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Lactate dehydrogenase activity and total protein as diagnostic markers for cavitary effusions and identification of neoplastic effusions in dogs and cats

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ABSTRACT: The analysis and classification of cavitary effusions play a crucial role in determining a patient's diagnosis and prognosis. This prospective study assed ed the activity of Lactate Dehydrogenase (LDH) and the concentration of Total Protein (TP) in serum, pleural, and peritoneal effusions in dogs and cats. Effusions were categorized into three groups: GI (low protein transudate), GII (high protein transudate), and GIII (exudate) following conventional classification. These groups, irrespective of species, were further divided into NPG (neoplastic) and NNG (non-neoplastic) groups. In dogs, significant differences were observed among groups GI, GII, and GIII in terms of effusion LDH activity/serum LDH (LDHR), effusion LDH activity, serum TP/effusion TP (TPR), and effusion TP. Increased LDH activity in effusion was associated with the presence of neoplasia. The groups organized based on the etiopathogenesis of the effusion exhibited varying values of serum TP, TPR, LDHR, effusion TP, and LDH activity in the effusion. Conversely, statistical analysis of the data from cats showed no differences between the groups for the parameters evaluated in this study. Compared to conventional classification, Light's criteria demonstrated greater sensitivity in distinguishing between transudates and exudates and higher specificity in identifying transudates. We propose the use of established biochemical analyses to discern the mechanism of cavitary effusion formation and advocate for LDH activity measurement in effusions as a complementary diagnostic tool for cavitary neoplasms, especially in cases where cytological analysis of the effusion yields inconclusive results.
Key words: effusion, LDH, total proteins, transudate, exudate.

Atividade da lactato desidrogenase e proteína total como marcadores diagnósticos de efusões cavitárias e identificação de efusões neoplásicas em cães e gatos

RESUMO: A análise e classificação das efusões cavitárias auxiliam a determinar o diagnóstico e o prognóstico do paciente. O objetivo deste estudo prospectivo foi avaliar a atividade da lactato desidrogenase (LDH) e a concentração de proteína total (PT) no soro e nos derrames pleurais e peritoneais de cães e gatos. As efusões foram organizadas em grupos GI (transudato de baixa proteína), GII (transudato de alta proteína) e GIII (exsudato) de acordo com a classificação convencional e distribuídos nos grupos NPG (neoplásica) e NNG (não neoplásica). Os cães dos grupos GI, GII e GIII apresentaram diferenças estatísticas em relação à atividade da LDH da efusão/LDH do soro (LDHR), da LDH na efusão, PT do soro/PT da efusão (TPR) e PT da efusão. Verificou-se associação do aumento da atividade da LDH na efusão com a presença de neoplasia. Os grupos organizados de acordo com a etiopatogenia apresentaram valores desiguais de PT sérico, TPR, LDHR, PT da efusão e LDH na efusão. Por outro lado, a análise estatística dos dados dos gatos não mostrou diferença entre os grupos para os parâmetros avaliados. Critérios de Light foram mais sensíveis para identificar transudatos e exsudatos e mais específicos para determinar transudatos em comparação com a classificação convencional. Propomos o uso de análises bioquímicas testadas para distinguir o mecanismo de formação de efusões cavitárias e medição da atividade de LDH em efusões como exame complementar no diagnóstico de neoplasias cavitárias, contribuindo nos casos em que a análise citológica da efusão é inconclusiva.
Palavras-chave: efusão, LDH, proteínas totais, transudato, exsudato.

INTRODUCTION

Cavitary effusions represent the accumulation of fluids within body cavities, resulting from various physiological or pathological processes (DEMPSEY & EWING, 2010). These processes encompass alterations in hydrostatic and oncotic pressures, vascular permeability, and impaired lymphatic drainage. Moreover, effusion formation can also arise from factors like the extravasation of blood, urine, or bile (AGUIRRE & ABENSUR, 2014). Consequently, laboratory analysis is indispensable for unraveling the pathophysiology of these effusions and guiding toward differential diagnoses (BOHN, 2017).

Traditionally, effusions have been categorized based on two key parameters: total nucleated cell count (TNCC) and total protein concentration (TP), distinguishing between transudates and exudates. Transudates typically emerge from disruptions in oncotic and hydrostatic pressures or impaired drainage mechanisms. Conversely, exudates result from increased vascular permeability due to inflammation. However, it is important to note that conditions such as neoplasms, lymphatic alterations, and hemorrhage may not

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consistently exhibit exudate characteristics. Still, they are often grouped within this category due to their higher cellular concentration. The cytological examination of effusions can provide additional insights, hinting at potential infections, hemorrhage, or neoplasia, often surpassing the information gleaned solely from numerical data (BOHN, 2017). An ongoing challenge in identifying neoplastic cells lies in their differentiation from reactive mesothelial cells (STOCKHAM & SCOTT, 2011; THOMPSON & REBAR, 2016).

In human medicine, an established method for distinguishing transudative from exudative effusions involves assessing Lactate Dehydrogenase (LDH) activity, employing Light's criteria (LIGHT et al., 1972). The measurement of LDH activity serves as an indicator of cell damage or death, often induced by pathological conditions (PANTEGHINI & BAIS, 2008). Determining the pathological causes behind cavity effusion formation necessitates reliable criteria. In veterinary medicine, the conventional approach sometimes falters in differentiating effusions based on TNCC and TP due to overlapping values between transudates and exudates (ROSATO et al., 2011; ROMERO-CANDEIRA et al., 2002). Furthermore, studies have indicated a positive correlation between increased serum LDH activity and LDH activity within the effusion and the presence of neoplastic effusions (NESTOR et al., 2004).

Consequently, this prospective study primarily assessed the LDH activity and TP concentration in serum, pleural, and peritoneal effusions. The goal is to demonstrate that these analyses can serve as a valuable alternative for classifying cavitary effusions into transudates and exudates. Additionally, we aimed to underline the utility of LDH activity in effusions as a diagnostic aid for identifying neoplastic effusions in dogs and cats.

MATERIALS AND METHODS

Sample selection: inclusion criteria

Blood samples and effusions (pleural and peritoneal) from dogs and cats of varying ages, breeds, and genders were prospectively collected and analyzed. Veterinarians sent these samples from the University Veterinary Hospital (HVU) to the Veterinary Clinical Laboratory (LCV) at the Federal University of Santa Maria (UFSM). They were collected in 2ml EDTA tubes (Descarpack, São Paulo, SP, Brazil) for effusions only and 4ml tubes without anticoagulant (Firstlab, São José dos Pinhais, Paraná, PR, Brazil) for blood samples and effusions during the period from February to November 2021. Effusion samples that did not meet the requirement of being collected in both types of tubes were excluded from the study.

Physical, chemical, and cytological analysis and effusion classification

The physical evaluation of effusion samples involved assessing color, turbidity, and density using a refractometer (Instrutherm-São Paulo, SP, Brazil). Chemical testing was conducted using reagent strips (Laborclin-Pinhais, PR, Brazil) to provide a semiquantitative reading of pH, glucose, and occult blood. Samples with EDTA were processed in a hematological BC-VET 2800 analyzer (Mindray-São Paulo, SP, Brazil) to determine TNCC, erythrocyte count, and hematocrit (Ht). Subsequently, each sample was prepared as a slide using the squash technique (MEYER, 2016). These slides were dried, stained with Romanowsky derived stain (Quick Panotic -Laborclin-Pinhais, PR, Brazil), and microscopically evaluated using 10x, 20x, 40x, and 100x objectives. The evaluation included differential counting of nucleated cells, expressed as a percentage of total cells. Observations were made regarding cell populations, the presence or absence of infectious agents, cytological interpretation, and other relevant findings. Effusions were classified as low-protein transudate (TNCC < 3000/µL and TP < 2.5 g/dL), high-protein transudate (TNCC < 3000/µL and TP ≥ 2.5 g/dL), or exudate (TNCC ≥ 3000/µL) based on these criteria (BOHN, 2017). Exudates were further categorized as aseptic exudate, septic exudate, or exfoliative neoplastic effusion (SMUTS et al., 2016).

For biochemical analysis, blood and effusion samples without anticoagulant were centrifuged at 900xg for 4 minutes using a Combat centrifuge (CELM-São Paulo, SP, Brazil) to separate serum and supernatant, respectively. Analyses of serum LDH and effusion, as well as serum and effusion TP, were performed using an automatic biochemistry analyzer (BS 120-Mindray-São Paulo, SP, Brazil). When necessary, samples were stored at -20 ºC for at least two days (KANEKO et al., 2008). Effusions were categorized as transudates or exudates based on Light's criteria, widely used in human medicine. Effusions with LDH activity > 200 IU/L, effusion LDH/serum LDH (LDHR) > 0.60, and serum TP effusion/TP (TPR) > 0.50 were classified as exudates (LIGHT et al. al., 1972).

Classification of special effusion types

Various specialized effusion types were classified based on cytological analysis or additional

biochemical tests using the automated equipment. Hemorrhagic effusions were identified by Ht > 3% and, if present, signs of chronic hemorrhage such as erythrophagocytosis or breakdown products of red blood cells like hemosiderin observed as a blackish green pigment inside macrophages and hematoidin crystal with a yellowish color and variable shape (STOCKHAM & SCOTT, 2011). Effusions with over 10% eosinophils in the nucleated cell differential count were categorized as eosinophilic effusions (THOMPSON & REBAR, 2016). Chylous effusions were identified by an effusion cholesterol/triglycerides ratio < 1.0 and/or effusion triglycerides concentration > 100mg/dL. Uroperitoneum was suspected based on serum creatinine and effusion analysis, with a creatinine effusion/serum creatinine ratio ranging from 0.7 to 1.2 in dogs and 0.8 to 2.4 in cats (STOCKHAM & SCOTT, 2011). In cats, effusion albumin/globulin ratio < 0.6 was indicative of effusions related to feline infectious peritonitis (FIP) (JEFFERY et al., 2012). In cases of suspected pancreatitis, effusion lipase activity twice as high as serum activity indicated an influence of acute pancreatitis on cavitary effusion formation (STOCKHAM & SCOTT, 2011).

Sample classification into groups

Based on effusion classification, samples from dogs and cats were separately categorized into three groups: Group I (GI) for low-protein transudates, Group II (GII) for high-protein transudates, and Group III (GIII) for exudates. Subsequently, two combined groups, regardless of species, were formed: one for potential neoplastic effusions (NPG) and another for non-neoplastic effusions (NNG). These samples were further organized into groups based on the mechanisms of effusion formation, including increased hydrostatic pressure (IHP), decreased oncotic pressure (DOP), hemorrhage (HEM), bacterial infection (SEP), neoplasia (NEO), and neoplasia associated with bacterial infection (NS).

Statistical analysis

The data collected were analyzed using IBM SPSS Statistics 23 software. Data distribution was assessed for normality using the Kolmogorov-Smirnov (K-S) and Shapiro-Wilk tests. ANOVA was employed to compare serum TP among the different groups (GI, GII, and GIII) of dogs, as well as serum TP, effusion TP, and TPR values among groups of cats. Nonparametric Kruskal-Wallis tests were used for comparing LDH activity in effusion, serum LDH, and LDHR among groups (GI, GII, and GIII) of dogs and cats, as well as TPR and TP of effusion among dog groups due to the non-normal distribution of these variables.

Further comparisons between each combination of groups (GI/GII, GI/GIII, and GII/GIII) were conducted using the Mann-Whitney test. The same test was utilized to compare serum and effusion LDH activity between NPG and NNG groups. Parameters were assessed between groups categorized by etiology using ANOVA (serum TP and TPR) and Kruskal-Wallis tests (LDHR, effusion LDH activity, serum LDH, and cavitary effusion TP). Pairwise tests (LDHR, LDH activity in effusion, and TP of cavitary effusion) and Bonferroni correction were applied to compare data for each combination of groups (DOP/IHP, DOP/NEO, DOP/HEM, DOP/NS, DOP/SEP, IHP/NEO, IHP/HEM, IHP/NS, IHP/SEP, NEO/HEM, NEO/NS, NEO/SEP, and HEM/NS).

Finally, the diagnostic utility of the conventional classification and Light's criteria in identifying the pathophysiological mechanisms of effusions was assessed by calculating sensitivity (%), specificity (%), and accuracy (%) (ZOIA et al., 2020), considering a significance level of $P < 0.05$.

RESULTS

In the study period, 67 cavitary effusion samples were observed, 49 (73.1%) from dogs and 18 (26.9%) from cats. These samples included peritoneal (47 – 70.1%) and pleural (20 – 29.9%) effusions. Based on the conventional classification, 14 (28.6%) of the 49 effusions from dogs were categorized as low-protein transudates, with clinical diagnoses such as liver disease (4 – 28.57%), hypoproteinemia-related changes (2 – 14.28%), liver neoplasia (2 – 14.28%), pulmonary edema (2 – 14.28%), heart and liver diseases (1 – 7.14%), intrathoracic neoplasia (1 – 7.14%), heart disease (1 – 7.14%) and drug gastritis (1 – 7.14%) as etiopathogenesis. Among the effusions classified as high-protein transudates (9 - 18.4%) were intracavitary neoplasia (4 - 44.44%), heart disease (2 - 22.22%), trauma (1 - 11.11%), heart disease associated with liver disease (1 – 11.11%), and one case (11.11%) not clinically diagnosed by the veterinarian. Twenty-six effusions (53.0%) were classified as exudates, with causes including intracavitary neoplasia (10 - 38.5%), trauma/hemorrhage (3 - 11.5%), bacterial pneumonia (2 - 7.8%), intestinal rupture due to neoplasm (2 – 7.8%), liver failure (2 – 7.8%), heart disease (1 – 3.8%), urinary bladder rupture (1 – 3.8%), liver cyst (1 – 3.8%), pancreatitis (1 – 3.8%), pleuritis (1 – 3.8%), peritonitis (1 – 3.8%), and thoracic duct rupture (1 –

3.8%). In addition, one low-protein transudate (7.1%) was classified as eosinophilic effusion; two high-protein transudates (22.2%) were identified as hemorrhagic effusions; and eight exudates (30.8%) were classified as septic (4 - 15.4%), hemorrhagic (1 - 3.8%), chylous (1 - 3.8%), and uroperitoneum (1 - 3.8%).

Similarly, among the 18 effusions from cats, 3 (16.7%) were classified as low-protein transudates, mainly attributed to heart disease (2 – 66.7%) and hepatic lipidosis/cholangitis/pancreatitis (1 – 33.3%). Four effusions (22.2%) were categorized as high-protein transudates, linked to clinical diagnoses of intracavitary neoplasia (2 - 50%), FIP (1 - 25%), and hepatic congestion (1 - 25%). Of the effusions classified as exudates (11 – 61.1%), 5 (45.4%) were associated with intracavitary neoplasms, 1 (9.1%) with FIP, 1 (9.1%) with bacterial pneumonia, 1 (9.1%) with lower urinary tract rupture, and three effusions of unknown origin (27.3%). Biochemical tests identified one low-protein transudate (33.3%) as effusion due to acute pancreatitis, two exudates (18.2%) as FIP, and others as septic (3 - 27.3%) and neoplastic effusions (1 – 9.1%) based on cytological analysis.

For dogs, the Kruskal-Wallis test revealed significant differences among GI, GII, and GIII concerning LDHR (P = 0.000), LDH activity in effusion (P = 0.000), TPR (P = 0.000), and TP of effusion (P = 0.000). Mann-Whitney test results also indicated differences between GI/GII (P = 0.003), GII/GIII (P = 0.021), and GI/GIII (P = 0.000) when evaluating LDH activity in effusion, and similar differences were observed for LDHR (GI/GII P = 0.010) (GII/GIII P = 0.033) (GI/GIII P = 0.000), as well as between GI/GII and GI/GIII for TP effusion parameters (GI/GII P = 0.002) (GI/GIII P = 0.000) and TPR (GI/GII P = 0.003) (GI/GIII P = 0.000). However, the ANOVA test reported no significant difference among the three groups for serum TP (P = 0.095). In contrast, statistical analysis of data from cats showed no significant differences between the groups for the parameters evaluated in the study.

In 59 out of 67 dog and cat effusions evaluated, the presence of possible cavitary neoplasia as an etiopathogenesis resulted in statistically higher LDH activity values in neoplastic effusions compared to those of non-neoplastic origin (P = 0.014). Among the 59 effusions, only 56 had sufficient serum samples for analysis, and LDH activity in these 56 sera did not produce statistically significant differences (P = 0.912).

When considering the formation mechanism for the 46 effusions with defined clinical diagnoses, disparities among the groups were observed for TPR (P = 0.000), serum TP (P = 0.039), LDHR (P = 0.000), LDH activity in the effusion (P = 0.000), and TP effusion (P = 0.001). Additionally, TPR values differed between IHP/NEO (P = 0.009), DOP/SEP (P = 0.000), DOP/NEO (P = 0.006), DOP/HEM (P = 0.013), and DOP/NS (P = 0.046). LDHR also exhibited differences between DOP/NS (P = 0.008), DOP/SEP (P = 0.000), and IHP/SEP (P = 0.018). LDH activity in cavitary effusion showed variations between DOP/NEO (P = 0.039), DOP/NS (P = 0.005), DOP/SEP (P = 0.000), DOP/HEM (P = 0.010), and IHP/SEP (P = 0.010). Finally, TP effusion values differed between DOP/NEO (P = 0.005), DOP/SEP (P = 0.002), and DOP/HEM (P = 0.031). Serum LDH activity did not significantly differ between groups (P = 0.466).

Considering effusions with clinical diagnoses related to decreased oncotic pressure and increased hydrostatic pressure classified as transudates, and cavitary effusions resulting from neoplasia and bacterial infection classified as exudates, the conventional classification correctly identified 12 out of 15 transudates (80%) and 19 out of 28 exudates (67.8%). This diagnostic method exhibited a sensitivity of 57%, specificity of 90%, and an accuracy of 77% for identifying transudates, as well as a sensitivity of 86%, specificity of 62%, and an accuracy of 74% for determining exudates. When evaluating Light's criteria, all transudates (100%) and 13 of the 26 exudates (50%) were accurately classified. This resulted in a sensitivity of 67%, specificity of 100%, and an accuracy of 74% for defining transudates, as well as 100% sensitivity, 53% specificity, and 68% accuracy for indicating exudates.

The distribution of the analyzed samples (48/67) based on the mechanism of cavitary effusion formation, along with their corresponding values of TP and LDH activity in serum and effusions, TPR, LDHR, and their classification using conventional methodology and Light criteria, is detailed in table 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

DISCUSSION

In veterinary medicine, the classification of cavitary effusions presents inherent limitations in pinpointing their underlying causes. This is primarily due to the overlapping levels of TP between transudates and exudates (ROMERO-CANDEIRA et al., 2002; ROSATO et al., 2011), despite TP being the standard biochemical parameter for effusion classification (VALENCIANO & RIZZI, 2020). Such discrepancies can lead to ambiguous interpretations, highlighting the need for more effective criteria for effusion classification. Our study revealed that Light's

Table 1 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
IHP	83.4	32.0	0.38	5.3	0.2	0.04	GII	T
	210.3	81.5	0.39	6.0	4.0	0.67	GIII	T
	66.4	73.0	1.10	6.8	2.5	0.37	GI	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; IHP: Increase in hydrostatic pressure; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 2 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
IHP	356.5	33.8	0.09	5.1	0.6	0.12	GI	T
	325.3	27.7	0.08	5.3	1.0	0.19	GI	T
	510.9	41.4	0.08	4.5	2.6	0.58	GII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; IHP: Increase in hydrostatic pressure; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 3 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
DOP	190.0	9.5	0.05	5.0	0.2	0.04	GI	T
	106.1	9.7	0.09	4.8	0.2	0.04	GI	T
	613.0	82.4	0.13	6.1	3.6	0.59	GII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; DOP: Decreased oncotic pressure; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 4 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
DOP	92.5	9.1	0.10	4.7	0.3	0.06	GI	T
	380.2	18.7	0.05	4.7	0.2	0.04	GI	T
	277.2	4.8	0.02	2.4	0	0	GI	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; DOP: Decreased oncotic pressure; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 5 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
DOP	331.0	7.5	0.02	4.2	0.2	0.05	GI	T
	223.2	51.1	0.23	4.1	1.0	0.24	GIII	T
	128.8	131.3	1.02	4.4	0.2	0.04	GIII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; DOP: Decreased oncotic pressure; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 6 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
HEM	325.9	1899.4	5.83	6.9	3.3	0.48	GII	*
	-	4220.5	-	7.4	5.1	0.69	GIII	*
	1017.1	1435.5	1.41	4.0	4.6	1.15	GIII	*

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; HEM: Bleeding; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate. -: Data not obtained; *Not sortable.

Table 7 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	327.0	530.1	1.62	4.0	0.8	0.2	GI	T
	265.3	25.5	0.10	5.4	0.6	0.11	GI	T
	89.3	135.6	1.52	5.5	1.9	0.34	GII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 8 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	881.7	244.7	0.28	5.6	4.0	0.71	GII	T
	335.7	48.5	0.14	5.5	3.4	0,6	GII	T
	108.5	339.8	3.13	6.8	3.6	0.52	GIII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 9 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	189.1	51.2	0.27	6.1	2.3	0.38	GII	T
	1667.1	164.2	0.10	6.9	3.8	0.55	GII	T
	275.8	1640.2	5.94	5.4	5.0	0.92	GIII	E

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate; E: Exudate.

Table 10 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	-	1204.5	-	-	4.7	-	GIII	-
	567.0	13521.5	23.85	7.7	4.2	0.54	GIII	E
	-	56.5	-	-	2.4	-	GI	-

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate; E: Exudate. -: Data not obtained.

Table 11 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	231.6	261.6	1.13	6.2	5.5	0.89	GII	E
	1357.5	798.5	0.59	4.8	3.2	0.67	GIII	T
	130.2	89.4	0.69	5.1	2.3	0.45	GIII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate; E: Exudate.

Table 12 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NEO	131.1	132.7	1.01	7.6	6.2	0.82	GIII	T
	206.1	254.0	1.23	5.6	4.2	0.75	GIII	E

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NEO: Neoplasm; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate; E: Exudate.

Table 13 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NS	624.7	1502.0	2.40	5.0	3.0	0.6	GIII	E
	309.4	4427.6	14.31	4.9	4.4	0.90	GIII	E
	315.3	3396.8	10.77	3.9	3.7	0.95	GIII	E

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NS: associated neoplasia to bacterial infection; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; E: Exudate.

Table 14 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
NS	474.7	260.6	0.55	5.6	0.7	0.12	GIII	T
	216.2	405.4	1.88	4.6	1.9	0.41	GIII	T

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; NS: associated neoplasia to bacterial infection; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; T: Transudate.

Table 15 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
SEP	283.3	17015	6.01	3.8	2.5	0.66	GIII	E
	119.4	208.2	1.74	4.3	6.8	1.58	GIII	E
	257.8	3619.0	14.03	6.8	4.6	0.68	GIII	E

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; SEP: bacterial infection; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; E: Exudate.

Table 16 - Distribution according to etiological frequency and their respective values of TP and LDH activity in serum and effusions, TPR, LDHR, and distributions in groups according to conventional classification and Light's Criteria of the cavitary effusions from dogs and cats, with defined clinical diagnosis.

Etiopathogenesis	sLDH (UI/L)	eLDH (UI/L)	LDHR	sTP (g/dL)	eTP (g/dL)	TPR	CC	LC
SEP	459.7	6257.7	13.61	5.5	4.6	0.84	GIII	E
	623.8	1701.7	2.73	6.3	4.5	0.71	GIII	E
	646.6	6887.0	10.65	3.7	3.6	0.97	GIII	E

CC: Conventional classification; eLDH: Lactate dehydrogenase activity in effusion; eTP: Total protein effusion; GI: Low protein transudate; GII: High protein transudate; GIII: Exudate; SEP: bacterial infection; LC: Classification according to Light's criteria; LDHR: Effusion/serum lactate dehydrogenase ratio; TPR: Total effusion/serum protein ratio; sLDH: Serum lactate dehydrogenase activity; sTP: Serum total protein; E: Exudate.

criteria exhibited greater specificity for identifying transudates and demonstrated higher sensitivity for diagnosing both transudates and exudates compared to conventional methods. However, the limited number of samples within each formation mechanism group posed a limitation in assessing the overall performance of the analysis. Consequently, this study focused solely on the diagnostic test analysis for transudates and exudates.

A previous study documented variations in effusion LDH activity, serum TP, and effusion TP in pleural effusions of dogs, differentiating between effusions caused by increased hydrostatic pressure and those resulting from decreased colloid osmotic pressure and exudative effusions (ZOIA et al., 2020). Another study also demonstrated LDH activity's ability to differentiate exudates (LIGHT et al., 1972). One hypothesis explaining elevated LDH activity in hyperprotein transudates suggests the presence of a significant amount of this enzyme in the heart muscle due to cardiomyopathies, a leading cause of increased hydrostatic pressure (SMUTS et al., 2016). While it may not be possible to classify transudates based solely on their formation mechanism, distinguishing between transudates and exudates is considered the initial step in determining the etiology of cavitary effusion accumulation. This differentiation allows for the subsequent determination of the underlying pathophysiological processes through TP and albumin (PORCEL & LIGHT, 2006; ZOIA & DRIGO, 2016), combined with patient history, physical examinations, and complementary tests. Cytological analysis and microbiological culture play vital roles in diagnosing and identifying exudates (ZOIA et al., 2020).

Statistical analysis revealed significant differences in LDH activity in effusion, LDHR and TPR between effusions caused by increased hydrostatic pressure and septic effusions, even though TPR values and effusion TP concentration did not differ between high-protein transudates and exudates. These results underscore the limitation of using TP concentration alone to differentiate high-protein transudates from exudates and emphasize the importance of employing other biochemical parameters investigated in this study for detecting septic exudates. However, this distinction was not observed when analyzing non-septic exudates.

Regarding the differentiation of transudates caused by increased hydrostatic pressure and hemorrhagic effusions, the influence of hemolysis cannot be ruled out. Hemolysis may lead to an increase in LDH activity due to the substantial presence of this enzyme in erythrocytes and platelets (PANTEGHINI & BAIS, 2008), despite the presence of blood in the effusion not affecting LDH measurement. The influence of hemolysis was mitigated in this study by promptly centrifuging and separating the supernatant from the samples upon their arrival at the laboratory, recommending analysis at the same time.

In the context of identifying neoplastic effusions through cytology, the diagnosis is limited to the observation of neoplastic cells. In their absence, effusions may be mistakenly classified as transudates or exudates (VALENCIANO & RIZZI, 2020). Neoplastic cells can be challenging to identify due to their cytological characteristics, which resemble reactive mesothelium (ZIMMERMAN, 2005; THOMPSON & REBAR, 2016). This highlighted the need for complementary diagnostic methods for accurate classification. In this study, a significant difference was observed in increased LDH activity in neoplastic effusions compared to non-neoplastic ones. However, neoplastic effusions did not exhibit different values when compared to septic and hemorrhagic effusions. Additionally, effusions resulting from

neoplasms were classified as transudates. These findings suggested a potential association between neoplastic effusions and undetected hemorrhagic and inflammatory processes in cytological analysis and raise the hypothesis that changes in the affected organ may contribute more significantly to effusion formation than the neoplasm itself.

Identifying exudative effusions through increased LDH activity is attributed to its release during cell death, particularly in the case of inflammatory cells (JOSEPH et al., 2001). This increase occurs in the final stages of anaerobic glycolysis (PANTEGHINI & BAIS, 2008). Hence, the elevated LDH levels in neoplastic effusions can be explained by the reliance of these cells on anaerobic glycolysis for energy production (NESTOR et al., 2004).

Statistical analysis revealed differences in LDHR, TPR, effusion TP, and LDH activity between peritoneal and pleural effusions in dogs categorized as low-protein transudate, high-protein transudate, and exudate. These findings correlated with classifications based on TNCC and effusion TP. The use of these biochemical tests in conformity with the conventional classification was previously reported in abdominal, pleural, and pericardial effusions of dogs (ROSATO et al., 2011). However, this positive result was not observed in the differentiation of high-protein transudates and exudates in canine peritoneal effusions using LDHR, or in peritoneal effusions from dogs using the parameters LDHR and LDH activity in effusion (JÚNIOR et al., 2011). It is worth noting that the limited number of cat samples may have contributed to the results obtained in this study, which is one of its limitations.

CONCLUSION

This study suggested the use of biochemical tests, including the measurement of LDH activity and TP levels, to aid in identifying the etiopathogenesis of cavitary effusions, especially those of neoplastic origin not always diagnosed through cytological analysis. The findings of this study have the potential to offer more efficient and expedited clinical guidance to patients based on the analysis of cavitary effusions.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Ethics Committee on Animal Use (CEUA) of the Universidade Federal de Santa Maria (UFSM), under registration No. 5739020221.

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