DOI: 10.1590/1809-6891v24e-74772E

Ciência Animal Brasileira
Brazilian Animal Science

e-ISSN 1809-6891

Section: Veterinary medicine
Research article

Epidemiological and anatomopathological findings of type 1 and 2 abomasum ulcers in cattle with different comorbidities

Achados epidemiológicos e anatomopatológicos de úlceras do abomaso tipo 1 e 2 em bovinos com diferentes comorbidades primárias

Adony Querubino de Andrade Neto[1*], José Ricardo Barboza Silva[2], Carla Lopes de Mendonça[1], Rodolfo José Cavalcanti[1], Jobson Filipe De Paula Cajueiro[1], Maria Izabel de Souza[1], Lilian Rose Marques de Sá[3], José Augusto Bastos Afonso[1]

[1]Universidade Federal Rural De Pernambuco (UFRPE), Garanhuns, Pernambuco, Brazil
[2]Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil
[3]Universidade de São Paulo (USP), São Paulo, São Paulo, Brazil
[*]Corresponding author: adony_neto@hotmail.com

Abstract
This study aimed to investigate the epidemiological and pathological findings of type 1 and 2 abomasal ulcers in cattle with different primary comorbidities. A total of 201 animals; 40/201 (20%) were young cattle under the age of two years and 161/201 (80%) were adult cattle over the age of two years, which were hospitalized for clinical care 152/201 (75,62%), 19/201 (9,45%) obstetric care, 17/201 (8,46%) surgical care and 13/201 (6,47%) for anatomopathological diagnosis, being euthanized or had natural death. The diagnosis of ulcers was based on the result of the *post-mortem* examination (macroscopic and histopathological analysis). Histopathological examination was performed on 201 fragments of ulcers and classified as type 1 or type 2. Of these, 193/201 (96.01%) corresponded to type 1 ulcers, of which 12/193 (5.97%) corresponded to subtype 1a lesions, 101/193 (50.25%) to subtype 1b, 77/ 193 (38.31%) to subtype 1c, 03/193 (1.49%) to subtype 1d, while 08/201 (3.98%) were type 2 ulcers. The ulcers were characterized by a focal, focally extensive, multifocal or diffuse inflammatory process, mainly by mononuclear cells. Abomasitis associated with ulcerated mucosa was found in 160/201 (79.60%). In 26/201 (12.93%) the abomasitis had diffuse foci of multifocal lymphocytic proliferation by atypical lymphocytes. Digestive and reproductive comorbidities were seen more frequently in cattle with type 1 or type 2 ulcers. The Subtype 1b focal ulcers and subtype 1a and 1b multifocal ulcers were more prevalent. In addition to the presence of comorbidities, most cases occur in the dry period, associated with feeding with higher amounts of concentrates and silages.
Key-words: dairy cattle; diseases of the abomasum; melena; ulcer; histopathology.

Resumo
Objetivou-se estudar os achados epidemiológico e anatomopatológico de úlceras do abomaso tipo 1 e 2 em bovinos com diferentes comorbidades primárias. Um total de 201 animais; 40/201 (20%) eram bovinos jovens com idade inferior a dois anos e 161/201 (80%) eram bovinos adultos com idade superior a dois anos, os quais foram internados para atendimento clínico 152/201 (75,62%), 19/201 (9,45%) obstétrico, 17/201 (8,46%) para atendimento clínico-cirúrgico e 13/201 (6,47%) para diagnóstico anatomopatológico, sendo eutanasiados ou tiveram morte natural. O diagnóstico das úlceras foi baseado no exame *post-mortem* (análise macroscópica e histopatológica). O exame histopatológico foi realizado em 201 fragmentos de úlceras e classificado como tipo 1 ou do tipo 2. Destes, 193/201 (96,01%) corresponderam a úlceras tipo 1, das quais, 12/193 (5,97%) corresponderam a lesões subtipo 1a, 101/193 (50,25%) a subtipo 1b, 77/193 (38,31%) a subtipo 1c, 03/193 (1,49%) ao subtipo 1d, enquanto 08/201 (3,98%) foram úlceras tipo 2. As úlceras foram caracterizadas por processo inflamatório focal, focalmente extenso, multifocais ou difusos, principalmente por células mononucleares. Abomasite associada à mucosa ulcerada foi encontrada em 160/201 (79,60%). Em 26/201 (12,93%) a abomasite apresentava focos difusos de proliferação linfocítica multifocal por linfócitos atípicos. As comorbidades digestivas e reprodutivas foram observadas com maior frequência em bovinos com úlceras tipo 1 ou tipo 2. As úlceras focais subtipo 1b e úlceras multifocais subtipo 1a e 1b foram mais prevalentes. Além da presença de comorbidades, a maioria dos casos ocorrerem no período seco, associados à alimentação com maiores aportes de concentrados e silagens.
Palavras Chave: bovinos leiteiros; doenças do abomaso; melena; úlcera; histopatologia.

1. Introduction

Abomasal ulcers are important causes of pain and consequent loss of animal productivity, being commonly found in cattle of all breeds, ages, and in all production systems. They have a multifactorial etiology, however, the causes are still little known, which suggests that they occur as a result of the imbalance between aggressive and protective factors of the abomasal mucosa [(1)]. In dairy cows, ulcers are more frequent shortly before calving and may be related to increased plasma cortisol, or in the first

Received: December 14, 2022. Accepted: March 8, 2023. Published: April 19, 2023.

4 to 6 weeks of lactation due to intensive feeding, and changing diets with high energy density feeds. Most cows with ulcer of the abomasum have primary disease, usually related to the transition period, due to stress or puerperal diseases such as retained fetal membranes, metritis, mastitis, ketosis, hypocalcemia, or displacement of the abomasum [2].

In males, ulcers occur particularly after long transport, prolonged surgical procedures, painful clinical conditions, or during confinement related to dietary management and stress [3]. In calves, ulcers or erosions of the abomasum are associated with age, climate, rearing system, feeding, mineral deficiencies, stress, and proliferation of microorganisms such as; *Aspergillus fumigatus*, *Mucor* spp., *Clostridium perfringens,* and *Campylobacter* spp. In addition to these, abrasion of the abomasal mucosa by sediment, phytobezoars, and comorbidities may be involved [4, 5].

From a clinical and anatomopathological point of view, abomasal ulcers were classified by Whitlock [6], modified by Smith et al. [7] into non-perforated and perforated ulcers. The former are classified as type 1, when the degree of erosion and hemorrhage is minimal and clinical signs are discrete and barely perceptible, or type 2, characterized by intense intraluminal hemorrhage, attributed to lesion of the left or right branches of the gastroepiploic artery in the submucosa, with obvious clinical signs of apathy, anorexia, melena, and pale mucous membranes. In the *post-mortem* anatomopathological examination, large clots are visualized in the lumen of the abomasum or mixed with ingesta, the contents of the organ are usually dark or blackish, and the abomasum may be distended by a large amount of blood [8, 9, 10].

Perforated ulcers are classified into; type 3, when the peritonitis is focally restricted to the ulcerated region, because the omental sheath and the adhesions formed with the adjacent peritoneum stop the leakage of the contents of the abomasum [11] , type 4 ulcer, when perforation usually occurs in the right wall, and organ contents rapidly spill into the peritoneal cavity, resulting in diffuse peritonitis [12], and type 5 ulcer, 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 [13]. For the classification of type 1 ulcers, Braun et al. [14] added 4 subtypes, based on macroscopic appearance, classifying them into subtypes 1a, 1b, 1c, and 1d. The prevalence of type 1 ulcers varies according to the epidemiological characteristics and the different diagnostic methods used in the examined population, reaching 65.9% in confined males, 20.5% to 84% in slaughtered cows, and 59.3% in calves. The prevalence of type 2 ulcers can range from 2.17% to 8.8% [8,15].

The clinical diagnosis of type 1 abomasal ulcers is difficult and in most cases can only be made at slaughter, necropsy, or during abomasotomy [16, 17]. In cattle affected by type 2 ulcers, the diagnosis is based mainly on clinical signs, associated with increased serum activity of pepsinogen and plasma gastrin, which has diagnostic value in hemorrhagic ulcers [18, 19]. The fecal occult blood test is another resource indicated to aid in the diagnosis when there is the suspicion of ulcer in cattle without melena, however, the test results present low sensitivity [20, 21]. of 80% and is crucial for distinguishing cows with type 2 ulcers from healthy cows or cows with other types of abomasal ulcers. In addition, hematocrit determination is indicated to assess the severity of anemia from blood loss in cows with melena [10].

The majority of ulcers are diagnosed at slaughter or at necropsy, as an incidental anatomopathological finding or as a cause of death. In the macroscopic examination of the abomasum, it is common to find multiple ulcers, with acute, chronic, or already healed evolution, and with more than one classification [5,22, 23, 24]. Few studies have addressed the relevance of type 1 abomasal ulcers in cattle, especially when associated with primary comorbidities [17, 21, 23, 24, 25,]. In Brazil, studies related to the occurrence of type 1 and 2 abomasal ulcers are scarce, mainly regarding the anatomopathological characteristics of these ulcers in animals with comorbidities. Given the clinical, scientific, and economic importance of these conditions in cattle, the current work aims to present the epidemiological findings and the anatomopathological characteristics of type 1 and type 2 abomasal ulcers in cattle with primary comorbidities.

2. Material and methods

2.1 Research location and animals

The work was developed 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 Animal Science of the University of São Paulo (FMVZ-USP). In total, 201 animals were used, with several primary disorders, of which; 40/201 (20%) were young animals less than two years old, 6/40 (15%) less than two months old and 34/40 (85%) more than two months old, and 161/201 (80 %) were animals older than two and a half years (considered adults for having given birth at least once), 152/201 (75.62%) animals were hospitalized for clinical care, 19/201 (9.45%) for obstetric care, 17/201 (8.46%) for clinical-surgical care, and 13/201 (6.47%) for anatomopathological diagnosis (dead on arrival at the institution). One hundred and four animals (55.31%) were euthanized with the owner's authorization following the euthanasia guidelines

recommended by Luna & Teixeira [26], and 84 (44.69%) died during treatment. The decision for euthanasia was taken based on the prognosis of the primary clinical disease, when the cost of treatment was unfeasible for clinical resolution, or when the response to treatment was ineffective, or even if during the surgical or obstetric procedure the treatment was found to be unfeasible due to peritonitis and established chronic infections. The diagnosis of type 1 and 2 ulcers was based on the results of the post-mortem examination.

2.2 Macroscopic and histopathological evaluation

The abomasum was separated from the stomach during necropsy, opened along the greater curvature, and its contents were evaluated for macroscopic aspects such as the presence of blood, edema, clots, geosediments, foreign bodies, and fibers, then immersed in water to remove them. Macroscopic examination of the abomasum was performed in 103 samples, in which the ulcers were analyzed regarding the degree of penetration into the mucosa, bleeding and topographical distribution by the affected region, cardiac, fundic, and pyloric, and then the photographic records were made of the abomasal mucosa. A single fragment was collected representing the ulcerated mucosa with a portion of the healthy mucosa, at least one type 1 or 2 ulcer, and for each abomasum only one type of ulcer was collected for histopathological analysis. For preparation, the ulcers were fixed in 10% buffered formalin, processed using a routine protocol and stained with hematoxylin-eosin (HE). Histopathological examination was performed on 201 ulcer fragments corresponding to each abomasum with a type 1 (n=193) or type 2 (n=8) ulcer. The macroscopic and histopathological evaluation of the ulcers was performed following the classification criteria described by Whitlock [6] and modified by Smith et al. [7], for type 1 and 2 ulcers. For type 1 ulcers, the classification was performed based on the subtypes according to the classification adopted by Braun et al. [14] (Chart 1).

Chart 1. Classification criteria for type 1 and 2 ulcers.

Type of ulcer	Macroscopic description	Histopathological description
Subtype 1a	Minimal defects and loss of mucosal wrinkles, mucosal discoloration (usually reddish).	Acute to subacute necrosis of the superficial mucosal epithelium with minimal damage to the mucosal cells, suggesting lack of mucus production.
Subtype 1b	Deeper lesions with discrete mucosal hemorrhage, demarcated by a depressed center.	Acute necrosis of the mucosal epithelium, often accompanied by discrete hemorrhage. Necrosis sometimes extends almost to the muscularis mucosa.
Subtype 1c	Craters lined with inflammatory debris or fibrin, depressed center, and raised and recessed margins.	Typical chronic ulcers with local destruction of all mucosal layers including submucosa and muscularis. Mucosa is replaced by necrotic debris, fibrosis, and granulation tissue.
Subtype 1d	Radial wrinkles with a central scarring point, affecting only gastric folds or fully perforated gastric folds.	Reduced number of tubular glands and with regenerated adenocytes. Marked increase in connective tissue, glandular portions are separated consisting almost exclusively of mucus produced by adventitial cells.
Ulcer type 2	Hemorrhagic ulcer with mucosal necrosis and penetration of a larger abomasal vessel and severe intraluminal hemorrhage, with formation of large clots and blackened contents.	Acute necrosis of the mucosa up to the submucosa region, with infiltration of inflammatory cells, fibrin and a lot of hemorrhage in the mucosa and submucosa, necrosis of the major vessels of the submucosa.

Source: Whitlock [6] and modified by Smith et al. [7]; Braun et al. [14].

2.3 Epidemiological information

Epidemiological risk information such as; sex, breed, age, rearing system, feeding, time of year, lactation stage and number of births was collected from the clinical records of the hospitalized animals.

2.4 Statistical analysis and ethics committee

Statistical analysis was performed descriptively and absolute and relative frequencies were determined for each variable (epidemiological and anatomopathological). The study was approved by the Ethics Committee on the use of animals of the Federal Rural University of Pernambuco, CEUA/UFRPE, under protocol No. 23082.017161/2017-21.

3. Results

3.1 Primary comorbidities

The main primary diseases diagnosed were related to the **digestive system**: 57/201 (28%); of these, 27/57 (47%) corresponded to traumatic reticulum peritonitis and sequelae. To the **reproductive system**: 37/201 (18%); of these, 18/37 (48%) corresponded to maternal-fetal dystocia. To the **respiratory system**:

25/201 (12%); of these, 19/25 (76%) corresponded to pneumonia. To the **nervous system**: 21/201 (10.44%); of these, 7/21 (33%) corresponded to malignant catarrhal fever. To the **musculoskeletal system**: 18/201 (9%); of these 4/18 (22%) corresponded to trauma. To the **hematopoietic system**: 17/201 (8%); of these, 8/17 (47%) corresponded to cases of Anaplasmosis. **Inconclusive cases**: 14/201 (7%). **Neoplasms:** 6/201 (3%); of these, 3/6 (50%) corresponded to lymphosarcoma (enzootic leukosis). To the **renal system**: 3/201 (1.5%); of these, 2/3 (66%) corresponded to cases of nephritis; To the **circulatory system**: 2/201 (1%); Of these, 1/2 (50%) corresponded to vena cava thrombosis. **Peritoneal cavity**: 1/201 (0.5%); corresponding to a **case of peritonitis** (Chart 2).

3.2 Epidemiological factors

The occurrence of ulcers according to sex, in relation to the age group of bovines with comorbidities, was higher in females for both young and adult animals. The predominant rearing system was semi-intensive for both young animals and adult animals and the predominant diet was based on forage and concentrates. The voluminous feed was based on corn silage, forage cactus (*Opuntia ficus indica*), elephant grass (*Pennisetum purpureum*), or sugarcane (*Saccharum officinarum)*. The concentrates consumed were based on corn, wheat, cotton, or soy. The predominant time of year was the dry season, corresponding to the months of October to March, both for young and adult animals. Of the adult animals, 106/150 (69%) were lactating, with an average of seventy days postpartum, and 81/108 (53%) of the animals were multiparous with two to ten deliveries (Table 1).

3.3 Macroscopic and histopathological evaluation

In the macroscopic evaluation, the presence of geosediment was observed in 08/103 (7.76%) of the examined abomasa, in addition to compaction by fibers in 03/103 (3%) of the abomasa, metallic foreign bodies in 04/103 (3.88%), phytobezoars in 02/103 (1.94%), lymphosarcoma in 03/103 (2.91%), swollen mucosa in 72/103 (69.90%), hemorrhagic content in 10/103 (9%), in which there was the formation of small and large clots in the lumen in six, and the presence of discrete hemorrhage in the ulcer bed in 52/103 (50%). As for the distribution of the ulcers, they were single or multiple and round, oval, or irregular in appearance. Twenty-two abomasum contained less than five ulcers, 45/103 (43%) abomasum contained between five and twenty ulcers, and 36/103 (35%) abomasum contained more than twenty ulcers. Most ulcers were located in both body and pyloric regions, both in young animals and in adult animals (Table 2).

Chart 2. Relative and absolute frequency of primary comorbidities diagnosed in 201 cattle, young (or aged < 2 years) and adult (or aged > 2 ½ years), associated with abomasal ulcer types 1 and 2.

Primary Comorbidities	No. od cases	%
Digestive System	57	28%
Traumatic reticuloperitonitis and sequelae	27	47%
Enteritis	7	12%
Intestinal Obstructions by phytobezoars	6	11%
Ruminal acidosis	4	7%
Compression of the stomach	3	5%
Displacement of the abomasum	3	5%
Vagal indigestion	2	4%
Intussusception	2	4%
Pyogranulomatous hepatitis	2	4%
Dilation of the abomasum	1	2%
Esophageal obstruction	1	2%
Reproductive System	37	18%
Maternal-fetal dystocia	18	49%
Mastitis	11	30%
Uterine rupture	4	11%
Dropsy	2	5%
metritis	1	3%
Uterine prolapse	1	3%
Respiratory System	25	12%
Pneumonia	19	76%
Tuberculosis	4	16%
Dictyocaulus	1	4%
Acute pulmonary emphysema	1	4%
Nervous System	21	10%
Malignant catarrhal fever	7	33%
Rabies	5	24%
Botulism	3	14%
Spinal cord trauma	3	14%
Herpetic encephalitis	1	5%
Radial nerve palsy	1	5%
Electric accident	1	5%
Musculoskeletal System	18	9%
Trauma	4	22%
Deficiency of selenium and vitamin E	2	11%
Downer cow syndrome	2	11%
Umbilical and perineal hernias	2	11%
Fractures	2	11%
Malnutrition	1	6%
Scapular abscess	1	6%
Gangrenous panniculitis	1	6%
Coxofemoral dislocation	1	6%
Enzootic calcinosis	1	6%
Omphalophlebitis	1	6%
Hematopoietic System	17	8%
Anaplasmosis	8	47%
Babesiosis	6	35%
Parasitic sadness	2	12%
Trypanosomiasis	1	6%
Inconclusive	14	7%
Neoplasms	6	3%
Enzootic leukosis	3	50%
Squamous cell carcinoma (base of horn)	1	17%
Cardiac	1	17%
Pituitary gland	1	17%
Renal System	3	1.5%
Nephritis	2	67%
Urolithiasis	1	33%
Circulatory System	2	1%
Vena cava thrombosis	1	50%
Endocarditis	1	50%
Peritoneal Cavity	1	0.5%
Peritonitis	1	100%
Total	**201**	**100%**

Table 1. Epidemiological factors of young and adult cattle with comorbidities with type 1 and type 2 abomasal ulcers.

Epidemiological Factors	(n)	Variables	Young cattle ≤ 2 years (n=40)	Adult cattle ≥ 2.5 years (n=161)
Sex	201	Female	28 (70%)	153 (95%)
		Male	12 (30%)	8 (5%)
Breed	201	Mixed-breed[1]	25 (63%)	99 (61%)
		Pure-breed[2]	15 (37%)	62 (39%)
Rearing System	196	Semi-intensive	15 (37%)	84 (52%)
		Intensive	13 (33%)	45 (28%)
		Extensive	12 (30%)	27 (17%)
Feeding	191	Volumoso[3]	9 (22%)	27 (17%)
		Voluminous and concentrated [4]	18 (45%)	129 (80%)
		Milk	8 (20%)	-
Season of year	201	Dry season[5]	25 (62%)	84 (52%)
		Rainy season[6]	15 (38%)	77 (48%)
Lactation stage	150	Lactating cows	-	106 (69%)
		Dry cows	-	44 (29%)
Number of births	108	Primiparous	-	27 (17%)
		Multiparous	-	81 (53%)

[1]Mixed Dutch, Jersey, Gir and Guzerat; [2] Dutch, Swiss Brown, Nelore, Jersey, Guzerat, Gir, Sindhi and Charolais.[3] Corn silage, forage palm (*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. Macroscopic findings of the abomasum of young and adult cattle with comorbidities with type 1 and type 2 abomasal ulcers.

Parameters	(n)	Macroscopic Findings	Young Cattle < 2 years (n=20)	Adult Cattle >2.5 years (n=83)
Content Findings	10	Hemorrhagic content [1]	2 (10%)	8 (9%)
	8	Geosediment	-	8 (9%)
	6	Blood clots [2]	1 (5%)	5 (6%)
	4	Metallic foreign bodies	1 (5%)	3 (3%)
	3	Fiber compression	1 (5%)	2 (2%)
	2	Phytobezoars	-	2 (2%)
Mucosal findings	72	Edematous mucosa	15 (75%)	57 (68%)
	52	Discrete bleeding [3]	11 (55%)	41 (49%)
	3	Tumor nodules	-	3 (3%)
Distribution of ulcers	48	Focal	12 (60%)	36 (43%)
	55	Multifocal	8 (40%)	47 (57%)
Number of ulcers	22	< 5 ulcers	2 (10%)	20 (24%)
	45	Between 5 and 20 ulcers	10 (50%)	35 (42%)
	36	> 20 ulcers	8 (40%)	28 (33%)
Topography of ulcers	1	Fundus	-	1
	29	Body	7 (35%)	22 (26%)
	25	Pylorus	4 (20%)	21 (25%)
	1	Fundus and body	-	1 (1%)
	2	Fundus and pylorus	-	2 (2%)
	39	Body and pylorus	8 (40%)	31 (37%)
	6	Fundus, body, and pylorus	1 (5%)	5 (6%)

[1] Content with macroscopic evidence of blood. [2]Presence of large and small blood clots in the abomasal content. [3]Discrete bleeding into the ulcer bed.

The frequency of abomasa with type 1 ulcers and type 2 ulcers, single or multiple, evaluated in young and adult cattle with comorbidities (Table 3) demonstrated that single ulcers of subtype **1b** and multiple ulcers of subtypes **1a** and **1b**, respectively, were the most commonly found.

Subtype **1a** ulcers were distributed over the body and pyloric region, however most were present in the pyloric region, and were characterized by superficial reddish lesions. Those of subtype **1b** were distributed in the fundus, body, and pylorus, with the majority distributed throughout the body and were characterized by depressed centers with hemorrhage, affecting the surfaces of the folds in the fundus, body, or the pyloric mucosa. Subtype **1**c ulcers were mostly present in the pyloric region, however they were also seen in the body and fundus of the abomasum. They had a cratered appearance, with depressed centers and retracted edges, with inflammatory debris and fibrin. Subtype **1d** ulcers were located in the body folds and were characterized by circular perforation areas in the folds, with hemorrhagic and retracted edges (Figure 1).

Table 3. Relative and absolute frequency of abomasa (n=103) with type 1 and 2 ulcers in young and adult cattle with comorbidities.

Distribution of Ulcers	No. of abomasa	Types of ulcers	Young cattle < 2 years (n=20)	Adult cattle >2.5 years (n=83)
Focal n= 48	6	Subtype 1a	3 (15%)	3 (4%)
	32	Subtype 1b	8 (40%)	24 (29%)
	5	Subtype 1c	1 (5%)	4 (5%)
	2	Subtype 1d	-	2 (2%
	3	Type 2	-	3 (4%)
Multifocal n= 55	22	Subtypes 1a and 1b	3 (15%)	19 (23%)
	1	Subtypes 1a and 1c	-	1 (1%)
	17	Subtypes 1b and 1c	2 (10%)	15 (18%)
	1	Subtype 1b and Type 2	-	1 (1%)
	10	Subtypes 1a, 1b, and 1c	2 (10%)	8 (10%)
	1	Subtypes 1a, 1b, and 1d	-	1 (1%)
	1	Subtypes 1a, 1b and Type 2	1 (5%)	-
	2	Subtypes 1b, 1c, and 1d	-	2 (2%)

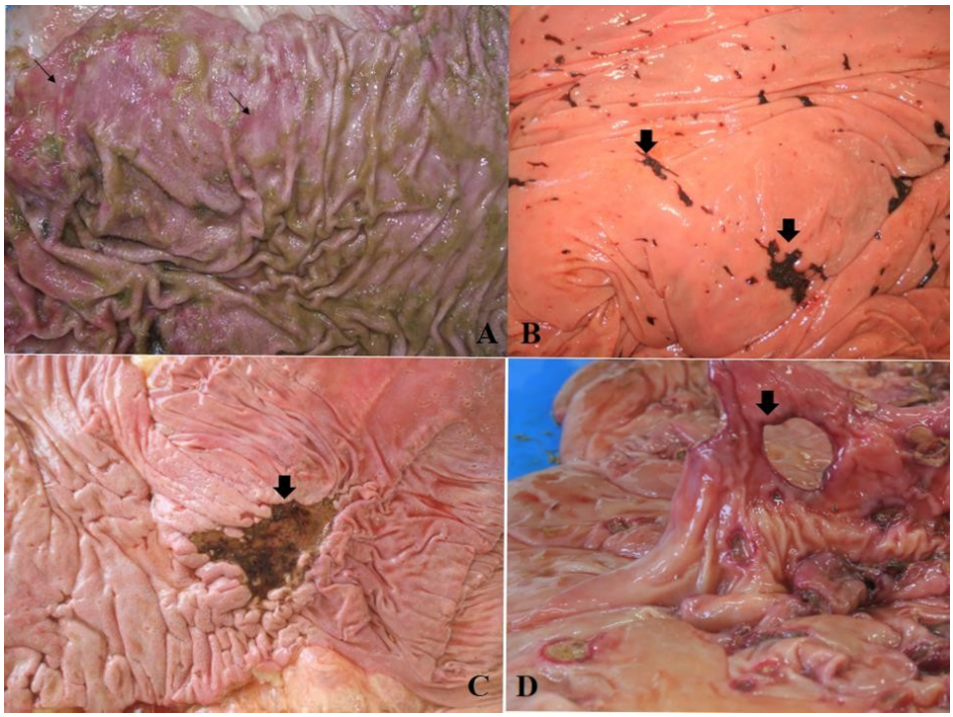

Figure.1. Macroscopy of type 1 ulcers. (A) Areas of subtype 1a erosions (arrows), reddish regions in the pyloric region. (B) Subtype 1b erosions (arrows), areas of deeper mucosal hemorrhage demarcated by a depressed center in the body of the abomasum. (C) Ulcer subtype 1c, chronic craters (arrow), with lining of inflammatory debris, deep center, raised and retracted margins with formation of fibrosis and granulation tissue. (D) Gastric fold of the body of the abomasum fully perforated in ulcer 1d (arrow).

Type 2 ulcers were mostly located in the body of the abomasum, although they were also seen in the pyloric region. They were characterized by deep hemorrhagic centers, protruding and irregular margins, the bed of the ulcer had adhered clots and fibrin, it was not possible to visualize branches of the gastroepiploic artery in the bed of the ulcer and the content of the abomasum had large clots of dark red or blackish color. Histopathologically, **type 2** ulcers were medium to deep, characterized by moderate to intense lymphocytic and neutrophilic infiltrate, with intense hemorrhage in the mucosa and submucosa, with the presence of fibrin and vascular alterations in the submucosa (Figure 2).

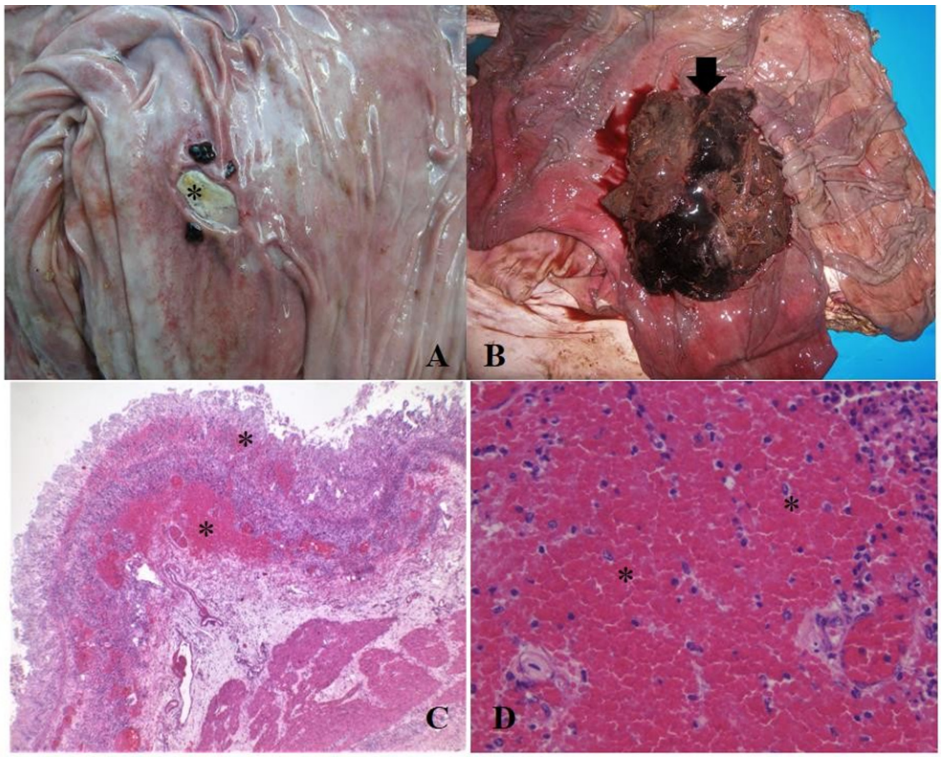

Figure 2. Macroscopy and microscopy of type 2 ulcers. (A) Deep ulcer in the body of the abomasum, with the presence of clots and fibrin in the ulcer bed (asterisk) (B) Lumen of the abomasum with formation of a large blood clot (arrow) as a result of a type 2 ulcer. (C) Extensive mucosal necrosis reaching the submucosa of the abomasum with intense hemorrhage (asterisk), 20x magnification, HE. (D) Submucosa of the abomasum with intense hemorrhage and inflammatory cells (asterisks), 40x magnification, HE.

In the histopathological examination, 201 fragments of ulcers were analyzed, 193 corresponded to type 1 ulcers, and of these 12/193 (5.97%) corresponded to subtype **1a** lesions, 101/193 (50.25%) to subtype **1b**, 77/193 (38.31%) subtype **1c**, and 3/193 (1.49%) subtype **1d**. **Type 2** ulcers were seen in 8/201 (3.98%). Subtype **1a** erosions were characterized by superficial necrosis of mucous cells, with discreet superficial and inflammatory alterations, sometimes the only evidence was loss of superficial mucus-producing cells. Subtype **1b** ulcers were characterized by acute superficial necrosis that extended to the muscularis mucosa due to expansion of connective tissue and necrosis of the lamina propria of the mucosa, with focal lymphocytic infiltrate and discreet hemorrhage. Subtype **1c** ulcers were characterized by chronic necrosis extending into the submucosa and muscle layer, forming three focally extensive inflammatory zones, with the ulcer bed represented by inflammatory cells and necrosis, a zone of granulation tissue below, followed by an area of dense connective tissue or fibrosis and vascular alterations in the submucosa. Subtype **1d** ulcers were characterized by superficial or medium lesions with necrosis and atrophy of the gastric glands and a mild inflammatory infiltrate in the submucosa (Figure 3).

Other alterations found in the ulcerated mucosa were alterations in the gastric glands in 182/201 (90.54%) of the cases, of which 46.77% presented glandular dilatation, 20.40% presented glandular atrophy, 11.94% presented hypertrophy, 9.45% presented gastric gland hyperplasia, and 1.99% presented glandular metaplasia. Abomasitis was found in 160/201 (79.60%), with lymphoid follicle hyperplasia in 104/201 (51.74%). In 26/201 (12.93%) the abomasitis showed diffuse foci of multifocal lymphocytic proliferation by atypical lymphocytes, with loss of the glandular structure of the mucosa**.** Foreign bodies of plant origin in the mucosa or submucosa were observed in 62/201 (30.85%). Fungal hyphae were found colonizing mucosal or submucosal vessels in 19/201 (9.45%) of the cases. Colonies of bacteria in the ulcer bed or foveolar epithelium were seen in 77/201 (38.50%) of the cases. The inflammatory response of each type of ulcer and of the abomasal mucosa is shown in Table 4.

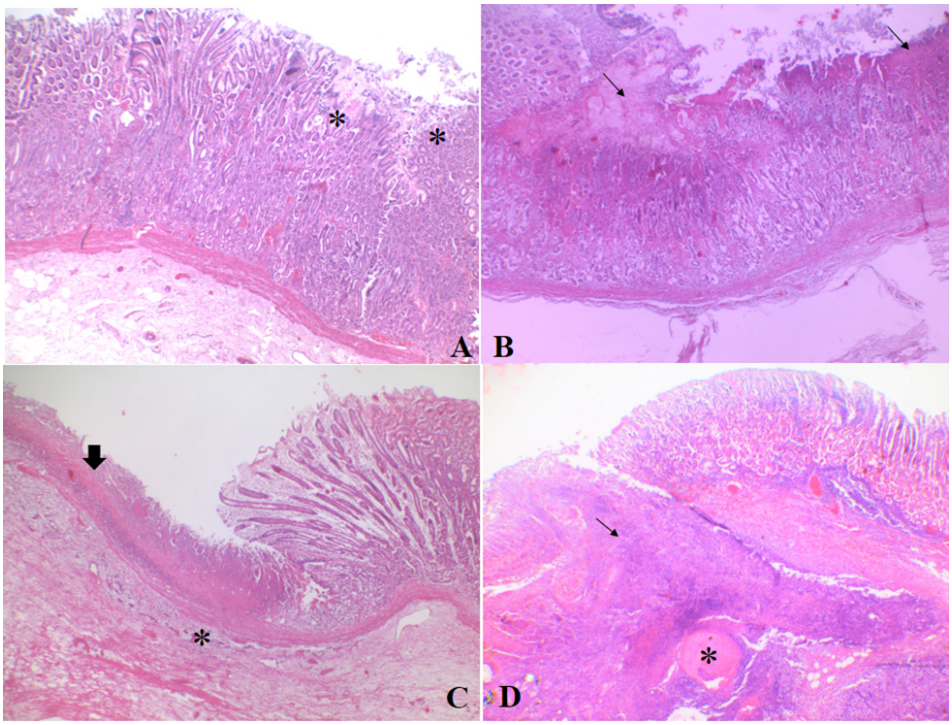

Figure 3. Microscopy of type 1 ulcers, staining (HE). (A) Areas of superficial mucosal erosions (asterisks), with loss of superficial mucus-producing cells in subtype 1a erosion, 20x magnification. (B) Areas of superficial necrosis in the mucosa with hemorrhage and connective tissue expansion in the lamina propria (arrows), in subtype 1b erosion, 20x magnification. (C) Extensive mucosal necrosis reaching the submucosa of the abomasum with fibrin and inflammatory cells in the ulcer bed (arrow), submucosa with granulation tissue (asterisk), in subtype 1c ulcer, 20x magnification. (D) Necrosis from the mucosa to the submucosa (arrow) with formation of granulation tissue, fibrosis and thrombi in vessels in the submucosa (asterisk) in subtype 1d ulcer, 20x magnification.

4. Discussion

In the current work, it was not possible to correlate a specific type of disease as the cause of type 1 or 2 ulcers. What could be inferred regarding comorbidities is that many diseases promote the release of pro-inflammatory factors, since endogenous and exogenous stress are associated with increased secretion of histamine, cortisol, hydrochloric acid, pepsin, and the decrease in the release of prostaglandin-E (PGE2), thus causing an imbalance of protective factors in the mucosa, linked to the pathogenesis of ulcers [25]. In the present study, 28% of the comorbidities were related to the digestive tract, with 47% of them being traumatic reticulum peritonitis and sequelae, 12% enteritis, and 11% cases of intestinal obstruction by phytobezoar, which leads to compromised transit as a result of adhesions, atony of the organs, obstructions, and direct damage to the abomasal mucosa, factors that predispose the prolonged action of hydrochloric acid and pepsin in the abomasal mucosa [27]. Braun et al. [17] found the displacement of the abomasum, vagal indigestion, and hepatic steatosis as the main digestive comorbidities in cattle related to type 1 ulcers. These diseases are more frequent in Europe in relation to the region of the present study, where diseases are often consequences of feed seasonality.

Abomasal ulcers are the most important and frequent cause of gastrointestinal bleeding in cattle, however their diagnosis is rarely performed when there is no evidence of melena on physical examination, as seen in cases of type 2 ulcers, which often differentiates them clinically from type 1 ulcers, [28]. Type 1 ulcers, although very frequent in post-mortem examinations, present nonspecific clinical signs, as even deep ulcers may remain unapparent until they are perforated and lead to peritonitis. They are usually secondary, resulting from stress or associated with comorbidities, as seen in the current study [17].

In this work, 20% of the animals were young, aged less than two years, and of these 15% were aged less than two months, while 80% were adults over two and a half years, the majority were females and were reared semi-intensively. According to Hund & Wittek [25] ulcers occur in cattle of all ages, sexes, breeds, and rearing systems. According to Jelinski et al. (29) the age distribution among calves with ulcers can be divided into two groups: calves in the pre-rumination stage (<3 weeks) and calves

Table 4. Histopathological characteristics of abomasal ulcers types 1 and 2.

Histopathological Parameters	Types of ulcers				
	Ulcer type 1 (n=193)			Ulcer type 2 (n= 8)	
	Subtype1a	Subtype 1b	Subtype 1c	Subtype 1d	Type 2
Ulcer depth					
Mucosa (superficial)	12 (100%)	93 (92%)	-	2 (67%)	-
Mucosa and submucosa (medium)	-	8 (8%)	38 (49%)	1 (33%)	5 (62%)
Mucosa to muscle (deep)	-	-	39 (50%)	-	3 (38%)
Type of Inflammatory Infiltrate					
Absent	9 (75%)	15 (15%)	-	-	-
Lymphocytic	2(17%)	39 (39%)	13 (17%)	1 (33%)	1 (12%)
Neutrophilic	-	11 (11%)	16 (21%)	-	2 (25%)
Plasmacytic	-	1 (1%)	1 (1%)	-	-
Lymphocytic and neutrophilic	-	16 (16%)	14 (18%)	1 (33%)	3 (38%)
Lymphocytic and granulomatous	-	-	4 (5%)	-	1 (13%)
Lymphocytic, neutrophilic and histiocytic	-	4 (4%)	18 (23%)	-	1 (12%)
Lymphoplasmacytic	-	14 (14%)	8 (10%)	1 (33%)	-
Degenerated cells	1 (8%)	1 (1%)	3 (4%)	-	-
Distribution of the inflammatory infiltrate					
Absent	9 (75%)	15 (14%)	-	-	-
Focal	1 (8%)	50 (49%)	13 (17%)	1 (33%)	2 (25%)
Focally extensive	-	8 (8%)	44 (57%)	1 (33%)	2 (25%)
Multifocal	2 (17%)	22 (22%)	9 (12%)	1 (33%)	3 (37%)
Diffuse	-	6 (6%)	11 (14%)	-	1 (13%)
Degree of inflammatory infiltrate					
Absent	9 (75%)	15 (15%)	-	-	-
Discreet	2 (17%)	49 (48%)	15 (19%)	2 (67%)	1 (12%)
Moderate	1 (8%)	35 (35%)	41 (53%)	-	4 (50%)
Intense	-	2 (2%)	21 (27%)	1 (33%)	3 (38%)

Depth of the ulcers: Superficial: lesion restricted to the mucosa; Medium: lesion reaches the submucosa; Deep; injury reaches the deep muscle layer. Type of inflammatory infiltrate: predominant cell type in the ulcerated and mucosal region. Distribution of the inflammatory infiltrate: Focal: restricted to ulcer; Focally extensive: inflammatory infiltrate along the mucosa and submucosa from the ulcer. Multifocal: in addition to the ulcer there are foci of inflammatory cells in the mucosa. Diffuse: all mucosa with inflammatory infiltrate. Degree of inflammatory infiltrate; mild (few cells in the ulcer and mucosa), moderate (more than one inflammatory focus in the ulcer and mucosa), intense (ulcer or mucosa region with many inflammatory foci).

in the transition phase (3 to 8 weeks) when the animal is more susceptible to the formation of ulcers, after this period there is a reduction in cases. However, in the present study, a greater occurrence was observed in young animals aged over two months. According to Akbari et al. [30] there is no significant difference between age and type 1 abomasal ulcers in cattle, but a significant relationship was found between sex and abomasal ulcers in slaughtered healthy cattle, with the chance of having an abomasal ulcer in females being 2.26 times greater than in males. Despite the results found in this work having a higher occurrence in females, it is difficult to state that sex is the risk factor, since these animals were submitted to different management practices, were from different regions, and the clinical casuistry must be considered, as the region of the study was a dairy basin, where the population of females is higher than that of males. However, Hund et al. [15], examining healthy cattle, found a significantly higher occurrence of type 1 ulcers when compared with cattle from farms where the owner admitted to noticing health problems in the animals. However, previous research has already shown that abomasal ulcers frequently occur in animals with comorbidities [31].

In the present study, there was a higher occurrence of cases in the dry season (October to March) for both young and adult animals, even when the animals were raised under different management practices and in different geographical locations. It should be noted that, in addition to the presence of comorbidities, most cases received feed based on concentrates and silages, highlighting that 69% of the cows were at the peak of lactation, with an average of 70 days in lactation, the same risk factors as noted by Palmer & Whitlock [9]. Aukema & Breukink [8] found that in cows grazing in the period from May to October the incidence of ulcers was higher than in winter, and although temperature and atmospheric pressure did not show any correlation with the incidence of ulcers, there was a positive correlation between high rainfall and the occurrence of ulcers. Akbari et al. [30],

observed a higher occurrence of type 1 ulcers in the summer than in other seasons, corroborating the results found by Hussain et al. [32] in buffalo.

In the current study, the frequency of single subtype 1b ulcers was higher in both young and adult animals. Subtypes 1a and 1b occurred more frequently in abomasa with multiple ulcers. According to Braun et al. [14] each type of ulcer has a topographical region of predilection, subtypes 1a and 1c occur mainly in the pyloric region, and subtypes 1b, 1d, and type 2 ulcers occur mainly in the fundic region, corroborating the findings of this study. The occurrence of multiple ulcers in this study was much more frequent in adult animals than in young animals, which had a higher frequency of isolated ulcers. This result corroborates the results of Hussain et al. [32] and Tajik et al. [33] who found a higher occurrence of multiple ulcers in adult animals.

Histopathological examination was used to characterize the ulcer in terms of the depth of involvement of the mucosa and wall of the abomasum, the presence of the etiological agent, and the inflammatory process, in order to avoid bias in the sub-classification of type 1 ulcers. Although the classification is macroscopic, this subtyping of type 1 ulcers and the difference between subtype 1c and type 2 ulcers raises questions, especially for professionals familiar with the description of ulcers adopted by Whitlock [6]. The results found in this study suggest that each subtype of ulcer has a different pathophysiological process. Ulcers located in the pyloric region are probably different from those in the fundic region, and subtype 1a and 1b erosions can be confused with the presence of mucosal hemorrhages, while subtype 1d ulcers may be underreported or have a false prevalence due to the specific area that occurs when the characteristics that identify them are not understood, as this subtype can form radial wrinkles that converge to a central point of healing or the gastric folds can be completely perforated [14; 15, 23]. In our work, subtype 1d had a low occurrence, and all of 1d ulcers presented as circular perforation areas in the gastric folds in the fundal region.

It is unlikely that the occurrence of ulcers in the body of the abomasum differs pathogenically from ulcers in the fundus of the abomasum, nor do ulcers in the antrum-pyloric mucosa differ from ulcers in the pyloric mucosa, disagreeing with Munch et al. [23], who divided the abomasal mucosa into zones, with zone 1 corresponding to the fundic region (body and fundus of the abomasum), zone 2 to the antro-pyloric mucosa, and zone 3 to the pyloric mucosa (both belonging to the aglandular pyloric region). The location of the ulcer subtypes can be explained by a possible difference in the effects of hydrochloric acid and pepsin in the fundic region in relation to the pyloric region, which is aglandular and does not produce hydrochloric acid or pepsin, but produces mucus that is freely permeable to hydrogen ions and intercellular junctions that are wider than the fundus region. Therefore, ulcers in the pyloric region tend to be provoked by the effect of hydrochloric acid and pepsin, and ulcers in the fundic region tend to be initiated by processes that cause structural damage, secondary to ischemia [34].

Histopathological examination demonstrated that abomasal ulcers were characterized by focal, focally extensive, multifocal, or diffuse inflammatory processes, mainly by mononuclear cells. Lymphocytes were predominantly found associated with other inflammatory cells with mild to intense infiltration in the mucosal lamina propria. An occurrence of abomasitis due to atypical lymphocytes was found in 12.93% of cases, associated with mucosa with subtype 1a and 1b erosions and subtype 1c ulcers, which suggests neoplastic infiltrate due to the Bovine Enzootic Leukosis virus, although in only three cases there was the presence of lymphosarcoma in the abomasum, corroborating the findings of Palmer & Whitlock [9] who reported lymphocytic infiltration in cases of leukosis associated with ulcerations, without evidence of lymphosarcoma.

It appears that the severity of the ulcer is related to the depth of the lesion, with subtype 1c and type 2 ulcers, which reach the submucosa and muscular layer, being consequently more serious than superficial ulcers, subtypes 1a, 1b, and 1d. According to Braun et al. [14], the age of the lesion can be estimated based on the inflammatory reaction: subtypes 1a and 1b are mainly acute or subacute, and subtypes 1c and 1d are always chronic, which corroborates the findings of the current work. It was found that type 2 ulcers are acute, differing from subtype 1c ulcers, which are chronic, formed by extensive necrosis, with areas of granulation tissue and fibrosis. There are no data available for cattle with abomasal lesions relating the time of formation of each ulcer. Gastric erosions in horses progressed to ulcers within 36-72 hours in Murray's study [35]. It is possible for subtype 1a erosions to progress to subtype 1b erosions or subtype 1c ulcers, subtype 1b erosions when located in the gastric folds to progress to subtype 1d ulcers, as well as type 2 ulcers to progress to subtype 1c ulcers, until they evolve into perforated ulcers by the action of agents that attack the mucosa or heal. According to Munch et al. [23] subtype 1a erosions can become subtype 1c ulcers as they are mostly located in the same regions.

The histopathological findings referring to alterations in the gastric glands associated with the ulcerated mucosa corroborate the findings in the literature [14; 32, 36]. Although little is known about the participation of fungi in the etiopathogenesis of abomasal ulcers, hyphae colonizing vessels, mucosa or submucosa associated with some ulcer subtypes were found in the present study. Colonies of bacteria were also found in pits and ulcer

beds. It is possible that these are secondary and opportunistic agents resulting from the loss of mucosal integrity.

5. Conclusions

The results of the current study emphasize the occurrence of type 1 abomasal ulcers in young and adult dairy cattle with comorbidities. Digestive diseases were the most frequent primary comorbidities associated with the presence of type 1 or 2 ulcers. Single ulcer subtype **1b** and multiple ulcer subtypes **1a** and **1b** were the most commonly found in the *post-mortem* anatomopathological examination. Regardless of the age of the animals, the predominant site for type 1 ulcers did not differ between the fundus or pylorus region. Cases occurred mostly in the dry season (October to March), both for young and adult animals, and most adult animals were cows in peak lactation. The ulcers were characterized by focal, extensive, multifocal, or diffuse inflammatory processes, predominantly by mononuclear cells, with associated abomasitis in most cases of the lymphocytic type. The economic effect of erosions or ulcers of the abomasum in ruminants has been little investigated and should be taken into account for future studies, especially when analyzing the effect of ulcers on the digestive functionality of the abomasum. To this end, earlier diagnostic tools also need to be developed in future studies, to avoid production losses and help veterinarians in the early treatment and prevention of these conditions.

Conflicts of interest

The authors have no conflicts of interest.

Authors contributions

Conceptualization: A.Q. de Andrade Neto, J. R. B. Silva, C. L. de Mendonça, R. J. C. Souto, J. F. D. P. Cajueiro, M. I. de Souza, L. R. M. de Sá and J. A. B. Afonso. *Data curation:* C. L. de Mendonça, L. R. M. de Sá and J. A. B. Afonso. *Formal analysis*: A. Q. de Andrade Neto, C. L. de Mendonça, L. R. M. de Sá and J. A. B. Afonso. *Research:* A.Q. de Andrade Neto, J. R. B. Silva, Souto, J. F. D. P. Cajueiro, M. I. de Souza and J. A. B. Afonso. *Visualization:* A.Q. de Andrade Neto; Resources; C.L. de Mendonça, L.R.M. de Sá and J.A.B. Afonso. *Supervision:* C. L. de Mendonça, L. R. M. de Sá and J. A. B. Afonso. *Validation*: C. L. de Mendonça, L. R. M. de Sá and J. A. B. Afonso. *Financing Acquisition and Project Management:* J. A. B. Afonso. *Writing (original draft, review & editing):* A. Q. de Andrade Neto.

Acknowledgements

To CAPES for granting the scholarship, to Resident Veterinarians at Clínica de Bovino-Garanhuns/UFRPE, who helped in performing the necropsies and collecting material from the animals studied. To the technicians of the histopathology laboratory of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo-FMVZ-USP, Mauro de Mattos and Cláudio Arroyo, and Prof. Lilian Marques de Sá for help with the histopathological diagnosis of the work.

References

1. Akbari H, Shahbazfar AA, Araghi-Sooreh A, Hassan-Nejad H, Zangisheh M, & Taheri M**.** Macroscopic and pathologic evaluation of cattle abomasal ulcers in Tabriz industrial slaughterhouse. Vet. Res. Biol. Prod**.** 2017;05(177):192-202.

2. Aukema JJ, & Breukink HJ. Abomasal ulcer in adult cattle with fatal haemorrhage. Cornell. Vet. 1974;64(2):303–317.

3. Braun U, Bretscher R, & Gerber D. Bleeding abomasal ulcers in dairy cows. Vet. Rec. 1991;129(13):279-284.

4. Braun U, Eicher R, & Ehrensperger F. Type 1 Abomasal Ulcers in Dairy Cattle. J. Vet. Med. 1991; 38(1–10):357–366.

5. Braun U, Gerspach C, Hilbe M, Devaux DJ, & Reif C. Clinical and laboratory findings in 60 cows with type-3 abomasal ulcer. Schweiz. Arch. Tierh. 2019;161(9):523–531.

6. Braun U, Gerspach C, Nuss K, Hässiga M, Hilbeb M, & Reif C. Clinical and laboratory findings, treatment and outcome in 145 cows with type-2 abomasal ulcer. Res. Vet. Sci. 2019;124:366–374.

7. Braun U, Gerspach C, Reif C, Hilbe M, & Nuss K. Clinical, laboratory and ultrasonographic findings in 94 cows with type-1 abomasal ulcer. Schweizer. Arch. Tierh. 2020;162(4):235–244.

8. Braun U, Reif C, Hilbe M, & Gerspach C. Type-5 abomasal ulcer and omental bursitis in 14 cows. Act. Vet. Scand. 2020;62(1):4.

9. Braun U, Reif C, Nuss K, Hilbe M., & Gerspach C. Clinical, laboratory and ultrasonographic findings in 87 cows with type-4 abomasal ulcer. BMC. Vet. Res. 2019;15(1):100.

10.Constable PD, Hinchcliff KW, Done SH, & Grünberg W. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11a. ed. Elsevier. St. Louis, Missouri. 2017. p.2356.

11. Ducharme NG, Desrochers A, Fubini SL, Pease AP, Mizer LA, Walker W, Trent AM, Roy JP, Rousseau M, Radcliffe RM, & Steiner A. Surgery of the bovine digestive system. In: Fubini SL, & Ducharme NG. (Eds). Farm Animal Surgery. 2a ed. Elsevier. St. Louis, Missouri; 2017. p.223-343.

12.Francoz D, & Guard CL. Abomasal Ulcers. In: Bradford PS, David CVM, & Nicola P. Large Animal Internal Medicine. 6a ed. Elsevier, St. Louis, Missouri; 2020. p. 889–893.

13.Fubini SL, Yeager AE, & Divers TJ. Noninfectious Diseases of the Gastrointestinal Tract. In: Peek SF, &. Divers TJ. (Eds). Rebhun's Diseases of Dairy Cattle**:** 3a. ed. Elsevier. St. Louis, Missouri; 2018. p. 168-245.

14. Hund A, & Wittek T. Labmagengeschwüre beim Rind. Tierarztl. Prax. Ausg. G. Grosstiere. Nutztiere. 2017;45(02):121–128.

15. Hund A, Beer T, & Wittek T. Abomasal ulcers in slaughtered cattle in Austria. Tierarztl. Prax. Ausg. G. Grosstiere. Nutztiere. 2016;44(5):279–285.

16. Hussain SA, Uppal SK, & Sood NK. Clinicopathological diagnosis of type-I abomasal ulceration in cattle and buffaloes. Indian. J. Vet. Pathol. 2015;39(3):239.

17.Hussain SA, Uppal SK, & Sood NK. The prevalence, frequency and topographic distribution of type 1-abomasal ulcers in water buffalo (*Bubalus bubalis*): A case control study. Vet. Arhiv. 2019; 89(3): 317-330.

18. Jassim A, Yousif AAR, & Kshash QH. Study on Abomasal Ulcer in Sheep in Iraq. Int. J. Adv. Res. 2014;2(1):342-349.

19. Jelinski MD, Ribble CS, Campbell JR, Janzen ED. Investigating the relationship between abomasal hairballs and perforating abomasal ulcers in unweaned beef calves. Canadian Vet. J. 1996; 37(37):23-26.

20.Jelinski MD, Ribble CS, Chirino-Trejo M, Clark EG, & Janzen ED. The relationship between the presence of *Helicobacter pylori*, *Clostridium perfringens* type A, *Campylobacter* spp, or fungi and fatal abomasal ulcers in unweaned beef calves. Canadan. Vet. J. 1995;36:379-382.

21. Jensen R, Pierson RE, Braddy PM, Saari DA, Benitez A, Lauerman LH, Horton DP, & McChesney AE. Abomasal erosions in feedlot cattle. Am. J. Vet. Res. 1992;53(1):110-115.

22. Luna SPL, & Teixeira MW. Eutanásia: considerações éticas e indicações técnicas. Revta. CFMV. 2007;13(41): 60-69.

23. Marshall TS. Abomasal Ulceration and Tympany of Calves. Vet. Clin. North Am. Food Anim. Pract. 2009; 25(1):209–220.

24. Mesarič M. Role of serum pepsinogen in detecting cows with abomasal ulcer. Vet. Arhiv. 2005;75(2):111–118.

25. Munch SL, Nielsen SS, Krogh MA, & Capion N. Prevalence of abomasal lesions in Danish Holstein cows at the time of slaughter. J. Dairy. Sci. 2019;102(6):5403–5409.

26. Murray MJ. Equine model of inducing ulceration in alimentary squamous epithelial mucosa. Dig. Dis. Sci. 1994; 39(12):2530-2535.

27. Nielsen SS, Krogh MA, Munch SL, & Capion N. Effect of non-perforating abomasal lesions on reproductive performance, milk yield and carcass weight at slaughter in Danish Holstein cows. Prev. Vet. Med. 2019;167(2):101–107.

28. Ok M, Sem I, Turgut K, & Irmak K. Plasma gastrin activity and the diagnosis of bleeding abomasal ulcers in cattle. J. Vet. Med**.** 2001;48(9):563–568.

29. Palmer J, Whitlock R. Perforated abomasal ulcers in adult dairy cows. J Am Vet Med Assoc 1984; 184: 171–174.

30.Palmer JE, & Whitlock RH. Bleeding abomasal ulcers in adult dairy cattle. J. Am. Vet. Med. Assoc. 1983;183(4):448–451.

31. Silva Filho AP, Afonso JAB, Souza JCA, Dantas AC, Costa NA, & Mendonça CL. Achados clínicos de bovinos com úlcera de abomaso. Vet. Zootec. 2012;19(2):196–206.

32.Smith DF, Munson L, & Erb HN. Abomasal ulcer disease in adult dairy cattle. Cornell Vet. 1983;73(3):213–224.

33.Souza LM, Assis RN, Rego RO, Santos JF, Coutinho LT, Souza JCA, Mendonça CL, Afonso JAB, & Souto RJC. Achados clínicos, laboratoriais e anatomopatológicos de bezerros com úlceras de abomaso. Ciênc. Vet. Tróp. 2017;19(3):20-28.

34. Tajik J, Khodakaram AT, Heidari M, & Babazadeh M. Prevalence, histopathological, and some epidemiological aspects of abomasal ulcers in water buffalo (*Bubalus bubalis*) in Iran. Comp. Clin. Path. 2013; 22(2):271-275.

35. Uzal FA, Plattner BL, & Hostetter JM. Alimentary System. In: Maxie MG. (Ed) Jubb, Kennedy, and Palmer's Pathology of Domestic animals. Vol. 2. 6a. ed. Elsevier, St Louis, Missouri. 2016; p.1-257.

36.Whitlock RH. Bovine stomach diseases. In: Anderson NV. (Ed). Veterinary Gastroenterology, Lea and Febiger, Philadelphia; 1980. p.425-428