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Glandular gastric disease prevalence in equids (horses, donkeys, and mules) and other gastric findings: post-mortem comparative study

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Abstract

Equine glandular gastric disease (EGGD) has limited prevalence studies and scarce understanding of the predisposing factors and pathophysiological mechanisms involved. The objectives of this study were to determine and compare the post-mortem prevalence of EGGD and other gastric lesions in Colombian equids, specifically horses, donkeys, and mules. The study utilized a sample of 300 equids stomachs, with 100 specimens from each group, collected immediately after slaughter. The post-mortem examination included a thorough inspection of the ventral fundus, adjacent area of the margo plicatus, and pyloric antrum of the glandular mucosa, carefully identifying any lesions or abnormalities. The results showed that the overall macroscopic presence of EGGD in the equid population was 68.30% on average, with specific rates of 45.00% in horses, 77.00% in donkeys, and 83.00% in mules, with lesions predominantly affecting the fundus and pyloric antrum regions. Inflammatory processes were observed to be similar between donkeys and horses, while mules exhibited distinct inflammatory patterns in the glandular mucosa. Additional gastric findings, such as gastritis (85.30%), gastric impaction (10.00%), and parasitic infections (13.30%), were also documented during the study. The findings suggested that EGGD was prevalent across all three groups of equids. Although the inflammatory conditions and their distribution were similar in horses and donkeys, differences in the location, frequency, and severity of mucosal lesions were observed among the three species.

Introduction

Prevalence studies of equine glandular gastric disease (EGGD) are relatively scarce and show considerable variability in terms of methodology and geographical representation. Reported prevalence rates range widely from 6.00 to 72.00%, depending on horses breed and type of sports or work activities.[1-5] In donkeys, prevalences between 2.60 and 100% have been described.[6,7] Studies in mules are scarce; however, an occurrence of up to 20.00% has been described.[8]

Predisposing factors of EGGD are not yet well described, although factors, such as breed, intensity and frequency of exercise, stress, changes in the microbiome, use of anti-inflammatories, and association with trainers, although controversial, have been pointed out to induce lesions of the glandular mucosa *via* altering its protective mechanisms.[4,9-12] Recently, a prevalence of 67.00% was reported in horses used in educational practices, indicating that psychological stress associated with student activities was a predisposing factor in this population.[13] Therefore, the pathophysiology of EGGD is considered multifactorial, with intensive management factors being the most studied compared to the different mechanisms of occurrence in equine squamous gastric disease (ESGD).[14-16]

Differences in prevalence studies, controversies in pathophysiology, and simultaneous presence of EGGD and ESGD in most horses indicate differences in defense mechanisms of each mucosa against variability in the intensity of ulcerogenic causes and management particularities according to the type of activity equids perform.[4,9] Therefore, conducting this study in different equid species in various contexts will contribute to the understanding of EGGD.

This study aimed to determine and compare the post-mortem prevalence of EGGD and other findings in the

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gastric lumen in equids (horses, donkeys, and mules) to identify and compare macroscopic and microscopic characteristics in horses *versus* donkeys *versus* mules.

Materials and Methods

Study design. Three hundred equids stomachs were collected immediately after slaughter, corresponding to 100 stomachs of each species (horses, donkeys, and mules). The animals were distributed by sex, body condition score (BCS),[17] and age, arranged into the corresponding age groups: Young (under 5 years), adults (6 - 14 years), and geriatric (over 15 years). All animals were of Creole breed. The equids originated from various geographical regions and were subjected to the long-distance transport (up to 24 hr), prolonged fasting, and stress. These conditions prevented us from accurately determining the management systems or identifying predisposing factors for gastric syndrome. In addition, they had to wait an average of 12 hr to be slaughtered and have their stomachs evaluated. Clinical signs consistent with equine gastric ulcer syndrome (EGUS) were considered during the initial examination.

Each stomach was incised along the greater curvature, from the cardia to the pyloric antrum (PA), to fully expose the gastric mucosa. The contents were then removed and the interior thoroughly rinsed with water. Following this, the glandular mucosa was examined, focusing on the ventral fundus, area adjacent to the margo plicatus, and PA. Lesions were described and classified according to the 0 - IV scale adapted from the EGUS Council consensus[18] and modified for EGGD.[19] Where 0 is mucosa without injury, I is mucosa without lesion but with hyperemic areas, II is one to five small, focal, or multifocal superficial lesions (up to 0.20 cm deep), III is five or more small, focal, or multifocal superficial lesions (up to 0.20 cm deep), and IV is extensive lesion with areas of deep ulceration (more than 0.20 cm deep).

Glandular mucosa samples were randomly taken from 10 stomachs of each group for conventional histo-pathological analysis with Hematoxylin and Eosin staining for the microscopic description and comparison of the EGGD lesions of each equid group. In addition, during the macroscopical inspection process of the glandular mucosa, all findings related or not to EGGD lesions were recorded, such as inflammatory processes (hyperaemia, edema, clots, and erosions) and findings in the luminal compartment (gastric masses, impaction, and parasites). Gastritis was considered in macroscopic evaluation as follows: Chronic: The presence of erosion and acute: The presence of hyperemia and edema, and microscopically, chronic: The presence of erosion and inflammatory cellular infiltrate (mainly macrophages) and acute: The presence of inflammatory cellular infiltrate (mainly neutrophils) and vascular congestion.[20,21]

Statistical analysis. Descriptive statistical methods were used to analyze all the data being presented as means and percentages in comparative frequency tables by species, location of the glandular mucosa, and macroscopic and microscopic descriptions. All data were analyzed using R (version 2023.06.0; RStudio Team, Boston, USA). The population was divided into three groups according to the species (horses, donkeys, and mules), with the same variables analyzed in each group and compared both within and among groups. Data on sex, age group, and body condition for each species were compared using the chi-square test. The Shapiro-Wilk test was used to determine the normality of the data. Frequencies of different gastric lesions among groups were compared using the chi-square test. A significance level of $p < 0.05$ was applied to all tests. For statistical comparison between prevalences, chi-square or Fisher exact tests were used, depending on the distribution of frequencies.

Results

Table 1 presents the characterization of the equine population included in the study, detailing the distribution of individuals according to the sex, age group, and BCS. Within the donkey group, a higher proportion of females and a lower proportion of males were observed compared to the horses ($p = 0.01$). In the mule group, geriatric animals were more frequent and young animals were less frequent compared to the donkeys ($p < 0.003$). The BCS was in values below the ideal condition and very similar in the population; however, the mean (± standard deviation) in horses (3.12 ± 3.30) was slightly higher than that for donkeys (3.00 ± 0.00) and mules (3.01 ± 2.20) with a statistically significant difference ($p = 0.0003$). In addition to the poor body condition, no other clinical signs were recorded.

Description and classification of the presence of EGGD, according to the mucosal region and in each equine population are shown in Table 2. Fundus was more impacted in mules (80.00%) compared to the donkeys (67.00%; $p = 0.04$) and both significantly higher than horses (24.00%; $p < 0.0001$). The margo plicatus was also more affected in donkeys (30.00%) and mules (29.00%) compared to the horses (2.00%; $p < 0.0001$). In mules, the fundus was the most prevalent region ($p < 0.0001$). In horses, both the fundus and PA were similarly and significantly more affected compared to the margo plicatus ($p < 0.0001$). The EGGD lesions of each group of equids are shown in Figure 1.

Presence and distribution of gastritis in the glandular mucosa are presented in Table 3. Gastritis was observed in 65.30% of the equids. Prevalence in horses (75.00%) was statistically higher ($p = 0.01$) compared to the donkeys (58.00%), but not different (p = 0.07) from mules (63.00%).

Table 1. Characterization of the equids population of the study, detailing the distribution of individuals according to the sex, age group, and body condition score (BCS) among Colombian horses, donkeys, and mules (n = 100) subjected to the post-mortem stomach evaluation.

Characteristics	Horses	Donkeys	Mules
Sex			
Female	62	44*	53
Male	38	56*	47
Age			
Young (under 5 years)	15	25	8†
Adult (6 - 14 years)	44	43	40
Geriatric (over 15 years)	41	32	52†
BCS			
Score 2	0	0	2
Score 3	88	100*	95
Score 4	12	0*	3

Mean BCS 1 - 9 for groups, according to the Henneke *et al.*[17] Only the scores found are reported.
*† Symbols indicate difference from horses or donkeys in the same row, respectively ($p < 0.05$).

Acute cases (40.70%) were more usual ($p = 0.01$) than chronic ones (30.70%). Acute cases were the highest ($p < 0.0001$) in horses, followed by donkeys (40.00%) and then mules (12.00%). Chronic cases were more frequent ($p = 0.03$) in mules (41.00%) than horses (27.00%) and donkeys (24.00%). The simultaneous presence of both types of gastritis was seen in almost one third of the mules (29.00%), being higher ($p < 0.0001$) than horses (5.00%) and mules (8.00%). Overall, fundus was the most affected region, followed by PA in all groups ($p < 0.01$). Histopathological study revealed several findings related to the EGGD in horses, donkeys, and mules, being shown in Figure 2 and Table 4. The cellularity in the inflammatory processes in this mucosa was similar in the three groups, and the lymphocytes (51.00%), plasma cells (30.00%), eosinophils (11.00%), and neutrophils (8.00%) were the most abundant cells. Regarding the rest of the findings, most were common among horses and donkeys, with the exception of necrosis that was not observed in the former, but several of the findings present in the other groups were not reported in mules. In general, all the findings indicated inflammatory processes, vascular changes, and loss and thickening of layers of the gastric surface. Also, the presence of nematode larvae was observed in the samples.

During the general evaluation of the stomachs, other findings were reported (Table 5 and Fig. 3), which showed the presence of gastric impaction and intra-gastric parasites identified as *Habronema spp*. in the three groups of equids. The presence of clots in ulcers in the glandular mucosa and ulcers across the antro-duodenal junction was observed in a mule. In all evaluated stomachs, no structures compatible with intra- or extra-luminal granulomatous or neoplastic cases were found, with only one case of serosal angiomatosis in a donkey.

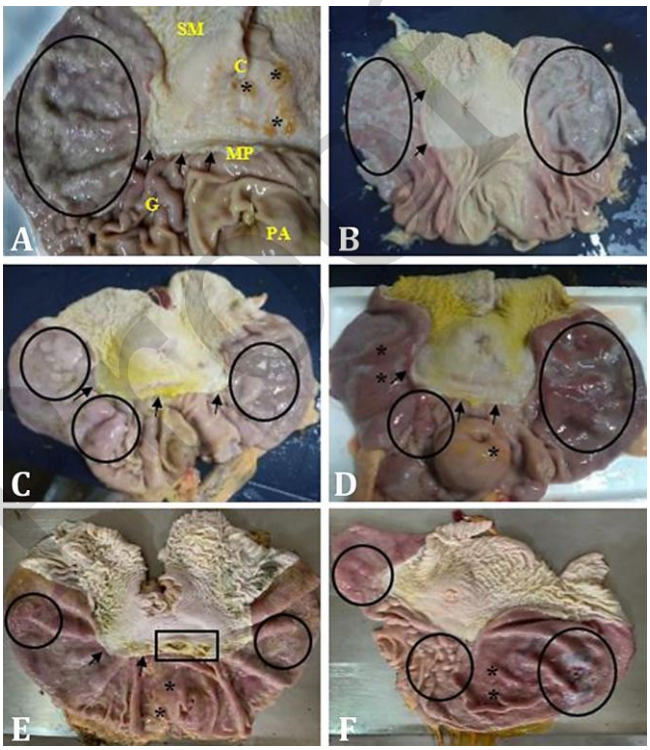

Fig. 1. Presence of equine glandular gastric disease in 300 equids examined. **A** and **B)** Lesions in the gastric mucosa of a horse: Grade IV ulcers in the glandular mucosa (G; circles), hyperkeratosis of margo plicatus (MP; arrows), and ulcerative lesions in the squamous mucosa (SM; *), C: Cardia, PA: Pyloric antrum; **C** and **D)** Lesions in the gastric mucosa of a donkey: Grade III and IV ulcers in the glandular mucosa (circles), hyperkeratosis of MP (arrows), and gastritis (*); **E** and **F)** Lesions in the gastric mucosa of a mule: Grade II and III ulcers in G (circles), gastritis (*), hyperkeratosis of MP (arrows), and ulcers in the SM (rectangle).

Table 2. Post-mortem prevalence of equine glandular gastric disease of the 300 Colombian equids.

Degree	Horses			Donkeys			Mules		
	F	MP	PA	F	MP	PA	F	MP	PA
0	76	98	69	33	70	54	20	71	62
I	1	0	0	0	0	1	1	1	0
II	12	2	17	32	13	29	16	6	6
III	7	0	11	22	12	12	34	13	19
Total positive	24[a]	2[b]	31[a]	67*	30*	46	80[a]*†	29[b]*	38[b]

F: Fundus; MP: Margo plicatus; PA: Pyloric antrum.
[ab] Prevalence rates followed by the same letter did not differ in the same group.
*† Symbols indicate difference from horses and donkeys at the same stomach site, respectively, as $p < 0.05$.

Table 3. Type and location of post-mortem gastritis in the glandular gastric mucosa of the 300 Colombian equids.

Degree	Horses			Donkeys			Mules		
	F	MP	PA	F	MP	PA	F	MP	PA
Acute gastritis	42^{Aa}	5^{c}	23^{Ab}	20^{Aa}	1^{b}	19^{Aa}	0^{Bb}	0^{b}	12^{Aa}
Chronic gastritis	24^{Ba}	3^{b}	1^{Bb}	21^{Aa}	0^{b}	3^{Bb}	33^{Aa}	1^{b}	6^{Ab}
Acute and chronic gastritis	4^{C}	0	1^{C}	8^{Ba}	0^{b}	0^{Bb}	24^{Aa}	2^{b}	3^{Bb}
Total	70^{a}	8^{c}	25^{b}	49^{a*}	1^{c*}	22^{b}	57^{a}	3^{c}	21^{b}

F: Fundus; MP: Margo plicatus; PA: Pyloric antrum.

$^{ABC, abc}$ Prevalence rates followed by the same letter (uppercase within columns and lowercase within rows) did not differ.

*† Symbols indicate difference from horses and donkeys at the same stomach site, respectively, as $p < 0.05$.

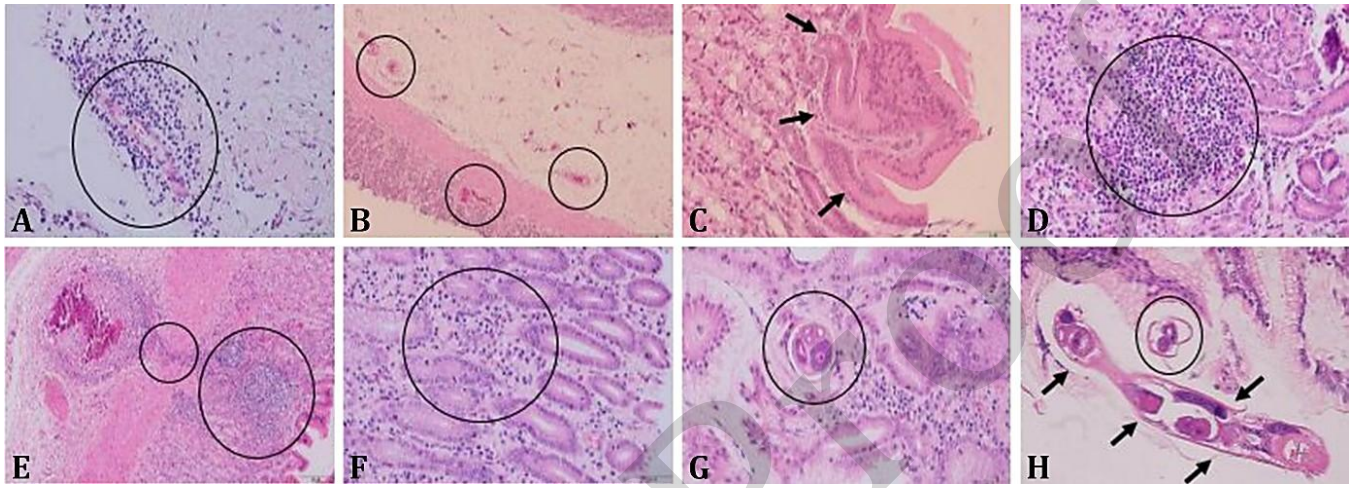

Fig. 2. Histopathological findings in the post-mortem gastric glandular mucosa samples from equids (Hematoxylin and Eosin staining, 10.00 and 40.00 × magnifications). **A)** Peri-vasculitis in the gastric mucosa of a horse (circle); **B)** Vascular congestion in the gastric surface of a horse (circles); **C)** Epithelial hyperplasia in a mule (arrows); **D)** Lymphocyte accumulation in the submucosa of a horse (circle); **E)** Inflammatory infiltrate in the submucosa in a mule (circles); **F)** Eosinophils in the mucosa of a donkey (circle); **G** and **H)** Parasitic form compatible with a nematode in the gastric mucosa of a mule (cross-section [circles] and longitudinal section [arrows]).

Table 4. Histopathological findings in the samples of the glandular mucosa of the stomachs selected from the three groups of equids analyzed.

Histopathological findings	Horses	Donkeys	Mules
Vascular congestion	+	+	+
Epithelial hyperplasia	+	+	-
Exocytosis	+	+	-
Peri-vasculitis	+	+	-
Parasites	+	+	+
Necrosis	-	+	-
Inflammatory cells			
Lymphocytes	+	+	+
Neutrophils	+	+	+
Eosinophils	+	+	+
Plasmocytes	+	+	+

+: Present; -: Absent.

Table 5. Other macroscopic findings in the equids stomachs evaluated in this study.

Findings	Horses	Donkeys	Mules
Gastric impaction	2	24	4
Parasites	11	15	14
Angiomatosis	0	1	0
Glandular mucosa blood clots	0	0	1
Ulcers in antro-duodenal junction	0	0	1

Discussion

Equine glandular gastric disease occurred in the three groups of equids. General prevalence of EGGD (68.30%) reported here was in the upper range of previously reported studies in different breeds of horses for sports and work activities.[4,5,14,15] Despite the high prevalence in this population, it did not exceed the rate of 83.30% lesions in the squamous mucosa reported in a previous work with the same population of equids.[22] Therefore, it is new evidence that the prevalence of EGGD did not exceed that of ESGD,[4,10] although recently higher rates of EGGD have been reported under certain particularities of sports management and training.[10,23,24]

Frequency of EGGD prevalence by groups was similar to that reported in horses (48.90%) and higher than that in mules (19.60%) by gastroscopy exam, and lower than that reported in donkeys (100%) by post-mortem exam from the same geographical region.[4,6,8] However, these studies were carried out at different times, with differences in gastric lesions classification criteria, and in animals with different purposes. In addition, the nature of the study also influences the frequencies of presentation, since a post-mortem approach allows more complete

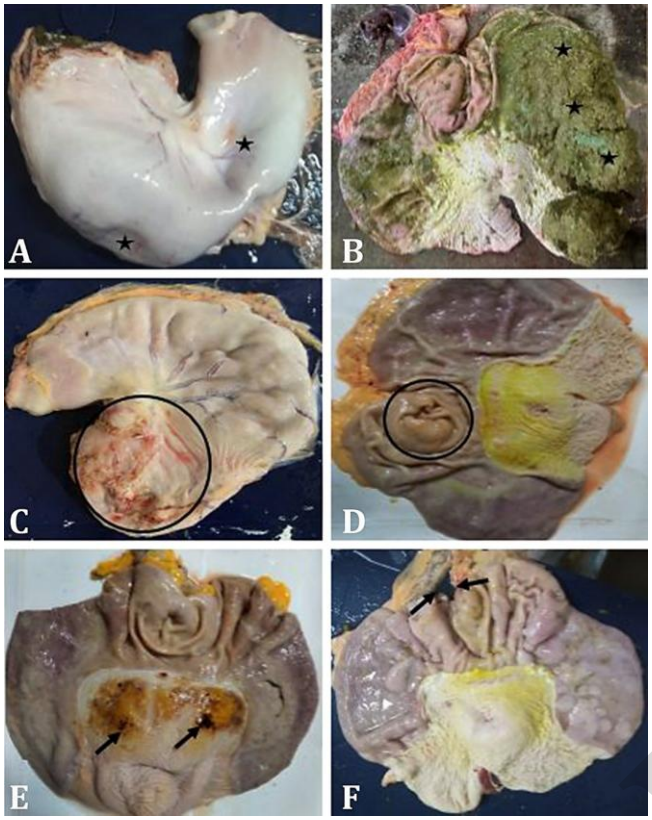

Fig. 3. Other macroscopic gastric findings in the evaluated equids. **A)** Gastric compaction in a donkey: Pasty consistency and digital impressions (★); **B)** Open stomach of a donkey: Compacted plant material (★); **C)** Stomach of a mule showing angiomatosis in the serosa (circle); **D)** Ulcers at the antro-duodenal junction in a mule (circle); **E)** Blood clot in the squamous gastric mucosa of a horse, located between the cardia and pyloric antrum (arrows); **F)** Blood clot in the glandular gastric mucosa of a horse, near the pyloric antrum (arrows).

evaluations than an ante-mortem one,[25-27] the latter characterizing previous studies in equids and mules. In the case of donkeys, both of which were carried out post-mortem and the prevalence was different, indicating that time and previous conditions may influence the results.

Donkeys (77.00%) and mules (83.00%) presented the highest number of glandular lesions, including lesions whose grades corresponded to the highest in the adopted classification. Thus, the severity and location in the fundus were similar between the two groups, consistent with studies carried out previously in these animals.[22] Horses participated with 45.00% of EGGD indicated that, compared to the other groups, there were fewer individuals with high degrees of disease. Also, the PA was the region with the highest number of ulcers, as reported in previous studies.[10,12] Therefore, under the study conditions, there were differences in susceptibility and localization of EGGD lesions among horses, donkeys, and mules.

Probably, the frequency and severity of ulcers in donkeys and mules can be associated with the activities carried out by these animals, since they are preferred for long journeys and carrying out agricultural activities due to their hardiness and efficiency.[8] It is believed that long labor journeys are a risk factor for EGGD development.[6-8] In addition, most of these individuals came from dry regions and far from the slaughter site; therefore, they were subjected to the long-distance travels and solid and liquid fasting, which constitute predisposing factors for EGUS.[28,29] In the case of horses, the low prevalence could be associated with the intensity level of the predisposing factors to which they were exposed,[14,15] since they traveled short distances and most of these animals were destined for other less intense activities.

A limitation of this kind of post-mortem study is that, it was not possible to evaluate the impact of the predisposing factors identified prior to slaughter due to the lack of knowledge about equids previous management, activities, and health conditions. This is particularly relevant in studying EGGD, which may improve, worsen, or resolve without medical intervention.[13] Unlike ESGD, the mechanisms of injury for EGGD still require clarification. However, the effects of stress have been directly related to its pathophysiology,[4] evidencing an increased cortisol response to new stimuli or exogenous adrenocorticotropic hormone in horses.[30,31] Although, this correlation has not been demonstrated in mules and donkeys for both gastric mucosae,[32] which despite being subjected to high stress factors have shown fewer gastric lesions,[8] possibly due to evolutionary differences between these two equids species.[33] In this case, the population was subjected to the various stressful events, involving long-distance transportation, nutritional restriction, heterogeneous groupings, and behavior linked to the aggression and social dominance. However, cortisol was not measured to verify such hypothesis. On the other hand, the effects of anti-inflammatories have been associated with the induction of glandular lesions.[5,23] Although no previous information regarding the use of these drugs was obtained, but due to the presence of chronic pathologies (musculoskeletal lesions) in some animals, it could be presumed that, at some point these drugs were used.

During the ante-mortem general inspection, no clinical signs described for gastric diseases were identified, although no association between clinical signs and EGGD has been reported,[32] in addition to being considered non-specific.[4,9,23,34] However, the BCS of the entire population was low (3.00/9.00), being related to the gastric disease, although more common in ESGD,[35] and in EGGD in severe cases,[36] given the high simultaneous presence of both. This confirmed the non-specificity of the sign. Conversely, the elevated proportion of geriatric individuals (*i.e.*, >15 years of age) might be accounted for the sub-optimal BCS and compromised coat quality observed in certain subjects, rather than being associated with the presence of gastric disease. Although all animals were managed under grazing

and without concentrate supplementation which could also impact the BCS of these animals, the quantity and nutritional quality of forage were not known. Furthermore, other conditions related to the reduced BCS were not investigated, within the scope of this study.

Acute gastritis in the glandular mucosa was more frequent in horses and donkeys; however, chronic gastritis cases in mules persisted as the most frequent, potentially as a result of the intense physical demands imposed on them.[8] Despite the differences in inflammatory reactions between groups, the presence of a greater number of chronic cases in the squamous mucosa reflects the high degree of susceptibility of this mucosa, derived from weak protection mechanisms.[28,37] In the case of the glandular mucosa, acute gastritis could reflect the exacerbation of predisposing factors in this population that exceeded the defense mechanisms,[5,23] including for example prolonged fasting and transportation periods, types of stress, and possibly the use of anti-inflammatories due to the presence of chronic underlying problems in some animals.

Gastric fundus and PA were the most affected sites of the glandular mucosa as previously reported.[5,9,23] The mules did not present macroscopically compatible alterations with acute gastritis in the fundus, indicating that the location and type of inflammatory process were similar between horses and donkeys, with certain differences in the mules. However, the macroscopic findings of acute gastritis did not coincide with the histopathological ones, since the presence of mononuclear cells (lymphocytes and plasma cells) was the most frequent, indicating chronic, focal, regional, and diffuse gastritis between mild, moderate, and severe, according to the density of the inflammatory infiltrate, as previously reported in natural cases of gastric lesions.[20] This showed the low correspondence of macroscopic lesions with microscopic ones, indicating the need to evaluate both aspects in determining the origin of gastric lesions.[21] In addition, the presence of neutrophils in some samples indicated chronic active conditions, probably due to the conditions described in these animals, and the eosinophils related to the presence of parasites both in the tissue and gastric lumen.

Regarding the other gastric findings, the presence of *Habronema spp*. in the lumen and larvae in the mucous membranes has been associated with EGGD lesions.[8,38-40] Such findings were identified herein in the three groups of equids; therefore, their participation in these lesions is possible, associated with the inflammatory pattern and larval findings in histopathology. However, previous works have reported the presence of other parasites, such as *Draschia megastoma*,[38] *Gasterophilus spp*.,[7,41] and *Trichostrongylus axei*[6] as ulcerogenic agents and generators of inflammatory responses. The finding of around 15.00% of *Habronema spp*. in this population could infer a deficient preventative health care, given the lack of history available about the anthelmintic use in the presented population.

Gastric impactions observed in equids most likely evidenced the long periods of fluid fasting prior to slaughter (24 hr at least), causing dehydration of the gastric content.[42] Association between gastric impaction and dental alterations and other gastrointestinal problems has been reported;[43-45] however, such information was not considered herein. Gastric impaction was more frequent in donkeys (24.00%), with percentages higher than those previously reported,[6,42] possibly because the animals came from drier regions in addition to prolonged fasting periods. The *Habronema spp*. and antro-duodenal junction were possibly the mechanical factors of the parasites and gastric impactions as potential ulcerogenic factors.[46] Finally, no lesions compatible with neoplasia were found in the 300 stomachs evaluated, being in accordance to previous studies reported a low prevalence.[47,48]

In conclusion, this study evidenced the presence of EGGD in the three groups of equids (68.30%). The inflammatory conditions and their location were similar between horses and donkeys, while the mucosa showed some differences in the location, frequency, and severity of the lesions between horses, donkeys, and mules. However, in the absence of prior clinical information about the animals involved in the study and the exaggerated submission to identified predisposing factors to EGUS, it was difficult to assess the dynamics of naturally induced EGGD; therefore, the prevalence was high for this population. Nevertheless, this study allowed a comparison of macroscopic and histopathological findings among the three groups.

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Conflict of interest

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

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