*FULL ARTICLE*

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# Activated charcoal application in gastric ulceration areas in horses: preliminary experimental study

## *Aplicação do carvão ativado em áreas de ulceração gástrica em cavalos: estudo experimental preliminar*

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**ABSTRACT**

Gastric ulceration is frequent in adult horses, bringing significant economic and welfare implications to these animals. Treatment may employ several drugs, but adsorbents, such as activated charcoal, are not mentioned. Activated charcoal may be a valuable therapeutic option mainly because of its few adverse effects, fecal elimination, and low cost. This study aimed to evaluate the use of activated charcoal in experimentally induced gastric ulceration in horses and perform the microscopic characterization of charcoal particles in the injured epithelium. Five adult, undefined breed horses underwent a gastric ulcer induction protocol based on alternating fasting and free food access periods. Next, we performed a gastroscopic examination and applied activated charcoal diluted in water at the ulcers through a probe passed through the working channel of the gastroscope. The gastric mucosa was washed with water after a contact period of 5 min until the complete lack of macroscopic product visualization. Then, we performed a biopsy of the aglandular mucosa in erosive to ulcerative injury sites and a microscopic assessment of the fragments. Macroscopically, all animals presented ulcerative lesions in the greater or lesser gastric curvature along the *margo plicatus* and hyperkeratosis in the aglandular portion. Microscopically, all animals had hyperplasia, hyperkeratosis, epithelial erosion, desquamated epithelial cells, inflammatory cells, and variable amounts of charcoal particles, either free or adhered to the epithelium. We concluded that activated charcoal remains adhered to aglandular gastric lesions in horses even after lavage with water and histological processing, allowing its microscopic visualization. Therefore, activated charcoal's effectiveness as a therapy for equine gastric lesions warrants evaluation.

**Keywords:** Adsorbent. Biopsy. Gastroscopy. Histopathological analysis. Stomach ulcer.

**RESUMO**

A ulceração gástrica é identificada com frequência em cavalos adultos, trazendo implicações econômicas e de bem-estar importantes a estes animais. Embora existam vários fármacos que possam ser empregados no tratamento, a utilização de adsorventes como o carvão ativado ainda não é citada, podendo ser de grande valia como opção terapêutica, principalmente por ter poucos efeitos adversos, ser eliminado nas fezes e ter baixo custo. O objetivo do estudo foi avaliar pela primeira vez o uso de carvão ativado em casos de ulceração gástrica induzida experimentalmente em equinos e a caracterização microscópica das partículas de carvão em relação ao epitélio lesionado. Foram utilizados cinco cavalos adultos, sem raça definida que foram submetidos a protocolo de indução de úlceras gástricas baseado em alternância entre jejum e acesso livre ao alimento. Após esse período, foi realizado exame gastroscópico seguido da aplicação sobre as úlceras do carvão ativado diluído em água através de uma sonda passada pelo canal de trabalho do gastroscópio. A mucosa gástrica foi lavada com água após cinco minutos de contato, até a completa ausência de visualização macroscópica do produto. Logo após foi realizada biópsia da mucosa aglandular, em locais em que era possível observar lesões erosivas a ulcerativas, e os fragmentos foram avaliados microscopicamente. Macroscopicamente, todos os animais apresentaram lesões ulcerativas em curvatura maior ou menor do estômago, ao longo da região de *margo plicatus*, com hiperqueratose na porção aglandular. Microscopicamente, foram observadas hiperplasia, hiperqueratose, erosão do epitélio, células epiteliais descamadas, células inflamatórias e partículas de carvão aderidas ao epitélio ou livres em quantidade variável em todos os animais. Concluímos que o carvão ativado permanece aderido às lesões gástricas aglandulares em equinos mesmo após a lavagem com água e após o processamento histológico, podendo ser observado microscopicamente, devendo-se agora avaliar sua efetividade como terapia para lesões gástricas em equinos.

**Palavras-chave:** Adsorvente. Biópsia. Gastroscopia. Histopatológico. Úlcera estomacal.

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## Introduction

Gastric ulceration in horses has multifactorial etiologies and causes several clinical manifestations, being therefore defined as equine gastric ulceration syndrome (EGUS) (Murray, 2006). This syndrome is frequent in foals and adult horses undergoing intense training and exercise (Tamzali et al., 2011). In addition to its significant economic implications, EGUS reduces the welfare of the affected animals since they may present colic, weight loss, and decreased performance (Silva et al., 2001; Lesimple, 2020). In a study from the United Kingdom, EGUS prevalence rates were approximately 65% in domesticated horses and 26% in wild horses (Ward et al., 2015). A study with horses from a slaughterhouse revealed that only 4.76% of subjects did not present macroscopic gastric mucosal changes (Souza et al., 2014).

The equine stomach has two distinct anatomical regions, the glandular and aglandular portions. Since the aglandular stomach presents only a squamous epithelium barrier for protection, it is more susceptible to ulcerative and erosive lesions (Nadeau & Andrews, 2009; Sykes & Jokisalo, 2014). The *margo plicatus* separates both portions and forms the lower border of the aglandular gastric mucosa. This region is similarly susceptible to erosive and ulcerative injuries (Martineau et al., 2009a). In contrast, the glandular mucosa has a bicarbonate-rich protective mucus layer to neutralize the acidity on the surface of the stomach epithelium (White et al., 2009). Although EGUS is often used to describe ulcerative diseases of the stomach, the terms equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD) refer more specifically to the involved anatomical regions and are proposed by the European College of Equine Internal Medicine Consensus Statement (Sykes et al., 2015). There are also differences regarding the pathophysiology of these clinical conditions. Exposure to the acidity of gastric juice is one of the leading causes of ESGD (Sykes & Jokisalo, 2015a). The major causes of EGGD include changes in the protective mechanisms of the glandular mucosa resulting from the excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) and opportunistic pathogens (Sykes & Jokisalo, 2015b).

Diagnosis of equine gastric ulceration in different anatomical stomach regions relies on gastroscopy, which also allows determining a severity score for macroscopic injuries. The Equine Gastric Ulcer Council grading system (The Equine Gastric Ulcer Council, 1999) considers the presence or absence of hyperemia and hyperkeratosis and the size, number, and depth of injuries, classified on a scale from 0 to 4. Microscopic evaluation of gastric mucosa fragments includes hyperplasia, hyperkeratosis, inflammatory infiltrate, hemorrhage, and injury extension and depth (Andrews et al., 2002; Martineau et al., 2009b).

The conventional gastric ulceration treatment consists of drugs decreasing hydrochloric acid production, such as histamine H2-receptor antagonists and proton pump inhibitors (Baron & Perrin, 1983; Nieto et al., 2010). Studies demonstrated the effectiveness of omeprazole in preventing and treating gastric ulcers in young horses undergoing intense training (Mason et al., 2019). Mucosal protectors to neutralize the acid secretion from the gastric glandular epithelium, such as sucralfate, may also play a role in treating the syndrome. These agents adhere to the ulcerated mucosa, forming a protein layer by stimulating prostaglandin E2 (PGE2) synthesis and mucus secretion (Zavoshti & Andrews, 2017). Several herbal medicines have an antiulcerogenic activity and a potential role in EGUS preventive management in horses under training (Sharifi-Rad et al., 2018).

Recently, studies on treating ulcerated skin wounds in humans with activated charcoal revealed good outcomes (Scheer et al., 2017). The most significant feature of activated charcoal is that it is an adsorbent, forming a stable complex with many substances to facilitate their elimination from the body in cases of oral poisoning. The first description of the adsorbent properties of charcoal dates from the 1700s, and charcoal has been studied in drinking water treatment, as a cosmetics component (Derlet & Albertson, 1986; Westphalen et al., 2016), and to treat ulcerated wounds (Scheer et al., 2017). Charcoal can adsorb drugs, chemicals, toxins, gastrointestinal enzymes, and nutrients (López & Camberos, 2006). It is mainly used for treating diarrhea and intoxication. Its effectiveness is not reduced by pH

variations in the digestive tract, allowing the elimination of the adsorbed material in the feces (Juurlink, 2015). Activated charcoal is a treatment for gastrointestinal bleeding resulting from adverse effects of anticoagulants in human patients (Cheung & Leung, 2017). It can also be an adjuvant in chronic kidney disease treatment by reducing hyperphosphatemia and vascular calcification (Gao et al., 2019). Recent studies also advocated activated charcoal to treat chronic, foul-smelling wounds in humans with good results (Murphy, 2016; Haynes, 2018). According to Kerihuel (2010), chronic ulcerated cutaneous wounds in humans treated for four weeks with silver-impregnated activated charcoal showed a significant reduction in size, edema, and exudation.

Although several drugs can treat gastric ulcerations in horses, adsorbents, such as activated charcoal, represent a compelling and potentially valuable therapeutic option in equine clinical medicine and surgery as it is cheap, highly effective, and readily eliminated in feces. Activated charcoal application in gastric injuries may have other benefits because of its superficial adhesion, forming an additional protective layer to adsorb cell debris and substances potentially interfering with tissue repair. In addition, directly applying activated charcoal to the gastric mucosa via the oral route is easy.

Thus, the initial experimental demonstration of the feasibility of oral or endoscopic (via the working gastroscope channel) application of activated charcoal in horses and the microscopic characterization of charcoal particles in the lumen or epithelial surface even after gastric lavage may represent a promising new treatment strategy for EGUS. In this context, this study aimed to evaluate, for the first time, the use of activated charcoal in experimentally induced gastric ulceration in horses and to perform the microscopic characterization of charcoal particles in the gastric epithelium.

## Materials and Methods

The Ethics Committee on Animal Use from the Faculty of Veterinary Medicine and Animal Sciences, University of São Paulo, approved this project under number 7602220715.

This study used five horses of undefined breed, with an average age of 3.2 years and an average weight of 288.5 ± 20.1 kg. All animals underwent a physical examination before the gastric ulceration induction protocol.

Gastric ulceration induction occurred as proposed by Murray (1994) and Murray et al. (2001), with the animals allocated to individual pens and undergoing alternating periods of 24 h fasting and 24 h of free access to food (ad libitum hay) for five days, totaling 72 h of fasting. Next, we performed a gastroscopic examination of the animal, which was sedated and standing up. Gastroscopy preparation included fasting for 12 to 18 h, sedation with xylazine (0.7 mg/kg IV), and physical restraint in a suitable environment. After gastric insufflation and distension, we proceeded to the macroscopic evaluation of the stomach, consisting of visualization of the entire aglandular mucosa, *margo plicatus*, the visible portion of the glandular mucosa, and the greater and lesser gastric curvatures. Aglandular mucosa lesions were evaluated, photographed, and classified per the Equine Gastric Ulcer Council grading system (The Equine Gastric Ulcer Council, 1999). We also assessed the glandular stomach using an estimated visualization percentage. Chart 1 describes the macroscopic scores used for gastric injury grading.

Captor® (Inovet®) includes activated charcoal, zeolite, inulin, pyridoxine, glutamic acid, and zinc chelate.

After this assessment, we applied 8 g of activated charcoal (Captor®, Inovet®, Rio de Janeiro, Brazil) diluted in 100 mL of water using a probe (300 cm long, 5 mm in diameter) through the working channel of the gastroscope (Olympus Corporation®, Tokyo, Japan). The total volume of the aqueous charcoal solution was administered at the erosions or ulcerations in a single application (Figure 1). After 5 min, we proceeded to mucosal lavage with water until the macroscopic disappearance of the particles. The injured aglandular gastric mucosa was biopsied via the working gastroscope channel using biopsy forceps (325 cm long, 2.5 mm in diameter) (Figure 2).

The fragments were fixed in a 10% buffered formalin solution for at least 48 h and sent for standard processing to prepare histological slides. Briefly, samples were embedded

Chart 1 – Description of the endoscopic injury scoring system proposed by the European Consensus on Equine Gastric Ulcers (The Equine Gastric Ulcer Council, 1999). São Paulo, 2021.

| Lesion degree | Macroscopic changes |
|---|---|
| 0 | Intact epithelium with no hyperemia (reddening) or hyperkeratosis (yellowish squamous mucosa). |
| 1 | The mucosa is intact, but there are areas of (squamous) hyperemia or hyperkeratosis. |
| 2 | Small, single, or multifocal lesions. |
| 3 | Large, single, or multifocal lesions or extensive superficial lesions. |
| 4 | Extensive lesions with areas of apparent deep ulceration. |

in paraffin, cut into 5 µm sections, and stained with hematoxylin-eosin. The pathologist evaluating the slides was unaware of the changes observed in the endoscopic gastric examination and the identification of each case.

The histological scoring of the lesions was modified and adapted from Andrews et al. (2002) and Martineau et al. (2009b) to characterize endoscopic fragments representing only part of the mucosa. The histological evaluation considered the presence of charcoal particles in the samples in terms of quantity, location, and distribution (H1), in addition to epithelial (H2) and inflammatory (H3) changes. Chart 2 presents the scores and semiquantitative histological evaluation criteria used. Each animal received a score calculated as the sum of charcoal particle presence (H1) and the degree of mucosal damage (H2 + H3). The charcoal particle score ranged from 0 to 4, with zero indicating the lack of particles and four referring to charcoal particle observation in the gastric lumen and cells. The lesion score ranged from 0 to 8 per involvement severity, lesion extent and distribution, and cell type; zero indicated the absence of histological lesions, and eight referred to moderate to marked mucosal lesions. The results were presented descriptively, along with individual scores for each horse. We performed a correlation analysis for the macroscopic score, charcoal amount, and microscopic score using the GraphPad Prism 6.0 software for non-parametric variables.

## Results

No horse exhibited clinical changes during the procedure or within the next 24 h. All animals undergoing the

Figure 1 – Small erosive to ulcerative lesions in the aglandular gastric mucosa (score 2).

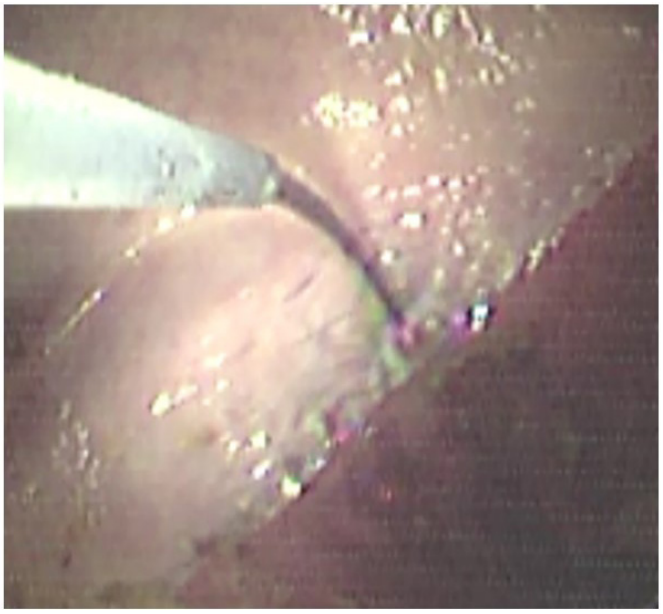

Figure 2 – Application of diluted activated charcoal to the aglandular gastric mucosa through the working channel of the gastroscope.

Chart 2 – Scores and criteria for semiquantitative microscopic assessment of the gastric mucosa for the presence of charcoal particles and epithelial and inflammatory morphological lesions. São Paulo, 2021.

| Parameter | Charcoal particles (H1 = 0-4) | Epithelial changes (H2 = 0-4) | Inflammatory changes (H3 = 0-4) |
|---|---|---|---|
| Score 0 | Absent | Absent | Absent |
| Score 1 | Few (< 3) fine-grained foci in the lumen at any magnification | Hyperplastic and intact epithelium; variable hyperkeratosis | Rare inflammatory cells |
| Score 2 | Up to 5 foci of variable granulation in the lumen and few epithelial cells | Hyperplastic and intact epithelium; evident hyperkeratosis | Few inflammatory cells |
| Score 3 | Up to 10 foci of variable granulation in the lumen and few epithelial cells | Hyperplastic and eroded epithelium, epithelial cell desquamation; evident hyperkeratosis | Moderate number of inflammatory cells mixed with epithelial and red blood cells |
| 4 | More than 10 foci of variable granulation in the lumen and few epithelial cells | Hyperplastic and ulcerated epithelium, epithelial and red blood cells in the lumen, evident hyperkeratosis | A large number of inflammatory cells mixed with epithelial and red blood cells |

experiment and gastroscopic examination had ulcerative lesions in the greater or lesser curvature of the stomach (5/5), next to the *margo plicatus*, and hyperkeratosis (4/5) in the aglandular portion. The score ranged from 2 (4/5) to 3 (1/5). Table 1 describes the changes and the macroscopic assessment scores. Horse 1 had the highest injury degree and extent (score 3). None of the animals showed changes in the glandular portion of the stomach. Figure 1 exemplifies the macroscopic changes in the aglandular mucosa.

The application of activated charcoal diluted in water (Figure 2) through the gastroscope's working channel, aided by a plastic probe (to avoid possible clogging and sedimentation of the charcoal inside the working channel), proved to be easy and enabled application in each individualized injury.

Table 2 shows the histological changes, and Figure 3 illustrates the microscopic changes. We observed epithelial hyperplasia and evident hyperkeratosis (5/5), epithelial erosion with neutrophilic exudation (3/5), epithelial cells, and desquamated inflammatory cells (5/5). All animals presented black charcoal particles in hematoxylin-eosin-stained slides. Charcoal amounts ranged from less than three (score 1) to more than 10 foci at 100x magnification (score 4). Horse 1 had the highest number of charcoal particles and injuries. However, the injury degree was similar among all horses, ranging from 5 to 6 (i.e., moderate injury degree) (Table 2). The correlation analysis revealed a strongly positive association between macroscopic and microscopic scores, with $R = 0.9484$ ($p<0.0001$, Spearman correlation test, GraphPad Prism 6.0). There was no correlation between the presence and quantity of charcoal particles and macroscopic and microscopic scores.

## Discussion

During the clinical evaluation period, none of the animals showed signs of pain, and the induced ulcers observed mainly presented mild severity, which corroborates the safety of the experimental protocol described.

The mucosal lesions caused by the 72 h fasting/feeding induction protocol were consistent with the expectations, allowing the study of the diluted activated charcoal application on mucosal surfaces and subsequent histological evaluation. The proposed design was satisfactory and showed that charcoal application by gastroscopy is feasible and allows for covering individual injured areas. We selected this

Table 1 – Description of mucosal changes and macroscopic lesion score at gastroscopic evaluation. São Paulo, 2021

| Animal | Gastroscopic evaluation description | Lesion score |
|---|---|---|
| 1 | A large ulcerative lesion in the aglandular portion of the greater curvature and several coalescent ulcerative lesions in the lesser curvature close to the *margo plicatus*; mild *margo plicatus* hyperkeratosis; unaltered glandular portion, with visualization of approximately 40% of the area. | 3 |
| 2 | Small ulcerative lesions in the aglandular portion close to the *margo plicatus* of the lesser curvature of the stomach; mild *margo plicatus* hyperkeratosis; normal glandular portion, with visualization of approximately 50% of the area. | 2 |
| 3* | Small, multifocal ulcerative lesions in the aglandular portion close to the *margo plicatus* of the lesser curvature of the stomach; moderate hyperkeratosis throughout the *margo plicatus*; normal glandular portion, with visualization of approximately 40% of the area. | 2 |
| 4 | Small ulcerative lesions in the aglandular portion close to the *margo plicatus* of the greater curvature of the stomach; mild *margo plicatus* hyperkeratosis; normal glandular portion, with visualization of approximately 30% of the area. | 2 |
| 5 | Small ulcerative lesions in the aglandular portion close to the *margo plicatus* of the greater curvature of the stomach; normal glandular portion, with visualization of approximately 40% of the area. | 2 |

*Ulcer border sampling was not feasible due to positioning issues, creating an ulcer by biopsy close to the *margo plicatus*.

Table 2 – Description of the histological analysis findings. São Paulo, 2021

| Animal | Histological analysis findings | H1 | H2 + H3 |
|---|---|---|---|
| 1 | Hyperplastic stratified squamous epithelium with areas of erosion and neutrophil exudation. Coal particles present in more than 10 foci (n*=2). | 4 | 6 |
| 2 | Hyperplastic stratified squamous epithelium, erosion focus, desquamated cells, and charcoal particles in less than 3 foci. Few neutrophils (n=3). | 1 | 5 |
| 3 | Hyperplastic stratified squamous epithelium with a discrete luminal infiltrate of intact and degenerated neutrophils, desquamated epithelium, and charcoal particles in less than 3 foci (n=2). | 1 | 5 |
| 4 | Hyperplastic stratified epithelium, moderate numbers of intact and degenerated neutrophils, and charcoal particles in up to 10 foci (n=1). | 3 | 6 |
| 5 | Hyperplastic stratified epithelium, neutrophil exudation, desquamated epithelium, and charcoal particles in up to 10 foci (n=4). | 3 | 5 |

*n = number of fragments.

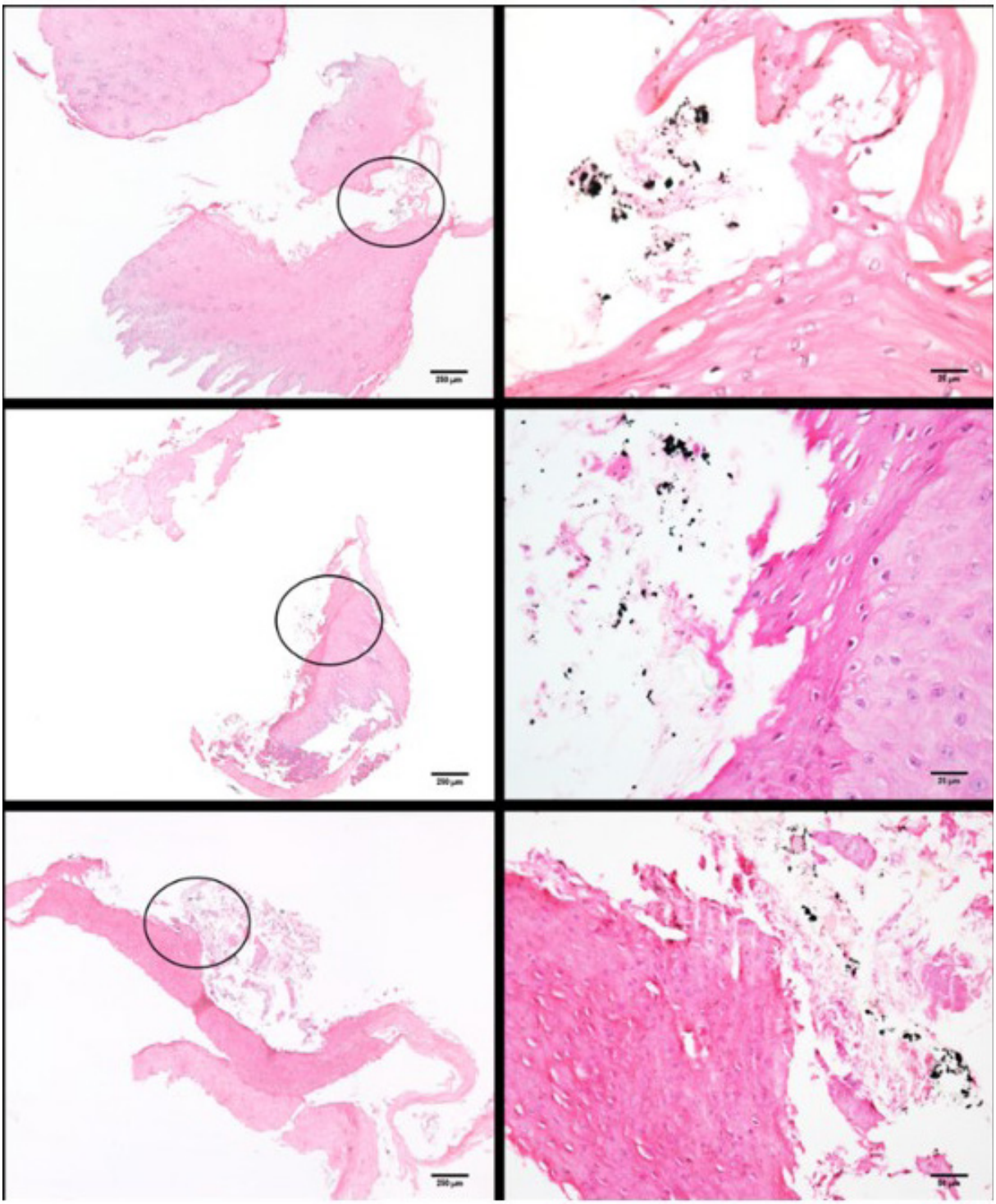

Figure 3. Photomicrographs of the aglandular epithelial surface of the equine stomach. A-C show charcoal particle foci with variable granulation in the lumen, intact (B), and degenerating (A and C) desquamated superficial epithelial cells—hematoxylin-eosin stain; bar in micrometers.

application procedure to ensure the product's contact with the lesions and test its technical feasibility. Therefore, initially, treatment can occur during the gastroscopic evaluation of the mucosa for diagnosis. We did not test the nasogastric application route, but, in principle, there is no known contraindication for its use.

Microscopic analysis of the fragments showed that the gastric mucosal injury induction was effective. It also revealed a relationship between injury visual inspection findings and the macroscopic score. Horse 1 had the highest macroscopic and microscopic scores (H2 + H3). Visualization of charcoal particles adhered to the epithelial surface or luminal content shows that charcoal application in lesions can be analyzed under microscopy and remains residual after the lavage and histological processing. The correlation between macroscopic and microscopic lesion scores was

positive and statistically significant. However, we did not detect any correlation with the amount of charcoal during the microscopic analysis.

The most striking result of this experiment was the presence of activated carbon particles in samples from all animals, even after gastric lavage with water. Although we do not know how long the product remains adhered to injured surfaces under practical conditions with no fasting, we emphasize that charcoal particle observation was possible even after the histological processing of the biopsy material, including an approximately 12 h bath in alcohol at increasing concentrations, xylene, and paraffin. This finding may indicate the proper charcoal adherence to injured areas, potentially benefiting the animal.

It is also worth noting that our results are valid only for the aglandular mucosa since this was the lesion assessment site. Further evaluation of activated charcoal adherence to glandular lesions and more severe gastric injuries is required.

The action of activated charcoal in healing gastric lesions and its interaction with them remains unknown in any animal species. Here, we demonstrated the permanence of activated charcoal on injured areas, a promising finding considering charcoal as a therapeutic strategy. Studies conducted with a control group may add interesting results and answers regarding the applicability of charcoal to treat equine gastric injuries, how long charcoal remains on lesions, its interaction with glandular ulcers, and whether there is a real benefit for their repair. As activated charcoal had good results in skin wounds (Murphy, 2016; Scheer et al., 2017; Haynes, 2018), we believe the same can happen in gastric ulcerations.

## Conclusion

We concluded that activated charcoal remains adhered to aglandular gastric lesions in horses even after water lavage and histological processing since it was observed microscopically. Now, its effectiveness in equine gastric lesions warrants evaluation.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

## Ethics Statement

We declare that the Ethics Committee on Animal Use from the Faculty of Veterinary Medicine and Animal Sciences, University of São Paulo, approved this project under number 7602220715.

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