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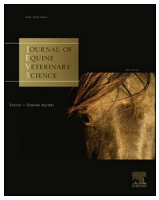

Prevalence of squamous gastric disease in Colombian equids at slaughter: A postmortem comparative study among horses, donkeys and mules

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ABSTRACT

Equine Gastric Ulcer Syndrome (EGUS) occurs with variable prevalence in horses, donkeys, and mules. Due to the particularities of the mucous membranes, the syndrome is made up of Squamous Gastric Disease (ESGD) and Glandular Gastric Disease (EGGD). Given the multifactorial nature and multiple classification systems of the syndrome, significant differences have been reported between prevalence studies performed *ante mortem*, which are even more remarkable when compared with postmortem evaluations. This study aimed to determine the presence and grade of squamous gastric disease in horses, donkeys and mules immediately after slaughter. The postmortem examination considered the inspection of the squamous region (cardia, dorsal fundus, and margo plicatus) and the classification of the observed lesions. The general prevalence of ESGD in the entire population of study was 83.3 % (78 %, 89 %, and 83 % for horses, donkeys, and mules, respectively), compromising the margo plicatus in all cases. 75 % had more than 5 lesions and 50 % had deep lesions, lesions of varying severity and/or evidence of recent/active bleeding. The prevalence of ESGD was similar in horses, donkeys, and mules subjected to similar handling conditions prior to slaughter, including long-distance traveling, fasting, and stress factors.

1. Introduction

Equine Gastric Ulcer Syndrome (EGUS) occurs in horses, donkeys, and mules and has been extensively reported in the worldwide literature [1,2]. Due to the particularities of each type of mucosa, the syndrome is divided into Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) [3]. Despite often presenting simultaneously there are differences in prevalence, epidemiology, pathogenesis, and treatment, and therefore, they are considered different diseases [4,5]. In this case, the presentation and characterization of ESGD in horses, donkeys, and mules destined for slaughter will be the focus of interest in this study.

ESGD has been extensively described in sport horses, with an increase in training periods, with prevalence ranging from 37 to 100 % and in different breeds [6-9]. In addition, it has been reported in horses used for other activities such as saddle, work, exhibition, police patrol, and working animals, with prevalence ranging from 11 to 79 % [10-15]. In live donkeys, an ESGD prevalence of 95 % has been reported [16]. In mules, the studies are scarce; however, a prevalence of 28 % has been reported by gastroscopic-based studies [17].

In general, the imbalance between the defense mechanisms of the gastric mucosa and both endogenous and exogenous injurious agents have been described as the cause of EGUS [18,19]. Specifically, about the pathophysiology of ESGD, the deficient defense mechanisms of this mucosa [20,21] added to the caustic effect of hydrochloric acid and the organic acids and volatile fatty acids may play an important role in the generation of lesions [18,20,22]. In addition, various predisposing and inducing factors for ulcers in this mucosa have been identified for horses and donkeys [23,24], but are poorly understood for mules [17].

Regardless of the predisposing factors, ESGD has an impact on equids due to the negative effects it causes such as postprandial colic, loss of body condition, decreased athletic performance and behavioral alterations of different degrees of complexity [25,26,27]. The high costs of management and pharmacological treatment of ulcers and the consequences that they can have on the animal's health, in addition to the high prevalence have made them become a relevant topic for the equine industry. In the case of the population of equids destined for slaughter, information is lacking, although they are exposed to several

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predisposing factors.

Given the absence of comparative studies, especially on the presence of ESGD in horses, donkeys, and mules subjected to similar conditions, various lesion classification systems, and the described limitations of gastroscopic studies, this study aimed to perform a postmortem evaluation of the stomachs of the three different types of equids to characterize similarities and differences in the presence of ESGD presentation.

2. Materials and methods

2.1. Ethical approval

All procedures were approved by the Ethics Committee for Experimentation with Animals of the University of Antioquia (No. 1472022), and carried out in accordance with the relevant laws and guidelines.

2.2. Methodology

A total of 300 equid stomachs from horses, donkeys and mules (100 from each group) were obtained immediately after slaughter. The number of individuals was selected at convenience. The equids came from various regions of the country. It was not possible to obtain the details of management systems from the information available. However, these animals are known to be subjected to long-distance traveling and fasting. Prior to slaughter, equids were classified by sex, body condition score [28] and age (<5 years or young, between 6 and 14 years or adult, and >15 years or geriatric), determined approximately by dental chronometry.

Each previously identified stomach was opened between the cardia and the pyloric antrum (PA) through the greater curvature, to fully expose the gastric surfaces and proceed with the removal of the contents and lavage with plenty of water. Next, the inspection of the squamous mucosa of the dorsal fundus, the cardia area, and the margo plicatus (MP) region was performed. The glandular mucosa was evaluated, inspecting the regions of the ventral fundus, the adjoining area of the margo plicatus, and the pyloric antrum.

For ESGD, a score was assigned according to number and severity. The classification by number of lesions was made from 0 to 4 as follows: 0 when there were no lesions, 1 when there were 1-2 localized lesions, 2 when there were 3-5 localized lesions, 3 when there were 6-10 lesions and 4 when there were >10 lesions or diffuse (or very large) lesions. Regarding severity, a score was assigned from 0 to 5, based on the following criteria: a 0) no lesions; 1) superficial lesions with only mucosal involvement and the interior of the lesion had a pink appearance; 2) lesions deeper than severity number 1 (these lesions had raised edges and the ulcer crater had a pink, granulation tissue-like appearance); 3) stomachs with multiple lesions of different severity; 4) deep and active mucosal lesions (hyperemic or darkened lesion crater, with necrotic appearance); 5) lesions with evidence of active bleeding and/or attached blood clots, in addition to an injury severity score of 4 [29].

Acute and chronic gastritis in the squamous mucosa was identified by the presence of hyperemia, edema, abrasions, and color changes in the mucosa, The classification was determined by the appearance, coloration, presence of coating and evidence of signs of chronicity in sites adjacent to the lesions (hyperkeratosis). Hyperkeratosis proximal to MP was graded according to the degree of distribution on a scale of mild when it was almost imperceptible, moderate when it was perceived in 50 % of tissue, and severe when it was in all tissue.

After inspection and evaluation of the gastric surfaces, samples from 10 stomachs of each group with similar lesions in the squamous mucosa were collected and submitted for conventional histopathological processing and hematoxylin and eosin (*H&E*) staining. Each sample was analyzed for the microscopic characterization from the inflammatory processes and the ulcerative lesions to allow a comparison among groups (i.e., horses, donkeys, and mules).

The type of hyperkeratosis was evaluated, which was determined by the increase in thickness of the superficial layer and the morphology of the observed nuclei, with normal nuclei indicative of orthokeratotic hyperkeratosis and pyknotic nuclei indicative of parakeratotic hyperkeratosis.

Inflammatory processes were identified through the presence of alterations such as edema, vascular congestion, and cellular infiltrates. The infiltrates were classified according to the type of cell, their quantity (mild, moderate, severe) and their distribution (focal, multifocal or diffuse). Necrosis was determined by the observation of dead cells with ample cytoplasm and processes of karyorrhexis, karyolysis or pyknosis in the nucleus. Ulcers were observed as a loss of mucosal continuity extending to the level of the lamina propria.

2.3. Statistic analysis

Data were analyzed by descriptive statistics. ANOVA and Kruskal-Wallis tests were used to compare body and ESGD scores among groups. The frequencies of sex, age group, body condition score were compared using the chi-squared test. The prevalence rates of different gastric lesions and sites among groups were compared using the chi-squared test. When significant differences were found, the prevalence ratio (PR) and the confidence interval (CI) were calculated. A significance level of $P < 0.05$ was applied to all tests.

3. Results

The characterization of the equids examined in each group is shown in Table 1, where the number of individuals by sex, age group, and body condition score are detailed. In the donkey group, females were more frequent, and males were less frequent when compared to horses ($P = 0.01$). In the mule group, geriatric equids were more frequent and young equids were less frequent when compared to donkeys ($P < 0.003$). The body condition score was in values below the ideal condition and very similar in the population; however, the mean (±SD) in horses (3.12 ± 3.3) was slightly higher ($P = 0.0003$) when compared to donkeys (3.0 ± 0.0) and mules (3.01 ± 2.2).

The prevalence of ulcers in the squamous mucosa according to classification is presented in Table 2. Ulcers were detected in 83.3 % of the subjects, without difference ($P = 0.26$) among groups (78 %, 89 %, and 83 % for horses, donkeys, and mules, respectively). According to the classification adopted and considering lesions with grades ≥2 by number, they exceeded 75 % in all groups, and according to severity, lesions with grades ≥2 exceeded 50 % in all groups, with the most affected being donkeys and mules. In all groups, ulcers were more frequent in the MP ($P<0.0001$). Comparing frequency rates among groups, ulcers were

Table 1
Absolute frequencies of sex, group age, and body condition score among Colombian horses, donkeys, and mules ($n = 100$ per each) subjected to postmortem stomach evaluation.

Characteristic	Equids		
	Horses	Donkeys	Mules
Sex			
Females	62	44*	53
Males	38	56*	47
Age			
Young (< 5 years)	15	25	8#
Adult (6–14 years)	44	43	40
Geriatric (> 15 years)	41	32	52#
Body condition score**			
Score 2	0	0	2
Score 3	88	100*	95
Score 4	12	0*	3

Markers indicate difference from horses (*) or donkeys (#) in the same row ($P < 0.05$).

** Average body conditions score 1-9 for groups, according to Henneke et al., (1983). Only the scores found are reported.

Table 2
Prevalence rates (%) of Equine Squamous Gastric Disease (ESGD), according to MacAllister et al (1997), in the equid population.

Classification	Equids								
	Horses			Donkeys			Mules		
Number (Grade)	F	MP	C	F	MP	C	F	MP	C
Negative (0)	90	24	72	86	12	48	87	19	60
1	0	7	0	0	5	0	2	6	1
2	0	28	4	4	26	17	2	24	7
3	3	21	11	7	34	18	1	25	12
4	7	20	13	3	23	17	8	26	20
Total	100	100	100	100	100	100	100	100	100
Positive total	10a	76c	28b	14a	88c	52b*	13a	81c	40b
Severity (Grade)	F	MP	C	F	MP	C	F	MP	C
Negative (0)	90	24	72	86	12	48b	87	19	60
1	0	12	0	0	6	0	2	11	1
2	0	32	10	6	27	19	2	29	15
3	3	16	7	6	30	16	3	21	13
4	2	5	1	0	11	5	2	8	3
5	5	11	10	2	14	12	4	12	8
Total	100	100	100	100	100	100	100	100	100
Positive total	10a	76c	28b	14a	88c	52b*	13a	81c	40b

F: Fundus, MP: margo plicatus, **C**: Cardia. Within groups, frequency rates followed by the same letter did not differ (P<0.0001).
* Differs from horses at the same stomach site ($P = 0.0025$).

more frequent in the cardia of donkeys compared to horses ($P = 0.0008$; PR:1.86, CI:1.30 to 2.70). The different grades, number and severity, can be seen in Fig. 1.

Macroscopic details of the inflammation types in the squamous mucosa are presented in Table 3. Chronic gastritis was observed more frequently in donkeys and mules than in horses. But overall gastritis occurrence was not different among groups. The fundus was more frequently affected in donkeys when compared to horses ($P = 0.004$; PR:2.55, CI:1.37 to 4.82) and mules ($P = 0.04$; PR:1.87, CI:1.08 to 3.28), and the cardia was less commonly affected in horses compared to donkeys ($P = 0.0004$; PR:0.29, CI:0.14 to 0.58) and mules ($P = 0.02$; PR:0.40, CI:0.19 to 0.84). During the assessment of the stomachs, were documented intragastric parasites identified as *Habronema spp*. across all three groups of equids (number of animals with presence of parasites: horses: 11, donkeys: 15, mules: 14).

Hyperkeratosis proximal to the MP was observed in the three groups of equids, as shown in Table 4. In all groups, moderate degrees are more frequent, followed by mild and severe ($P < 0.001$). Severe hyperkeratosis was more prevalent in mules compared to horses and donkeys ($P = 0.04$; PR:3.33, CI:1.03 to 11.01).

Histopathological findings of the stomachs selected for each group are presented in Table 5 and are shown comparatively in Fig. 2. Cellularity presented similarly in all groups, with distribution patterns between mild, moderate, or severe. Lymphocytes, plasma cells, neutrophils, and eosinophils were common in mucosa; however, plasma cells were not reported in horses. Microscopic evaluation corroborated the presence of mucosal hyperkeratosis, vascular changes, presence of ulcers and tissue necrosis, although exocytosis was not observed in donkeys and mules, meanwhile, no perivasculitis was found in horses.

Table 3
Prevalence (%) of gastritis in the equid population.

Findings	Equids								
	Horses (n = 100)			Donkeys (n = 100)			Mules (n = 100)		
Classification	F	MP	C	F	MP	C	F	MP	C
AG	7	9	6	1	1	0	0	1	1
CG	4	8	2	27	25	28	15	14	19
AG+CG	0	0	0	0	0	0	0	0	0
Total	11	17	8	28*	26	28*	15#	15	20*

AG: Acute gastritis, **CG**: Chronic gastritis, **F**: Fundus, MP: margo plicatus, **C**: Cardia.
* Differs from horses at the same stomach site (P<0.01).
# Differs from donkeys at the same stomach site ($P = 0.03$)

4. Discussion

The present study showed that the prevalence of ESGD in the three

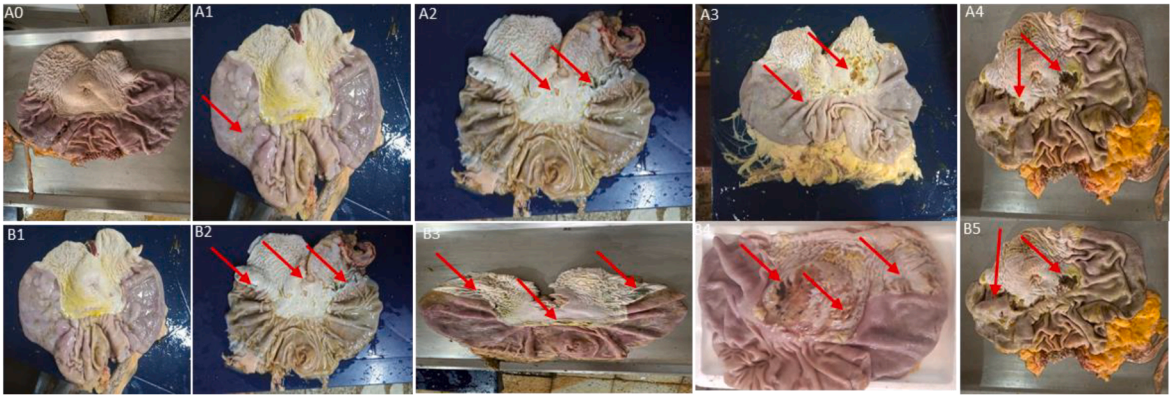

Fig. 1. Classification of lesions by number and severity for the squamous mucosa of the equids studied, according to MacAllister et al. (1997). Number (A): Score 0 (A0), score 1(A1), score 2 (A2), score 3 (A3) and score 4 (A4). Severity (B): Score 1(B1), score 2 (B2), score 3 (B3), score 4 (B4) and score 5 (B5).

Table 4
Prevalence (%) of hyperkeratosis in the squamous mucosa proximal to margo plicatus (MP) of the equid population.

MP	Equids		
	Horses (n = 100)	Donkeys (n = 100)	Mules (n = 100)
Mild	29 B	21 B	31 B
Moderate	56 A	61 A	54 A
Severe	3 Cb	3 Cb	10 Ca
Total	88	85	95

Prevalence followed by the same letter (capital within columns and lower case within rows) did not differ (P<0.0001).

Table 5
Histopathological findings in the samples of the squamous mucosa of the stomachs selected from the three groups of the equid population.

Variables	Equids		
	Horses	Donkeys	Mules
	Histological findings		
PH	X	X	X
Ulcers	X	X	X
Necrosis	X	X	X
VC	X	X	X
Exocytosis	X	—	—
Peri-vasculitis	—	X	X
	Inflammatory cells		
Lymphocytes	X	X	X
Neutrophils	X	X	X
Eosinophils	X	X	X
Plasmocytes	—	X	X

PH: Parakeratotic hyperkeratosis, **VC**: Vascular congestion

groups of equids was similar to that reported for various horse breeds used for sport [6-9], but higher than found by gastroscopy in mules and horses used for various other types of work [14,17,30]. However, the prevalence in donkeys was similar to that reported in *postmortem* studies of the same breed [31], but notably higher than in studies carried out in other countries [32,33,34]. No previous *postmortem* studies were found in relation to mules; however, it was higher than the prevalence reported by gastroscopy [17]. Although in the present study it was not possible to verify the influence of predisposing factors of ESGD, the high susceptibility of injury to this mucosa was reaffirmed [18].

All the animals were subjected to long-distance travel (up to 24 hours) and fasting of solids and liquids prior to slaughter (average of 12 hours). Long-distance travel has been described as a predisposing factor for ESGD [24,26,35], once the mucosa is exposed to gastric juice, aggravated by the fact that equines are often fasted before the trip. This causes the loss of the protective role of food in the stomach, which consists of absorbing gastric secretions or duodenal reflux to avoid contact with the mucosa [35]. Therefore, it can be inferred that the combination of these two factors influenced the occurrence of ESGD in the study population.

During the general inspection, no clinical signs commonly associated with ESGD were identified, such signs being considered nonspecific in the literature [1,2,3,5,26]. However, the body condition score of the entire population was low (3/9), a condition related to ESGD [26]. Poor BCS has not been directly associated with ESGD, and the low BCS is probably related to other factors (workload, diet, age (advanced), although some impact of ESGD cannot be ruled out. The high percentage of geriatric individuals (over 15 years of age) could influence the body condition and poor coat quality observed in some individuals, rather than being associated with gastric disease. Animals were managed at pasture without supplementation could also affect BCS, although, in these animals, the quantity and quality of forage was unknown. Other conditions related to low body condition were not explored.

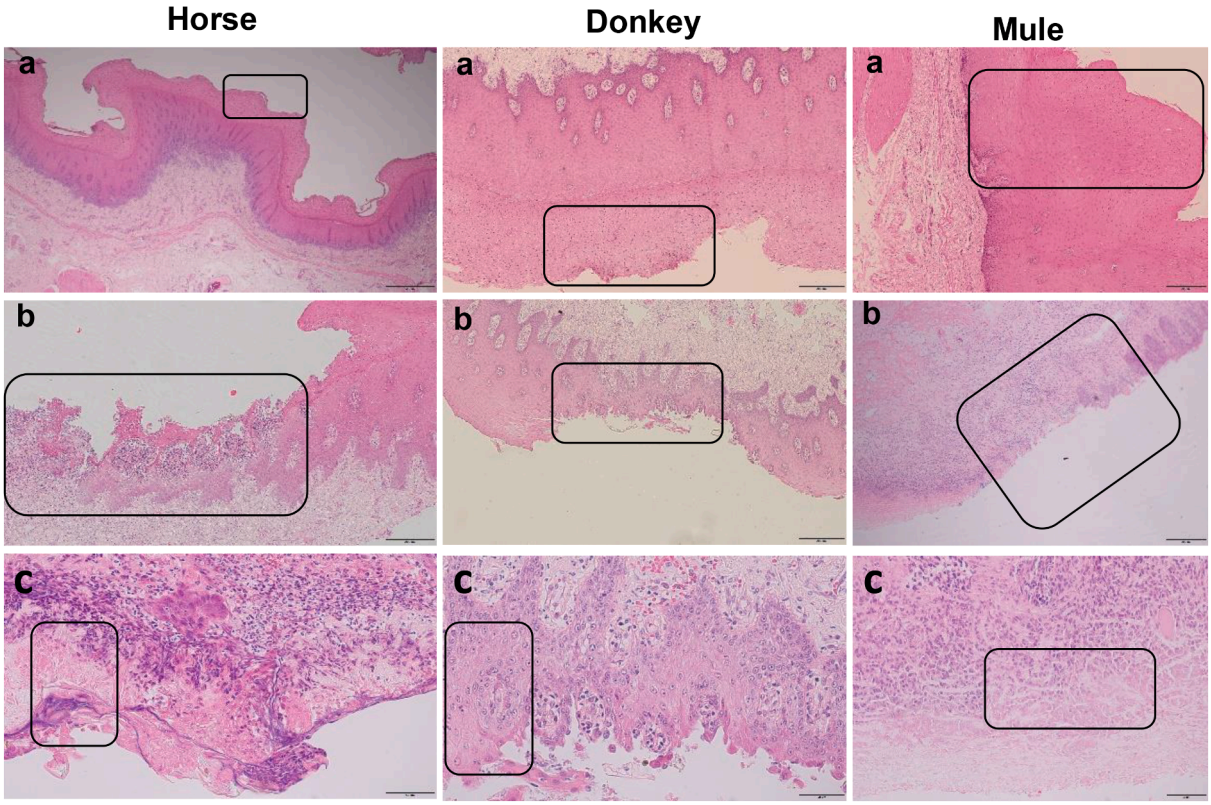

Fig. 2. Comparison of histopathological findings in the squamous mucosa of the stomachs selected for each equid groups. (a) parakeratotic hyperkeratosis, (b) ulcer, (c) necrosis. H&E, 10X and 40X.

It was presumed that most of these equids were grazing, where green forage has been controversially considered as a protective factor in ESGD in previous studies [11,22,36]. However, the level of non-structural carbohydrates is important due to the ulcerative process derived from fermentation [22,37]. Nevertheless, in the climatic conditions of these animals, this has little relevance since tropical forages have low levels of soluble carbohydrates (<6 %) [38]; therefore, the amount of fiber encourages chewing and saliva production, fulfilling its buffer function in the stomach. Consequently, the ESGD in the animals of study was probably initiated and aggravated by the caustic effect of the acid in the gastric fluid, exacerbated by transport and fasting.

Stressful activities in equids induce gastric ulceration [26,39], including long journeys, prolonged fasting, mixtures of animals from different places, aggressive behaviors and expression of dominance among animals. These events were identified in the population prior to slaughter; it is likely that stress contributed to the development of ESGD, although this factor has been more associated with EGGD [26,40,41]. The association of ESGD with chronic stress has reported an inverse relationship between cortisol and degrees of ulceration [42]. Unfortunately, the absence of a clinical history of everyone did not allow us to determine the type and intensity of activity or exercise, feeding and management practices, previous medical conditions, and presence of stereotypies, which have been specifically related to ESGD [27].

Chronic gastritis was more frequent in donkeys and mules, possibly related to the type of activity and management to which these animals are subjected. The degree of hyperkeratosis is compatible with the reaction of gastric tissue to damaging factors over time; in addition, most of the animals were adults and geriatrics, where chronic changes are more common observed. However, the presence of acute gastritis in all three groups could reflect the effect of fasting and transport undergone prior to slaughter, although incidence studies to confirm this observation have not been performed, as have been done in horses before and after exercise [9,43,44].

Microscopic findings showed similarity in cellularity and distribution patterns among the three groups, and to those described in studies conducted in horses [45,46,47]. The lesions found in the squamous mucosa were similar to those described by other authors [46,48]. However, histologically, the ulceration degree observed macroscopically was not evidenced in some cases, indicating that the number of samples taken was not representative of this gastric surface, since at least six samples per mucosa have been recommended [47]; therefore, this fact is a limitation of this study.

Chronic gastritis was histologically determined due to the abundance of mononuclear cells, thus demonstrating correspondence with the macroscopic findings. However, in horses there was no evidence of the presence of plasma cells, possibly due to a greater number of cases of acute gastritis in these specimens; however, the number of samples was not sufficient to confirm such a finding [47]. In the case of donkeys and mules, the presence of hyperkeratosis in the MP reflected chronicity associated with tissue reaction. The neutrophils present indicated active inflammatory conditions, possibly induced by the identified predisposing factors. In addition, the accumulation of eosinophils was considered to be due to the presence of parasites reported to induce lesions on the gastric surface [33,49,50] conforming was found in several equids (13.3 %).

The prevalence determined in this population was high compared to several endoscopic studies. This can be due to the *postmortem* evaluation exceeding the limitations of gastroscopy since it allows inspection of the entire gastric luminal surface [22,45,47]. In addition, the management of these animals does not represent the natural evolution of ESGD, since it is induced by exposure to predisposing factors. The way in which the activities are carried out in the slaughterhouse did not allow to access the history of each individual. Another limitation of this type of study was the inability to determine exactly the degrees of severity associated with the presence of clots or active bleeding, since they can be removed during the gastric lavage process.

Despite the above, this work allowed to demonstrate the high frequency of the squamous mucosa ulcers in horses, donkeys, and mules, and it was possible to determine that chronic gastritis was more frequent in donkeys and mules than in horses.

CRediT authorship contribution statement

Angie L. Medina B.: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rafael R. Faleiros:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **José R. Martínez A.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no know competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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