluated. A cavernous growth was found in 71% (10/14) of cases, capillary 22% (3/14), solid 7% (1/14). Cellular atypia was severe in 43% (6/14), moderate in 43% (6/14) and mild in 14% (2/14) of the organs evaluated. Histological grades were assigned as grade I in 21% (3/14) and grade II in 79% (11/14) of the organs evaluated (Table 7).

CLINICAL STAGE	n	AVERAGE
Stage I	1	6%
Stage II	6	35%
Stage III	10	59%
n	17	100%
HISTOLOGIC GRADE		
Grade I	3	21%
Grade II	11	79%
Grade III	0	0%
n	14	100%
ATIPIA		
Slight	1	10%
Moderate	5	50%
Severa	6	43%
n	14	100%
HISTOLOGIC PATTERN		
Cavernous	10	71%
Capillary	3	22%
Solid	1	7%
n	14	100%

Table 7. Clinical stage, histologic grade, cellular atypia and type of HSC. Laboratory of veterinary pathology. La Salle University. 2010-2019.

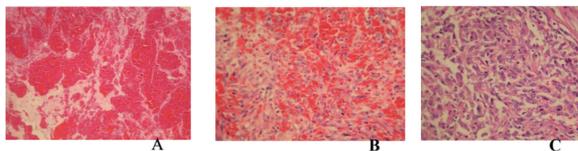


Figure 1. Histological types of canine hemangiosarcoma (HSC).

A. Cavernous hemangiosarcoma. H&E. 5X. B. Capillary hemangiosarcoma. H&E. 5X. C. Solid hemangiosarcoma. H&E. 5X. (Source: Author. Laboratory of Veterinary Pathology. La Salle University, 2023)

IMMUNOHISTOCHEMISTRY

Immunolabeling was performed in ten of the cases studied (10/17). With the *c-kit* marker, a mild marker was obtained in 60% (6/10) of the cases; there was no marker in 40% (4/10). With the Ki67 marker, a slight marking was obtained in 60% (6/10) of the cases; moderate in 30% (3/10) of the cases and no marking was observed in 10% (1/10). With the SOX2 marker, a mild marker was obtained in 50% (5/10) of the cases and no marker was observed in 50% (5/10) of the cases (Table 8).

MEDICAL RECORDS AND PARACLINICAL EXAMINATIONS

Medical records were obtained for 53% (9/17) of the patients who were diagnosed by necropsy with HSC and who were attended at the Small Animal Clinic of La Salle University. The behavior of the patients was perceived as calm 67% (6/9), nervous 22% (2/9), hyperactive 11% (1/9).

The type of feeding they received was recorded in the clinical history: 44% (4/9) were fed concentrate, 44% (4/9) were fed a mixture of home-made food and concentrate, 12% (1/9) were fed home-made food.

The most frequent reason for consultation was decay 55% (5/9), low back pain 44% (4/9), syncope 22% (2/9), weight loss 22% (2/9), poor appetite 22% (2/9) and others such as cough, bloody and/or mucous diarrhea, claudication, vomiting, convulsions and hematuria (Table 9).

A blood count was performed in 78% (7/9) of the patients. In the hemogram anemia was found in 85.7% (6/7) of the patients. The anemias were of the normochromic normocytic type in 66% (4/6), hemolytic anemia in 17% (1/6), normochromic anemia microcytic in 17% (1/6). The leukogram was altered in 85.7% (6/7) of the cases with the presence of neutrophilic leukocytosis in 66% (4/6) and lymphopenia in 34% (2/6). Thrombocytogram was altered in 85.7% (6/7) of cases with thrombocytopenia in 83% (5/6) and thrombocytosis in 17% (1/6). Serum for blood chemistry analysis was taken from 55.5% (5/9) of the patients and increased plasma proteins were observed in 80% (4/5), increased globulins in 60% (3/5), increased creatinine in 40% (2/5), increased ALT in 40% (2/5) and increased alkaline phosphatase (AP) in 20% (1/5) (Table 10).

CASE	BREED	LOCATION	HISTOLOGICAL GROWTH PATTERN	CELL ATIPIA	HISTOLOGIC GRADE	CD117	Ki67	SOX-2
2	CRIOLLO	BREAST, SKIN	CAVERNOSO/CAPILLARY	2	I -II	0	2	0
3	ALASKA	LIVER	CAVERNOSO	2	I	0	0	0
6	GOLDEN	SPLEEN, HEART AD	CAPILLARY	2	II	0	2	0
7	SCHNAUZER	LUNG	CAVERNOSO	3	II	0	2	0
10	BASSET HOUND	LIVER, SPLEEN, INTES-TINAL SEROSA, SKIN	SOLID	3	II	1	1	0
12	BIG DANISH	SKIN, LUNG	CAVERNOSO	3	II	1	1	1
13	GOLDEN	SPLEEN, THORAX, LUNG, KIDNEY, DIAPHRAGM	CAVERNOSO	2	II	1	1	1
14	WEIMARANER	BAZO	CAVERNOSO	2	II	1	1	1
15	CRIOLLO	MUSC. ABDOMINAL OBLIQUE, PERITONEUM, PERITONEAL	CAPILLARY	1	II	1	1	1
17	GOLDEN	CD-LUNG-SPLEEN	CAVERNOSO	3	II	1	1	1

Table 8. Histological characteristics and expression of CD117, ki67 and SOX2 immunomarkers in cases of HSC. Veterinary Pathology Laboratory. La Salle University. 2010-2019.

Cellular atypia: 1-Leve; 2-moderate, 3-severe. Immunohistochemistry: 0-Negative; 1-mild; 2-moderate; 3-high.

n	No.	BREED	AGE	SEX	TEMPERAMENT	FOOD	REASON FOR CONSULTATION
1	6	GOLDEN	7 YEARS	MALE WHOLE	QUIET	CONCENTRATE	SYNCOPE, FAINTING, LOSS OF APPETITE, VOMITING
2	7	SCHNAZER	16 YEARS	NEUTERED MALE	QUIET	MIXED	LOW BACK PAIN, POOR APPETITE
3	10	BASSET HOUND	11 YEARS	FEMALE	NERVOUS	MIXED	DROOPY, HEMATURIA, VOMITING, VOMITING
4	11	CRIOLLO	6 YEARS	MALE WHOLE	QUIET	CONCENTRATE	DECAÍDO
5	12	BIG DANES	9 YEARS	MALE WHOLE	QUIET	MIXED	MPI CLAUDICATION BY MASS
6	14	WEIMARANER	15 YEARS	MALE WHOLE	HYPERACTIVE	MIXED	SEIZURES, COUGH, WEIGHT LOSS
7	15	CRIOLLO	10 YEARS	MALE WHOLE	NERVOUS	CONCENTRATE	ABDOMINAL AND LUMBAR PAIN, BLOODY DIARRHEA
8	16	SPRINGER SPANIEL	13 YEARS	MALE WHOLE	QUIET	CASERA	DIARRHEA, WEIGHT LOSS, DROOPY, DYSPNEA, DYSPNEA
9	17	GOLDEN	12 YEARS	FEMALE	QUIET	CONCENTRATE	LOW BACK PAIN, LOW BACK PAIN

Table 9. Temperament, type of feeding and reason for consultation. HSC cases. Small animal veterinary clinic. La Salle University. 2010-2019.

n	NO. CASE	BREED	AGE	SEX	HEMOGRAM	PLAQUE-TOGRAM	LEUCOGRAM	BLOOD CHEMISTRY
1	6	GOLDEN	7 years	MALE WHOLE	Normochromic microcytic anemia	Thrombocytopenia	Leukocytosis with neutrophilia	ALT elevated 4 times
2	7	SCHNAZER	16 years old	NEUTERED MALE	NR	NR	NR	NR
3	10	BASSET HOUND	11 years	FEMALE	Normocytic Anemia Normochromic	Normal	Leukocytosis with neutrophilia	NR
4	11	CRIOLLO	6 years	MALE WHOLE	Anemia Hemolytic	Thrombocytopenia	Leukocytosis with neutrophilia	Elevated ALT, CREA, PP and Globulins
5	12	BIG DANES	9 years	MALE WHOLE	Normal	Thrombocytopenia	Normal	NR
6	14	WEIMARANER	15 years	MALE WHOLE	Normocytic Anemia Normochromic	Thrombocytosis	Lymphopenia	PP & Globulins, PA HIGH 369 U/L elevated
7	15	CRIOLLO	10 years	MALE WHOLE	Normocytic Anemia Normochromic	Thrombocytopenia	Lymphopenia	Elevated AST, PP and Globulins
8	16	SPRINGER SPANIEL	13 years	MALE WHOLE	Normocytic Anemia Normochromic	Thrombocytopenia	Leukocytosis with neutrophilia	CREA Y PP elevated
9	17	GOLDEN	12 years	FEMALE	NR	NR	NR	NI

Table 10. Laboratory tests. HSC cases. Small animal veterinary clinic. La Salle University. 2010-2019.

NR: Not performed

Radiographs were taken in 44% (4/9) of the cases. Chest radiographs showed cardiomegaly 25% (1/4) and bronchial pattern 25% (1/4); abdominal and spine radiographs showed the presence of abdominal masses in 50% (2/4) of the patients and deforming disc-spondyloarthrosis in 50% (2/4) of the cases

Ultrasound was performed in 44% (4/9) of the patients and echocardiography in 11% (1/9) of the patients. Echocardiography showed evidence of cardiomegaly. Abdominal ultrasound showed the presence of splenic masses with peritoneal effusion in 50% (2/4) and the presence of prostatic neoplasia was suggested in 50% (2/4) of the cases.

Concomitant diseases were found in the clinical history in 66% (6/9) of the patients. Degenerative osteoarthritis was reported in 100% (9/9) and one (1/9) of them, endocardiosis. None received chemotherapy treatment.

DISCUSSION

The results obtained in this work confirm several literature reports on the subject and allow us to make observations related to the diagnosis, clinical management, prognosis and possibilities for research on this type of neoplasm.

Despite being in a tropical geographic area, the presentation of hemangiosarcoma in the cases studied shares many of the clinical characteristics reported in other regions. The average age was 11 years in an age range between 3 and 16 years, with the average reported age being between 6 and 17 years (2), with an average of 10 years (7,13), although cases have been reported in canines younger than 3 years (1). Although it is reported to frequently affect neutered male dogs, over 25 kg, from middle age to geronts (7), there was a predilection for males with 82%, however, the largest number of cases studied corresponded to entire males (74%), contrary to what was reported, which indicates the need to expand the study population.

The most affected breeds were the Golden retriever followed by Creole dogs, as reported (2,7); although some authors include the Creole dog within the low risk breeds (6). These results suggest the importance of heredity in the presentation of the disease, but on the other hand, the popularity of the breed at a given time must be considered.

Weight was an important factor. A higher presentation of splenic HSC has been reported in patients over 27.5 kg in weight compared to dogs under this weight (30). In this study, 53% were canines weighing more than 25 kg. Similarly, overweight and obesity have been considered risk factors for cancer in humans; however, in dogs only obesity in young animals has been associated with a predisposition to develop mammary tumors in adulthood and osteosarcoma (31). Adipose tissue behaves as an endocrine organ that secretes proinflammatory cytokines and promotes chronic inflammation and carcinogenesis (32). In the study, the patients were overweight and in normal weight condition in 82% of the cases; likewise, no loss of body condition was observed in any of the cases, suggesting the chronicity of the neoplasm with mild presentation of clinical signs due to the affected organs, particularly the spleen.

It has been reported that mortality of CAH is high when its location is visceral (7). Hemoabdomen and hemopericardium have been reported as frequent findings and cause of death by hypovolemic shock in patients with visceral HSC (1,7,15,16); 70% of cases of hemoperitoneum in the dog are associated with HSC in the spleen (1). Patients with cardiac CAH mostly died naturally from cardiac tamponade although cardiac CAH has been reported in canines of the Golden Retriever breed with absence of pericardial effusion (33). In most cases, euthanasia, despite the necropsy findings, was performed for reasons unrelated to the neoplasm, since in most cases,

it had not been diagnosed. Clinical stage III was the predominant stage at necropsy with 71%; however, euthanasia was performed due to chronic and painful concomitant diseases such as osteoarthritis affecting the elderly, the presence of another neoplasm or the clinical presentation of jaundice, with poor prognosis. This confirms the silent nature of the disease.

The most frequent site of metastasis of HSC is the lung (1); however, in one of the cases it was the only organ affected, considering its primary origin in this organ. However, the literature reports that the first organ where HSC originates is the spleen (1,2,34,35), followed by the heart, as observed in the cases studied. The greater involvement of the lung is attributed to metastasis rather than to the primary origin of the mass, according to the histopathological study. Low lymph node involvement was evidenced; only two cases presented lymphadenomegaly at necropsy with evidence of metastasis to these organs on histopathology. In a study of canines, it was established that abdominal masses in canines were 5.8 times more likely to be malignant when involved in the GIT (36); however, in this study, this system was not affected, despite its malignancy.

Clinical signs were varied and, in many cases, non-specific; pale and subicteric mucous membranes were frequent and associated with hemoperitoneum; as reported in the literature (7), neutrophilic leukocytosis, anemia and thrombocytopenia (1,7,30) were the most frequent findings. In the case of anemia, hemolytic anemia is reported as a frequent finding and is attributed to vasculitis, liver failure and reticuloendothelial system deficiency (1,7); however, in the cases presented, normocytic normochromic anemia, typical of chronic disease, was the predominant type of anemia. It is important to note that creatinine was a parameter that was also found to be elevated; however, there are no reports of

other paraclinical tests that could guide us to rule out chronic kidney disease (CKD), taking into account the age of the patients. Neutrophilic leukocytosis was frequently observed, it is a paraneoplastic syndrome and is attributed to necrosis and suppression of antitumor innate immunity (7). Similarly, thrombocytopenia was a frequent finding and is attributed to sequestration and utilization due to tumor hemorrhage, destruction of the tumor vasculature and in cases of disseminated intravascular coagulation (DIC) (1,7,34,35).

In the histopathologic study, the highest percentage of cases were found in grade II, which means an intermediate aggressiveness that can be associated with longer survival times, with mild clinical signs and slow evolution of the disease; however, survival time was not part of this study. No correlation was observed between clinical stage and histologic grade, but a greater association was observed between stage III and severe cellular atypia. Cellular atypia is useful to identify levels of differentiation in cases of HSC (19) and this coincides with those found in the histopathological study. The histological type the cavernous pattern was the most frequent and can be related to the presence of hemopericardium and hemoperitoneum.

Routine H&E staining was sufficient to give the diagnosis; however, three immunomarkers with different target were used. Ki67 is a prognostic immunomarker that indicates cell proliferation (2), in the present study it was expressed in 90% of the cases with mild and moderate intensity, which may be related to the decrease in patient survival. Patients with moderate expression presented stages I and II, moderate and severe atypia, with the presence of masses in the lung and heart. Garcia-Iglesias et al. (38) found a high expression of mutated TP53 associated with high proliferative activity of Ki67, suggesting the association of the gene mutation with the development of

neoplasia (2). The c-kit as a therapeutic target immunomarker was mildly expressed in 60% of the cases where we would expect the response to tyrosine kinase inhibitor drugs to be less effective. Finally, the immunomarker SOX2 has been implicated in embryonic stem cells and adult stem cells, some of them of mesenchymal origin, and has been associated with the initiation, proliferation, invasion and migration of neoplastic cells in different types of neoplasms in canines including HSC (22,39); In the present study the expression was slight in 50% of the cases submitted to this procedure, which indicates that it may occur that it is not expressed in all organs, that it is not related, that it is another type of protein on which we can investigate as is the case of SOX7 which has greater expression in endothelial cells (40). On the other hand, it is important to take into account that the blocks of the cases that were included in this retrospective study are material of which we do not know the quality of the samples for immunohistochemistry.

CONCLUSIONS

HSC is a systemic, chronic, silent, systemic neoplasm of multifactorial etiology, generally affecting large breed dogs over 25 kg. Body condition is not commonly affected. Hematologic alterations are frequent presenting normocytic normochromic anemia, thrombocytopenia and neutrophilic leukocytosis. Histopathological diagnosis is necessary. However, in necropsy cases, the association between histological grade II and clinical stage III indicates the chronicity of the disease. Histological type was not relevant, but is associated with the presence of hemopericardium and hemoperitoneum. It is important to keep in mind that the results of this study are supported by a small number of cases, so it is necessary to validate them with a larger sample size. However, due to the frequency of presentation, the evolution of the clinical picture and

the aggressiveness of the neoplasm, it is necessary to strengthen the clinical and histopathological diagnosis and to continue working on the pathobiology of the disease and to focus future research that contributes to the prevention, early diagnosis, prognosis and adequate treatment of the disease worldwide.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in carrying out the study.

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