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Clinical and anatomopathological diagnosis in bovines affected by perforated abomasal ulcers and with comorbities

Diagnóstico clínico e anatomopatológico em bovinos acometidos por úlceras do abomaso perfuradas e com comorbidades

Adony Querubino de Andrade Neto [1]*, José Ricardo Barboza Silva [2], Carla Lopes de Mendonça [1], Rodolfo José Cavalcanti Souto[1], Lilian Rose Marquez de Sá[3], José Augusto Bastos Afonso[1]

ABSTRACT: This study describes the findings of the clinical and anatomopathological diagnosis in cattle affected by perforated abomasal ulcers and with comorbidities. Twenty-five cattle were evaluated, young (n=8, aged less than two years) and adults (n=17, aged greater than two and a half years), diagnosed with perforated abomasal ulcers. Epidemiological information was retrieved from the medical records. Blood, ruminal fluid, and peritoneal fluid (PI) samples were collected for laboratory examination. Ultrasound examination, exploratory laparotomy, and post mortem examinations were performed for diagnostic purposes. The main clinical findings of perforated ulcers in the present study were: apathy, dehydration, intestinal hypomotility, diarrheal stools, hypomotile or atonic rumen, tachycardia, tachypnea, abdominal distention with bilateral bulging, and increased abdominal tension. The most frequent abnormal hematological findings were hypoproteinemia and neutrophilic leukocytosis with a left shift. The peritoneal fluid (PL) showed an inflammatory reaction in the cytology, characteristic of an exudate. A confirmatory diagnosis of peritonitis by ultrasonography was possible in five cases, and in two cases at exploratory laparotomy. In the post mortem examination, the ulcers were characterized as type 3, 4, or 5, with comorbidities present in 68% of the cases. It is necessary to combine ultrasonography with abdominal centesis and exploratory laparotomy procedures to suggest perforated ulcers. However, post mortem examination better characterizes the type of perforated ulcer present, particularly type 5.

KEYWORDS: Abomasopathies, Ulcerative Lesions, Peritonitis in cattle, Ultrasonography.

RESUMO: Este estudo descreve os achados do diagnóstico clínico e anatomopatológico em bovinos acometidos por úlceras do abomaso perfurada e com comorbidades. Foram avaliados 25 bovinos, jovens (n=8, idade inferior a dois anos) e adultos (n=17, idade superior a dois anos e meio), diagnosticados com úlceras do abomaso perfuradas. As informações epidemiológicas foram resgatadas de prontuários de atendimento. Amostras de sangue, fluido rumenal e de líquido peritoneal (LP) foram coletadas para exame laboratorial. Procedeu-se exame ultrassonográfico, laparotomia exploratória e exame post mortem para fins de diagnóstico. Os principais achados clínicos de úlceras perfuradas do presente estudo foram: apatia, desidratação, hipomotilidade intestinal, fezes diarreicas, rúmen hipomotílico ou atônico, taquicardia, taquipneia, distensão abdominal com abaulamento bilateral e tensão abdominal aumentada. Os achados hematológicos anormais mais frequentes foram hipoproteinemia e leucocitose por neutrofilia com desvio a esquerda. O líquido peritoneal (LP), evidenciou reação inflamatória, na citologia, característica de exsudato. O diagnóstico de peritonite por ultrassonografia foi possível em cinco casos, e em dois casos na laparotomia exploratória. No exame post mortem as úlceras caracterizaram-se como do tipo 3, 4 ou 5, com comorbidades presentes em 68% dos casos. Aliar a ultrassonografia com a centese abdominal e laparotomia exploratória, são ferramentas necessárias para predizer úlceras perfuradas. No entanto, o exame post mortem caracteriza melhor o tipo de úlcera perfurada presente, principalmente a do tipo 5.

PALAVRAS-CHAVE: Abomasopatias, Lesões Ulcerativas, Peritonite em bovinos, Ultrassonografia.

[1] Universidade Federal Rural de Pernambuco, Garanhuns/PE, Brasil
[2] Universidade Federal de Viçosa, Viçosa/MG, Brasil
[3] Universidade de São Paulo, São Paulo/SP, Brasil
*Corresponding author: adony_neto@hotmail.com
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INTRODUCTION

Abomasal ulcer is common in cattle, affecting all age groups, and is characterized as an important disease of the digestive system, since it causes discomfort and pain for the animal, in addition to significant economic losses, especially in intensive livestock farming (Munch *et al.*, 2019; Silva Filho *et al.*, 2012; Souza *et al.*, 2017). Cattle can present multifocal or focal ulcers, that can perforate the abomasum or form permanent scars in the organ after reparative processes (Ducharme *et al.*, 2017). Fox (1980) and Whitlock (1980) initially classified abomasal ulcers into four types. Later, Smith; Munson; Erb (1983), based on the depth of the ulceration and the degree of hemorrhage, classified them as non-perforating and perforating.

Type 1 indicates non-perforating erosions or ulcers with minimal bleeding; type 2 is an unperforated ulcer that causes profuse intraluminal hemorrhage due to erosion of branches of the gastroepiploic artery into the submucosa (Braun et al., 2019b); type 3 is a perforated ulcer and the peritonitis is confined focally to the ulcerated region because the omental sheath and adhesions formed with the adjacent peritoneum stop leakage of contents from the abomasum (Braun *et al.*, 2019a). In type 4 ulcer, perforation usually occurs in the right-sided wall, and the organ contents rapidly spill into the peritoneal cavity, resulting in diffuse peritonitis (Braun et al., 2019b; Hund; Wittek, 2018).

More recently, a new classification grouped ulcers into five types, with the addition of subtypes 1a, 1b, 1c, and 1d, in the classification of type 1 ulcers (Braun; Eicher; Ehrensperger *et al.*, 1991; Hund; Wittek, 2017). Perforation of the abomasum in the omental bursa associated with omental bursitis, previously considered a subtype of type 3 ulcer (Fubini; Yeager; Divers et al., 2018), was designated as type 5 ulcer (Constable, 2014). Type 5 ulcer occurs when perforation occurs in the left wall of the abomasum, allowing the contents to leak into the omental bursa, causing omental bursitis, accompanied by empyema in the omental sac, between the two serous layers of the bursa (Braun et al., 2020b).

Perforated abomasal ulcers are ulcers of chronic evolution and negative outcome, with low prevalence, ranging from 0.63% to 1.6% (Palmer; Whitlock, 1984). Clinical, laboratory, and sonographic findings vary widely and have been described in detail in recent papers (Braun *et al.*, 2019a; Gerspach *et al.*, 2020).

Type 3 perforated ulcers present the following clinical signs: abdominal pain, anorexia, decreased ruminal motility, ruminal distention, abdominal tension, and kyphosis, signs similar to traumatic reticulum peritonitis. Types 4 and 5 perforated ulcers present more severe clinical signs, are considered medical emergencies, and are usually fatal within 24 to 48 hours. Clinical signs are typical of septic shock and diffuse peritonitis such as; tachycardia, tachypnea, dehydration, abdominal pain, depression, ruminal stasis, cold body extremities, and a severely altered general status. On transrectal palpation, negative intra-abdominal pressure is absent, and the peritoneum may appear rough and crackling, with fibrin debris (Braun *et al.*, 2020a; Palmer; Whitlock, 1984, Smith; Munson; Erb, 1986).

The diagnosis of perforated abomasal ulcer requires a comprehensive examination by an experienced clinician and it is not always possible to establish the diagnosis based on clinical signs alone. Therefore, additional diagnostic procedures are required, including evaluation of blood count, abdominal ultrasonography, analysis of peritoneal fluid, or exploratory laparotomy. In addition, in some cases the diagnosis is only possible in the post-mortem examination (Braun et al., 2019b). Local or diffuse peritonitis can be confirmed by finding bacteria and toxic changes on cytological examination after abdominocentesis (Constable *et al.*, 2017, Santos *et al.*, 2021).

Ultrasonography is a valuable technique and its findings in cows with peritonitis vary considerably (Gerspach *et al.*, 2020). Therefore, focal fibrinous peritonitis can be observed, characterized by echogenic deposits in the peritoneum and on the surface of the organs (Gouda *et al.*, 2020), there may be accumulation of anechoic to hypoechoic fluid (inflammatory ascites) interspersed with fibrin strands between organs, that may be seen in generalized peritonitis (Braun *et al.*, 2019b; 2020b). In some situations, ingesta can be seen as an echogenic coating on various abdominal organs and the omentum (Braun *et al.*, 2019a).

Animals with peritonitis resulting from type 4 and type 5 perforated ulcers have a poor prognosis and do not respond to treatment (Braun *et al.*, 2019b; 2020b). On the other hand, animals with type 3 ulcer also have a poor prognosis, but 20% of cases respond to treatment (Braun *et al.*, 2019a). As a result, additional diagnostic procedures beyond the physical examination almost always lead to a correct diagnosis and may predict untreatable peritonitis in 76% of cows with type 4 ulcer and 36% of cows with type 5 ulcer. For this reason, timely euthanasia of the animal is recommended, avoiding further suffering and unnecessary costs with medicines (Braun *et al.*, 2019b; 2020b, Gerspach *et al.*, 2020). In view of this, and given the scarcity of studies regarding these clinical entities, the current work aims to present the clinical and anatomopathological findings in cattle affected with perforated abomasal ulcer and comorbidities, and thus provide criteria to support the diagnosis and prognosis of abomasopathies.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on the use of animals of the Federal Rural University of Pernambuco, CEUA/UFRPE, protocol No. 23082.017161/2017-21.

The work was carried out at the Clínica de Bovinos de Garanhuns, *Campus* of the Federal Rural University of Pernambuco (CBG-UFRPE) in partnership with the Department of Pathology of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo (FMVZ-USP).

Twenty-five bovines were included, young (n=8, aged less than two years) and adults (n=17, aged greater than two and a half years), male and female, diagnosed with perforated abomasal ulcers, based on results of physical examination, ultrasound, or exploratory laparotomy, and proven on postmortem examination.

Epidemiological information such as: sex, breed, age, type of rearing, feeding, time of year, lactation stage, and number of deliveries was recorded in the clinical medical records.

Young and adult cattle were submitted to clinical examination according to Dirksen; Grunder; Stöber, (1993). Attitude, behavior, body temperature, color of the ocular conjunctiva, and degree of dehydration were observed. The vital parameters, such as heart rate and respiratory rate were also measured, as well as abdominal shape and abdominal tension. The rumen was evaluated for motility, stratifications, and tympany. Intestinal peristalsis and stool consistency were also evaluated. All parameters were recovered from the clinical records of animal care between 2014 and 2019.

Blood samples were collected in all animals in EDTA, by jugular venipuncture, in siliconized tubes with a vacuum system (BD-Vacutainer system®), for hematological analysis. Hematological analysis included determination of hematocrit (HT), red blood cells (He), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total and differential leukocyte count, total plasma protein (TPP), and plasma fibrinogen (PF) according to Harvey (2012). Ruminal fluid was collected from 22 animals using a Schambye and Sorensen probe, as per Dirksen; Grunder; Stöber, (1993) and evaluated for color, odor, consistency, pH, percentage of live infusoria, and density and motility of infusoria. In addition, the methylene blue reduction time and chloride concentration were determined. The peritoneal fluid (PL) was evaluated, obtained by abdominocentesis, in eight animals (n=2, age less than two years), (n=6, age greater than two and a half years). The collection was carried out using a 40x12 mm needle, and the PL was placed in sterile tubes (BD-Vacutainer system®) with and without anticoagulant (EDTA) for analysis of volume, color, odor, appearance, protein by refractometry, leukocytes by quantitative analysis, blood by dipstick, and cytological analysis, according to Valenciano; Arndt; Rizzi (2014).

The ultrasound examination was performed in seven animals, using a portable ultrasound device (Mindray®, model Z6, São Paulo, Brazil) with a 3.5 Mhz convex transducer, with the animals in a quadrupedal position. The evaluated area consisted of the reticular region at the height of the scapulohumeral joint on the left and right sides. The examination began in the left cranioventral region, followed by the left side (caudocranial direction) through the intercostal spaces, in the middle third of the scapula, and ending on the right side. During the examination, the motility of the reticulum and the presence of adhesions between the reticulum and adjacent organs were investigated by measuring the displacement of the reticulum from the abdominal cavity in the craniodorsal direction during the biphasic contraction. Impaired motility was identified by observing hyperechoic adhesions adjacent to the reticulum, and the abdominal cavity was explored for the presence of multiple fibrin deposits, intra-abdominal fluid, abscesses, and altered organ position, according to Braun (2005).

Exploratory laparotomy was performed in the right paralumbar fossa in seven animals (n = 1 young and n = 6 adults), because the results of the physical and ultrasonographic examination were suggestive of a differential diagnosis with the following diseases: intestinal obstruction by phytobezoar, displacement of the abomasum to the right, and peritonitis, the technique was performed according to Ducharme *et al*. (2017).

All 25 animals (n=8 juveniles; n=17 adults) died or were euthanized, according to Luna; Teixeira, (2007), with the authorization of the owner, or died naturally (they did not respond to treatment) after clinical or clinical-surgical examination. The decision for euthanasia was taken when the treatment was unfeasible for clinical resolution or when the response to treatment was not satisfactory. On postmortem examination, the peritoneal cavity was photographed and evaluated for the presence of focal or diffuse peritonitis, adhesions, and omental bursitis. The abomasum was separated from the rumen, opened along the greater curvature, and its contents were evaluated for macroscopic aspects, such as the presence of blood, clots, geosediment, foreign bodies, and fibers, and then immersed in water to remove them pré-estômagos, Ulcers were analyzed for; bleeding and topographic distribution by affected region, cardiac, fundic, or pyloric, and then the mucosa of the abomasum was photographed. Fragments were collected that represented the ulcerated mucosa, with a portion of the healthy mucosa, and at least one perforated ulcer. For histopathological analysis, ulcers were fixed in 10% buffered formalin, processed per the routine protocol and stained with hematoxylin-eosin (HE). The macroscopic evaluation of perforated ulcers was performed following the classification criteria described by Whitlock (1980) and modified by Smith; Munson; Erb (1983), and Constable (2014).

Statistical analysis was performed descriptively and absolute and relative frequencies were determined for each variable (epidemiological, laboratory, and anatomopathological). Variables were calculated by measures of central tendency, mean and standard deviation, for normal variables; laboratory [hematocrit (Ht), red blood cells (He), hemoglobin (Hb), mean corpuscular volume (MCV), corpuscular hemoglobin concentration (MCHC), total plasma protein (TPP), plasma fibrinogen (PF), rumen chloride concentration, peritoneal fluid volume, and peritoneal fluid protein concentration], and clinical tests (heart rate, respiratory rate, and body temperature). For non-normal variables, the median (leukocyte count in blood and peritoneal fluid) was used. Data analysis was performed using the BioEstat 5.4 program.

RESULTS AND DISCUSSION

In the current work, (6/8) 75% and (15/17) 88% of the animals affected with perforated ulcer were young and adult females, reared semi-intensively and intensively, respectively. According to Hund; Wittek, (2017) ulcers occur in cattle of all ages, sex, breeds, and rearing systems. Perforations of the abomasum have a low prevalence, are usually the cause of death in 1.6% of necropsied cattle (Jensen *et al.*, 1992), and correspond to 0.43% of cases in hospitalized animals (Palmer; Whitlock, 1984). The epidemiological information of young and adult cattle with perforated abomasal ulcers and patients with comorbidities is shown in (Table 1).

The main clinical findings of cattle with perforated ulcers in the present study were apathy, dehydration, hypomotile or atonic rumen, intestinal hypomotility, tachycardia, tachypnea, abdominal distension with bilateral bulging, increased abdominal tension, and diarrheal stools. The most common clinical findings of young and adult cattle affected by the types of perforated ulcers and carriers of comorbidities are shown in (Table 2).

According to Braun *et al.* (2019a), type 3 ulcers present the main clinical signs of anorexia, abducted limbs, cold extremities, congested scleral vessels, abdominal tension, tachypnea, fever, and tachycardia, which are similar to cows with types 4 and 5 abomasal ulcers. It is, therefore, not possible to differentiate them based on clinical signs, although these are generally more severe in cows with diffuse peritonitis and omental bursitis. The definitive diagnosis, based on clinical examination, with complementary laboratory and ultrasonographic examinations in perforated ulcers, is difficult and is almost always made impossible by the broad similarity of clinical signs associated with other digestive diseases that lead to peritonitis, mainly traumatic reticulum peritonitis (TRP), and intestinal diseases, such as obstructions, intussusception, and rupture (Braun *et al.*, 2019b, 2020b, Gerspach et al., 2020).

The two animals with type 5 ulcer presented abdominal tension considered normal. According to the studies of Braun *et al.* (2022), various clinical findings, such as abnormal behavior, heart rate greater than 100 bpm, colic, ruminal atony, melena, lordosis, and bruxism have high positive predictive values and can be used to differentiate diseased cows with TRP or abomasal ulcer from healthy cows. However, when comparing these signs in differentiating cows with TRP from cows with perforated abomasal ulcers, all of them showed low sensitivity for perforated ulcers, with the exception of increased abdominal tension, with sensitivity of 61% for type 3 ulcers, 81% for type 4 ulcers, and 100% for type 5 ulcers. Therefore, based on this study, type 5 ulcers can be ruled out in the absence of a tense abdominal wall, which was not verified in our study for animals with type 5 ulcer. In this regard, the differentiation between these diseases cannot be based only on the findings of the individual physical examination, there is a need for a detailed history and clinical examination, with complementary laboratory tests, ultrasound, exploratory laparotomy, and a comprehensive evaluation of all the information available to define the diagnosis of perforated ulcer.

Table 1. Epidemiological information of young and adult cattle with perforated abomasal ulcers and carriers of comorbidities.

Epidemiological Indicators	n	Variables	Young cattle ≤ 2 years (n=8)	Adult cattle ≥ 2.5 years (n=17)
Sex	25	Female	6 (75%)	15 (88%)
		Male	2 (25%)	2 (12%)
Breed	25	Mestiça[1]	5 (62%)	11 (65%)
		Pure[2]	3 (28%)	6 (35%)
Rearing System	25	Semi-intensive	6 (75%)	7 (41%)
		Intensive	2 (25%)	9 (53%)
		Extensive	-	1 (6%)
Feed	25	Bulky[3]	1 (13%)	1 (6%)
		Bulky and concentrated[4]	4 (50%)	16 (94%)
		Milk	3 (37%)	-
Time of the year	25	Dry season[5]	8 (100%)	9 (53%)
		Rainy season[6]	-	8 (47%)
Stage of lactation	15	Lactating cows	-	11 (73%)
		Dry cows	-	4 (27%)
Number of deliveries	11	Primiparous	-	5 (45%)
		Multiparous	-	6 (55%)

[1] Crossbred Dutch, Jersey, Swiss and Gir; [2]Dutch, Brown Swiss, Nelore. [3]Corn silage, cactus pear (*Opuntia ficus indica*), elephant grass (*Pennisetum purpureum*), and sugarcane (*Saccharum officinarum*). [4] Concentrates: corn, wheat, cotton and soy. [5] October to March. [6] April to September

Table 2. Clinical findings of young (n=8) and adult (n=17) cattle with perforated abomasal ulcers and comorbidities.

Clinical Parameters	Findings	n	Young cattle ≤ 2 years (n=8)	Adult cattle ≥ 2.5 years (n=17)	Type of perforated ulcer		
					Type 3	Type 4	Type 5
Posture	Standing	15	2 (25%)	13 (75%)	6	8	1
	Decubitus	10	6 (75%)	4 (25%)	4	5	1
Temperature (°C)	Physiological (37.5-39.5°C)	19	5 (26%)	14 (74%)	7	10	2
	Hypothermia (<37.5°C)	1	-	1 (100%)	1	-	-
	Hyperthermia (>39.5°C)	5	3 (60%)	2 (40%)	2	3	-
Behavior	Calm	8	4 (50%)	4 (50%)	4	4	-
	Agitated	5	1 (20%)	4 (80%)	2	3	-
	Apathetic	12	3 (25%)	9 (75%)	4	6	2
Degree of dehydration [1]	Physiological	4	1 (25%)	3 (75%)	1	2	1
	Mild (Grade I)	11	3 (27%)	8 (73%)	3	7	1
	Moderate (Grade II)	8	4 (50%)	4 (50%)	5	3	-
	Severe (Grade III)	2	-	2 (100%)	1	1	-
Respiratory frequency (rpm)[2]	Physiological (24-36)	12	3 (25%)	9 (75%)	3	7	2
	Bradypnea (< 24)	5	1 (20%)	4 (80%)	4	1	-
	Tachypnea (>36)	8	4 (50%)	4 (50%)	3	5	-
Heart Rate (bpm)[3]	Physiological (60-80)	9	1 (11%)	8 (89%)	5	4	-
	Bradycardia (<60)	2	-	2 (100%)	-	2	-
	Tachycardia (>80)	14	7 (50%)	7 (50%)	5	7	2
Abdomen shape	Physiological	12	3 (25%)	9 (75%)	5	5	2
	Bulging	13	5 (38%)	8 (62%)	5	8	-
Abdominal tension	Physiological	12	5 (42%)	7 (58%)	5	5	2
	Increased	13	3 (23%)	10 (77%)	5	8	-
Stratification of the Rumen	Defined	10	3 (30%)	7 (70%)	4	5	1
	Undefined	15	5 (33%)	10 (77%)	6	8	1
Tympany	Absent	16	3 (19%)	13 (81%)	5	9	2
	Present	9	5 (55%)	4 (45%)	5	4	-
Intestinal peristalsis	Atonic	1	1 (100%)	-	-	1	-
	Physiological	5	1 (20%)	4 (80%)	2	3	-
	Hypomotile	17	4 (23%)	13 (77%)	6	9	2
	Hypermotile	2	2 (100%)	-	2	-	-
Feces	Pasty	6	1 (17%)	5 (83%)	2	3	1
	Diarrheal	5	3 (60%)	2 (40%)	2	3	-
	Dry and mucus	2	1 (50%)	1 (50%)	1	1	-
	Melena	5	-	5 (100%)	-	5	-
	Absent	7	3 (43%)	4 (57%)	5	1	1

[1] Degree of dehydration: Mild up to 5%, Moderate 5% to 10%, Severe over 10%.[2]rpm: respiratory movements per minute.[3] bpm: beats per minute

Comorbidities associated with perforated ulcers were present in 17/25 (68%) of the cases. Of these, 12/17 (70%) were related to the digestive system, with compaction of the abomasum 3/12 (25%), displacement of the abomasum to the right 2/12 (17%), intestinal obstruction by phytobezoars 2/12 (17%), vagal indigestion 2/12 (17%), displacement of the abomasum to the left 1/12 (8%), intussusception 1/12 (8%), and rumen drinking syndrome 1/12 (8%) the most frequent; to the nervous system 2/17 (12%); of these rabies 1/2 (50%) and polioencephalomalacia 1/2 (50%). In addition to these, there were 1/17 (6%) cases of pneumonia, 1/17 (8%) cases of anaplasmosis, and 1/17 (8%) cases of mastitis.

In other studies, comorbidities were also observed in 86%, 33%, and 21% of cattle with perforated ulcers types 3, 4, and 5 respectively. Among these comorbidities, displacement of the abomasum was also responsible for the occurrence of perforated ulcers in cattle in previous studies (Braun *et al.*, 2019a, 2019b, Hund; Wittek, 2017). In impactions of the abomasum, rupture of the abomasum may occur, particularly in primary impaction, associated with fibrous feeding, resulting in diffuse peritonitis, which must be differentiated from perforated ulcers, which tend to be chronic lesions with the presence of fibrosis. In cases of impaction, rupture occurs at the ends of the organ close to the omaso-abomasal orifice or

pyloric end (Constable *et al.*, 2017). Comorbidities are predisposing factors for perforated ulcers, as they are responsible for increased cortisol secretion, decreased abomasal transit, increased hydrochloric acid and pepsin, and also result in reduced secretion of prostaglandin E, which protects the abomasal mucosa (Hund; Wittek, 2018).

The compromised laboratory findings of the hemogram were leukocytosis due to neutrophilia with a left shift. In addition to these findings, hypoproteinemia was also observed. Hypoproteinemia is indicative of loss or active secretion of protein-rich fluid into the peritoneal cavity. It is an important laboratory finding in cattle with typical clinical signs of peritonitis and reflects the massive sequestration of fluid and protein into the peritoneal cavity as a result of generalized peritonitis (Fecteau *et al.*, 2018, Santos *et al.*, 2021). The hematological findings, found both in young and adult animals with perforated abomasal ulcers and carriers of comorbidities, are shown in (Table 3).

The animals mostly presented mild to moderate degrees of dehydration, not interfering in general in the hematocrit. According to Braun *et al.* (2019b), in cases of severe peritonitis hypoproteinemia is usually associated with elevated hematocrit, as seen in cases of type 4 perforated ulcer. Leukocytosis is a laboratory finding of relative value in differentiating cows with TRP from perforated abomasal ulcers. According to Braun *et al.* (2019a, 2019b, 2020a) only 7%, 19%, and 25% of cows with ulcer types 5, 4, and 3 had leukocytosis, respectively. Leukocytosis reflects the severity of the inflammatory process in cases of perforated ulcers. Usually, leukopenia is also present in cases of perforated ulcers, being observed in 35%, 12%, and 7% of cows with type 4, 3, and 5 ulcers, respectively (Braun *et al.*, 2019a, 2019b, 2020a).

Leukopenia in cows with perforated ulcers can be interpreted as a sequel to sepsis and leukocyte sequestration to the focus of inflammation. Reports by Braun *et al.* (2022) report that the leukocyte count has low sensitivity to establish the precise cause of peritonitis when compared to TRP animals with abomasal ulcers. In most cases, a mild to moderate inflammatory process is observed, characterized by neutrophilic leukocytosis in type 3 and 5 perforated ulcers, due to the delimitation of the inflammation, generally circumscribed, focal in type 3 ulcers, and moderate in type 5 ulcers, whose delimitation of the inflammatory process is the omental bursa. In type 4 perforated ulcers, peritonitis is more severe and generalized, with septicemia, as the content leaks into the peritoneal cavity, presenting a shift of neutrophils to the left, corroborating the results found in this study (Fecteau *et al.*, 2018).

Altered rumen fluid findings are expected for cows with perforated abomasal ulcers. Among these findings, the most important is the chloride concentration, which in the present study was elevated (>30mEq/L) in 72% of the animals with perforated ulcer (Table 4).

An elevated ruminal chloride concentration was observed in 66%, 34%, and 60% of cows with type 3, 4, and 5 ulcers, respectively (Braun *et al.*, 2019a, 2019b, 2020a). The likelihood of an increase in ruminal chloride occurring in a cow with an abomasal ulcer is 14 to 27 times more likely than in a healthy cow without an ulcer (Braun et al., 2022). Cows with TRP presented ruminal chloride concentrations less than 40 mmol/l, significantly less often than cows with type 3 ulcers. The usefulness of ruminal chloride in differentiating diseases that promote elevation of this

Table 3. Mean value, standard deviation (x ± s), and overall mean of hematological variables observed in young (n=8) and adult (n=17) cattle with perforated abomasal ulcers and with comorbidities.

Hematological variables	Perforated abomasal ulcer		General mean
	Young cattle ≤ 2 years (n=8)	Adult cattle ≥ 2.5 years (n=17)	
Hematocrit (%)	31.00±9.00	31.00±10.00	31.00
Red blood cells(x106/µl)	7.21±1.24	6.62±2.47	7.00
Hemoglobin (g/dl)	8.87±2.25	10.0±3.53	9.60
MCV (fl) [a]	41.86±7.18	49.23±7.57	46.77
MCHC (%) [b]	33.06±1.28	32.77±2.75	32.86
Leucocytes (x106/µl)	14725.0±5843	15100±10351	14975
Lymphocytes (/µl)	5196.00±1767	5119±3401	5144.66
Neutrophils (/µl)	9191.00±5270	9457±9212	9368.54
Eosinophils (/µl)	111.00±0.0	257.0±340.47	68.87
Monocytes (/µl)	240.00±171.0	241.0±186.48	190.12
Rods (/µl)	222.00±0.00	386.0±472.08	202.29
TPP (g/dl) [c]	6.20±1.32	7.22±1.91	6.87
PF (mg/dl) [d]	587.50±269.59	706.25±312.98	666.66

[a]MCV: mean corpuscular volume; [b]MCHC: Mean corpuscular hemoglobin concentration; [c]TPP: Total plasma protein; [d]PF: Plasma Fibrinogen.

parameter, mainly displacement of the abomasum and anterior intestinal obstruction, from ulcers of the abomasum, is limited. However, from a practical point of view, a marked increase in ruminal chloride concentration >40mmol/l in TRP is unlikely, and serves to differentiate it from abomasal ulcers, when causes such as displacement of the abomasum and obstructive bowel diseases are ruled out (Braun *et al.*, 2022).

In all samples of peritoneal fluid (PL), in the eight animals analyzed, there were characteristics of inflammatory processes typical of peritonitis. Alterations included a yellowish color, fetid odor, cloudy appearance, and high leukocyte count, and cytological examination indicated the presence of large amounts of bacteria and inflammatory cells. The protein concentration by refractometry was on average 3.0g/dl, and two animals with type 4 ulcer had protein concentrations above 4.0g/dl, values considered altered in comparison with reference values for cattle (Dirksen; Gründer; Stöber, 1993, Wittek *et al.*, 2010, Fecteau *et al.*, 2018) (Table 5).

Table 4. Ruminal fluid findings from young (n=8) and adult (n=17) cattle with perforated abomasal ulcers and comorbidities.

Ruminal fluid variables	Findings	n	(%)	Young cattle ≤ 2 years (n=8)	Adult cattle ≥ 2.5 years (n=17)
Color	Normal	22	100%	7	15
	Altered	-	-	-	-
Odor	Normal	6	27%	3	3
	Altered	16	73%	4	12
Consistency	Normal	13	59%	3	10
	Altered	9	41%	4	5
pH	Normal	16	80%	4	12
	Altered	4	20%	2	2
Infusoria (%)	Normal	3	14%	1	2
	Altered	19	86%	6	13
Density	Normal	10	45%	3	7
	Altered	12	55%	4	8
Motility	Normal	7	32%	1	6
	Altered	15	68%	6	9
RPMB[a]	Normal	5	26%	2	3
	Altered	14	74%	3	11
Chlorides (mEq/L)	Normal	5	28%	1	4
	Altered	13	72%	4	9

[a]RPMB: reduction potential of methylene blue.

Table 5. Peritoneal fluid (PL) analysis of young (n=2) and adult (n=6) cattle with perforated ulcer and comorbidities (n=8).

Variables	Findings	n
Volume (ml)	15.0± 9.0	8
Color	Light amber	2
	Yellow	6
Odor	Fetid	4
	Odorless	4
Aspect	Cloudy	8
Proteín[1]	3.0 ± 1.16g/dl	8
Blood[2]	High	8
pH[2]	7.75± 0.27	8
Protein [2]	High	8
Leucocytes	Median = 1900 cells/ml	8
Cytology(PL)	**Findings**	**8**
Type 3 ulcer	Predominance of polymorphonuclear cells (80% to 90%), bacteria (+++).	1
Type 4 ulcer	Predominance of polymorphonuclear cells (80% to 90%), presence of mononuclear and mesothelial cells, bacteria (++++) and few red blood cells, coagulation, and fibrin.	6
Type 5 ulcer	Bacteria (++++) and many red blood cells, few mononuclear and polymorphonuclear.	1

[1]Source: Dirksen et al. (1993). [1] Refractometry; [2] Labtest reagent strip.

Santos *et al.* (2021), observed in cattle with peritonitis as a consequence of intestinal diseases and TRP, values of protein concentration in the PL higher than 3.0g/dl, the same as observed by Dezfouli *et al.* (2012) in cattle with peritonitis. Alterations in the PL of cattle with perforated ulcer with exudate characteristics were observed in 26%, 98%, and 100% of cattle with ulcer types 3, 4, and 5, respectively (Braun *et al.*, 2019a, 2019b, 2020a).

An exploratory laparotomy was performed in seven animals (n = 1 young and n = 6 adults). In one case of perforated ulcer type 3, the abomasum was adhered to the peritoneum, dilated, with focal peritonitis, and with the formation of fibrosis and abscesses in the serosa. In another case with diffuse peritonitis, suggestive of type 4 ulcer, the abomasum was distended with compaction and contents leaking into the peritoneal cavity. The other findings were not consistent with perforated ulcers, only being evidenced in the postmortem examination (Table 6).

It was possible to establish a diagnosis of perforated ulcers in two cases with exploratory laparotomy, which showed areas of adhesions with fibrosis, located mainly in the right abdomen. The abomasum with limited motility and with a focal area of adhesions more firmly to the floor of the abdomen, with formation of abscesses and fibrosis, a case of perforated ulcer type 3, and a case with diffuse peritonitis resulting from perforated ulcer type 4. Exploratory laparotomy is a valuable auxiliary diagnostic procedure in ruminants, with perforated ulcers of types 3, 4, and 5 being possible in 46%, 14%, and 7% of cows, respectively (Braun *et al.*, 2019a, 2019b, 2020a, Fecteau *et al.*, 2018).

Information obtained from physical examination and laboratory data usually indicates a diagnosis of peritonitis but does not provide a specific cause. As a result, excellent knowledge of bovine abdominal anatomy and good understanding of the pathophysiology of perforated ulcers are required for diagnosis during laparotomy (Fecteau *et al.,* 2018).

Ultrasonography was useful for visualizing and evaluating peritonitis in five cases out of seven analyzed, and in three cases the cause of peritonitis was suggestive of perforated ulcers. Abdominal ultrasonography in the evaluated animals constituted an important diagnostic possibility to evaluate perforated ulcers, especially when the changes were more evident on the right side of the abdomen. On ultrasound, fibrinous alterations in the serosa of the abomasum were seen in seven cows; these changes and more extensive inflammatory lesions in the abomasal region provide evidence of a type 4 ulcer (Braun *et al.*, 2019b). A diagnosis of focal fibrinous peritonitis attributable to type 3 abomasal ulcer as a consequence of displacement of the abomasum to the right was possible based on sonographic findings in a cow (Gerspach *et al.*, 2020). The cases with type 4 ulcers, which were suggestive in the ultrasound examination, showed more evident alterations in diffuse peritonitis on the right side of the abdomen. However, type 4 ulcers may not be visualized by ultrasonography, as the lesions are obscured by the inflammatory reaction resulting from diffuse peritonitis. A comparison of sonographic findings with postmortem examination in seven animals with perforated ulcers is shown in (Table 7).

Subjective signs of perforated abomasal ulcers, such as lesions associated with peritonitis, were observed by ultrasonography in cattle with type 3, type 4, and type 5 ulcers (Braun *et al.*, 2019a, 2019b, 2020a). Ultrasound examination of 46 cows with type 3 ulcers showed fibrin deposits in the serosa of the abomasum in five cows and free abdominal fluid in the abomasal region in six cows (Braun *et al.*, 2019a). In cows with type 4 ulcer, abdominal ultrasonography showed evidence of peritonitis in 87% (Braun *et al.*, 2019b).

The ultrasonographic evaluation of one of the animals with type 5 ulcer showed dorsally displaced omentum, with increased echogenicity and the presence of a moderate amount of anechoic content and fibrin filaments, forming images

Table 6. Exploratory laparotomy findings in young (n=1) and adult (n=6) cattle with perforated ulcer and comorbidities (n=7).

Local	Findings	n	%
Abdominal cavity	Focal peritonitis	1	14%
	Diffuse peritonitis	2	28%
	Organ adhesions to the peritoneum	2	28%
Reticulum and rumen	Adhesions to the peritoneum	2	28%
	Distended	4	57%
Abomasum	Displaced	2	28%
	Hypomotile or atonic	2	28%
	Adhesions to the peritoneum	2	28%
	Abscesses and fibrosis	1	14%
	Evidence of perforated ulcers	2	28%
	Distension	5	71%
Intestines	Distended or obstructed	2	28%

with a net aspect (fibrin). The same finding was observed by Gouda *et al.* (2020) in buffaloes with type 5 ulcer. With the ultrasound examination it is possible to differentiate inflammatory alterations that are inside and outside the omental bursa and thus improve the diagnosis of type 5 ulcers. For this, an association of ultrasonography with abdominocentesis and exploratory laparotomy is necessary in cows with signs of peritonitis. Changes associated with omental bursitis primarily involve the left side of the abdomen (Braun *et al.*, 2020a).

Focal peritonitis, diffuse peritonitis, and omental bursitis were found in all cases of perforated ulcer types 3, 4, and 5, respectively. In addition to perforated ulcers, other alterations were observed in the abomasum (Table 8).

Perforations of the abomasum occurred more frequently in the greater curvature, the fundal region of the body. In the postmortem examination ten animals were diagnosed with type 3 ulcers, with focal peritonitis; four in young animals and six in adult animals (Figure 1).

Perforation of the ulcer on the right wall of the abomasum leads to a type 4 ulcer and the contents leak into the peritoneal cavity, causing diffuse peritonitis (Braun *et al.*, 2020a), which corroborates the present study, in which thirteen animals with type 4 ulcers, had diffuse peritonitis (Figure 2).

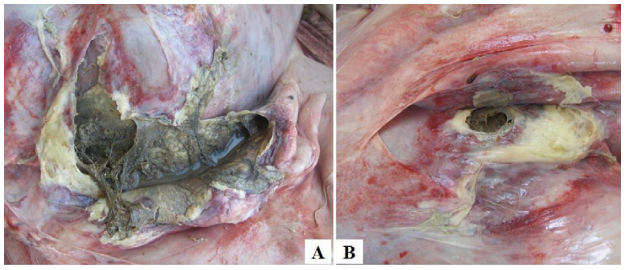
Studies with perforated ulcers in cattle have shown focal or omental adhesions, with necrosis, and abomasal content spread diffusely in the abdominal cavity or widely infiltrated in the omental fat, forming a pocket filled with fibrinonecrotic

Table 7. Comparative ultrasound and postmortem findings in cattle (n=7) with perforated abomasal ulcer.

Type of ulcer	Findings	
	Ultrasound	
Type 3	Cranioventral region of abdomen with reticulum and rumen displaced to the right side. Anechoic contents with echogenic fibrin filaments, with the presence of pockets in the cranioventral portions of the right abdomen.	Peritoneal fluid increased, cloudy and with fibrin. Multifocal adhesions of parietal peritoneum with organ surfaces, most evident on the right side. Abomasum with ovoid pocket, with a volume of approximately 3 liters.
Type 3	Reticle with irregular outline and displaced by 14 cm by structure measuring 10 x 10.4 cm, delimited by hyperechoic capsule, with hypoechoic content; in the center presence of more echogenic filaments. Between the reticulum and the abomasum, there is a heterogeneous and disorganized structure.	Focal adhesions of abdominal organs to the parietal peritoneum. Abomasum with extensive adhesions, mucosa with healed ulcer, and fistulous tract connecting the area of adhesion to the scar site.
Type 4	Increased, hypoechogenic peritoneal fluid with peritoneum 5.9 cm distant from the greater omentum. Reticulum displaced craniodorsally by 12 cm, with contractions. In the right latero-ventral region (intestinal recess) abomasum with heterogeneous echogenicity. Diffuse peritonitis with probable cause outside the omental recess.	Diffusely spread food content covering viscera and omentum. Dilated and compacted abomasum, with surface covered with necrotic fibrin debris. At the junction with the omasum an extensive perforated ulcer
Type 4	Slight increase in peritoneal fluid. It was not possible to visualize the reticulum because the abomasum was displaced to the right and dilated.	Peritoneal fluid increased translucent and with clots. The abomasum was pale, swollen, and distended.
Type 4	Reticle displaced dorsally 8.5 cm. Hyperechogenic, irregular and thick omentum. Presence of isolated liquid between the omentum and the abdominal wall, with echogenic filaments. Images suggest involvement of the right side indicative of peritonitis.	Increased, cloudy peritoneal fluid with free fibrin and the presence of a purulent exudate pocket between the diaphragm, liver, and abomasum. Abomasum with presence of type 4 ulcer, draining purulent secretion. Presence of diffuse peritonitis.
Type 4	Reticulum resting on the diaphragm, with smooth ventral contour, with four contractions in three minutes, inferior displacement of 5 cm. Images of the abdominal cavity are unclear as to evidence of peritonitis.	Digestive content in moderate amount in the cavity, fetid, dark and finding generalized serofibrinous peritonitis.
Type 5	Omentum displaced dorsally, with increased echogenicity and the presence of a moderate amount of anechoic content and echogenic filaments, forming images with a net aspect (fibrin). Intestine with little motility and increased echogenicity, it was not possible to visualize the abomasum.	Increased peritoneal fluid. Abomasum completely filled with fibrous material. Type 5 ulcer in the pyloric region, approximately 4 cm in diameter, forming a pocket of digestive contents and inflammatory exudate between the rumen and the greater omentum.

Table 8. Macroscopic findings observed in the abomasum of young (n=8) and adult (n=17) cattle with perforated ulcer and comorbidities.

Variable	Anatomopathological findings	n	Young cattle < 2 years	Adult cattle >2.5 years
Abdominal cavity findings	Focal peritonitis	10	4	6
	Diffuse peritonitis	13	4	9
	Omental bursitis	2	-	2
Findings of the content and mucosa of the abomasum	Geosediment	7	2	5
	Fiber compression	3	-	3
	Phytobezoar	1	1	-
	Mucosal fibrosis	22	8	14
	Edematous mucosa	18	6	12
	Non-perforated ulcers	19	7	12
	Hemorrhage in the mucosa	20	7	13
Topographic region of ulcer perforation	Perforation at the Bottom	5	1	4
	Perforation in the Body	17	5	12
	Perforation in the Pylorus	3	2	1



Source: author's collection.

Figure 1. Type 3 perforated ulcer in a 10-year-old cow. (A) Focal peritonitis between the fundus of the abomasum at the junction with the omasum. Presence of a fibrinonecrotic plug in the serosa of the abomasum in the perforation area. (B) Type 3 perforated ulcer covered with yellowish amorphous material.

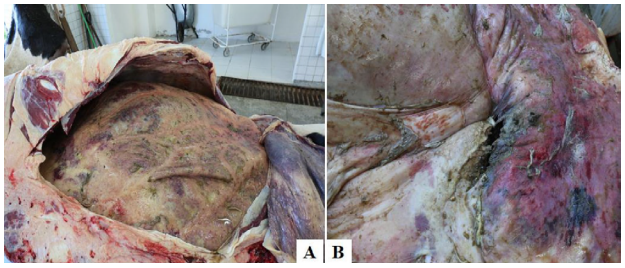


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Figure 2. Type 4 perforated ulcer in a seven-year-old cow. (A) Diffuse peritonitis, contents of the abomasum scattered, covering the viscera and omentum. (B) Abomasum with a surface covered with fibrinonecrotic debris and, at the junction with the omasum, an extensive type 4 ulcer proximal to the pylorus at the insertion of the omental recess.

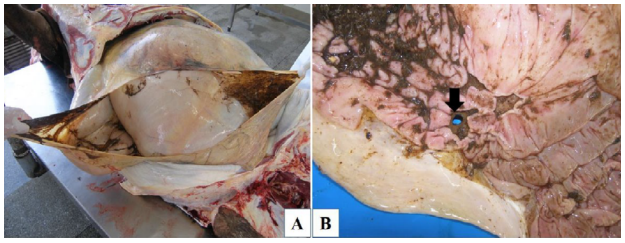
content, in cases of ulcer types 3, 4, and 5, respectively (Braun *et al.* 2019a, 2019b, 2020a).

In cases of perforation of the abomasal wall, in the left portion, the contents of the organ leak into the omental bursa, causing omental bursitis in cases of type 5 ulcer (Braun *et al.*, 2020a), corroborating with what was found in two animals diagnosed with this type of ulcer (Figure 3). Histopathologically, type 3 and 4 perforated ulcers were characterized by areas of extensive necrosis with fibrosis (Figure 4). Type 5 ulcers were characterized by extensive necrosis from the mucous layer to the serous layer with loss of glandular and vascular structure (Figure 5). The most common inflammatory changes found in the histopathology of perforated ulcers are shown in (Table 9).

According to Braun *et al.* 2019b and Hund; Wittek, 2017, fibrinonecrotic alterations of the organ with extensive inflammatory lesions, with fibrosis, affecting the mucosa, submucosa, muscle and serosa of the abomasum, provide direct evidence of perforated ulcers, corroborating the histopathological findings of this study.

CONCLUSION

Cattle affected by perforated abomasal ulcer, mostly presented associated comorbidities, with the digestive nature being the most frequent. The cases occurred more frequently in females, in the dry period, which received a diet based on roughage and concentrate. Ultrasonography, exploratory laparotomy, and abdominal centesis were useful to demonstrate the peritonitis resulting from the perforated ulcer, and to determine the prognosis of the affected animals. However, the anatomopathological findings were more consistent in establishing the definitive diagnosis of the type of perforated ulcer involved, and highlighting the inflammatory alterations that made the prognosis unfavorable, especially in animals affected by ulcer types 4 and 5. Therefore, a reliable diagnosis of perforated ulcers in cattle based only on physical examination is difficult, and it is almost always necessary for the veterinarian to use complementary tests, such as ultrasonography, abdominal centesis,



Source: author's collection.

Figure 3. Type 5 perforated ulcer in a 3-year-old male bovine. (A) Omental bursa filled with blackened, fibrin, necrotic content and amorphous material, adhered to the serosa of the abomasum, omasum, rumen, and reticulum (omental bursitis), note the absence of abomasal content outside the omental recess. (B) Presence of perforated ulcer (arrow) on the greater curvature in the region of the body of the abomasum.

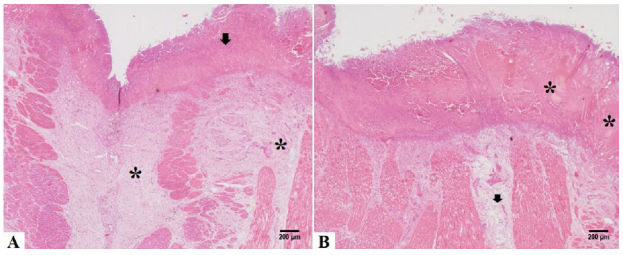


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Figure 4. Histopathology of perforated ulcers, 20x magnification, HE. (A) Type 3 ulcer, extensive necrosis of the superficial epithelium (arrow), with loss of epithelial vascular and glandular structure with extensive fibrosis (asterisks) in the submucosal and muscular layer of the abomasum. (B) Ulcer type 4, necrosis of the superficial epithelium with loss of epithelial and glandular structure, with formation of thrombi (asterisks) and fibrosis in the submucosal and muscular layer (arrow).

and exploratory laparotomy for decision making, thus avoiding more animal suffering and unnecessary treatment costs.

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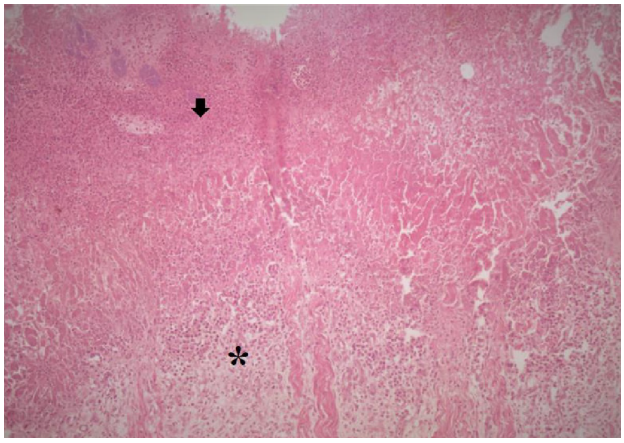

Source: author's collection.

Figure 5. Type 5 perforated ulcer, 20x magnification, HE. Presence of extensive necrosis of the mucosa (arrow), reaching the submucosal and muscular layers with loss of glandular and vascular structure, with the presence of lymphocytic and neutrophilic exudate (asterisks).

Table 9. Histopathological findings of perforated abomasal ulcers in young and adult cattle.

Inflammatory alterations	**Types of ulcers**			
Epithelium findings	**Type 3**	**Type 4**	**Type 5**	**n**
Fibrosis	10	10	2	22
Granulation tissue	8	10	1	19
Vasculitis	3	6	2	11
Thrombi in vessels	7	9	2	18
Abomasite	8	13	2	23
Inflammatory Response				
Lymphocytic	2	1	1	4
Neutrophilic	1	5	-	6
Lymphocytic and neutrophilic	4	2	-	6
Lymphocytic, neutrophilic and Histiocytic	2	2	-	4
lymphoplasmacytic	-	1	1	2
degenerated cells	1	2	-	3

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