

Histopathological Detection of *Helicobacter* Like Organisms in Gastric Mucosa of Spanish Horses

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ABSTRACT

The aim of this study was to detect the presence histopathological of *Helicobacter* (HLO: *Helicobacter* Like Organism) in gastric mucosa of Spanish horses. Were studied 12 horses with clinical signs for equine gastric ulcers syndrome in the Department of Anatomy and Comparative Anatomic Pathology, College of Veterinary Medicine. University of Cordoba, Spain. All horses were necropsies. Samples of gastric tissue were collected. The tissue samples fixed in formalin were processed by conventional histological techniques. Additionally, the special staining procedure of Warthin-Starry, were also carried out. Bacteriology culture from stomach performed. All cases were examined by histopathology. Of the 12 horses studied, 8% Grade 0 Epithelium is intact throughout; no hyperemia, no hyperkeratosis (yellowish color, sloughing). Grade 1 Mucosa is intact but there are areas of hyperemia and/or hyperkeratosis 33%. Grade 2 Small, single or multi-focal erosions or ulcer s 42%. Grade 3 Large, single or multi-focal

ulcers, or extensive erosions and sloughing 8%. Grade 4 Extensive ulcers, with areas of deep submucosal penetration 8%. Using the Warthin-Starry special stain, spiral-shaped bacteria in the gastric mucosa glandular were found in 8/12 (67%), Grade 1: 75%, Grade 2: 60%, Grade 3: 100%, Grade 4: 100%. There was no bacterial growth in either case. To conclude, we detected the presence histopathological of *Helicobacter* Like Organisms in the gastric mucosa with Equine Gastric Ulcer Syndrome of Spain horses.

INTRODUCTION

Helicobacter pylori and *Helicobacter*-associated organisms (HLO: *Helicobacter* like organisms) are organisms or cocos spiral-curved, Gram-negative, inhabitants of the gastric glands, parietal cells and mucus stomach. These bacteria are associated with inflammatory disease and ulceration of the gastric mucosa (acute gastritis, chronic gastritis, gastric ulceration and gastropathies). The number of species of the genus *Helicobacter* has expanded rapidly over the past decade (Fox, 2002). The genus now includes at least 24 formal names of species as well

as numerous species of *Helicobacter* waiting for its formal identification (Fox, 2002). They have been classified based on their sequence 16rRNA, DNA hybridization and morphology in electron microscopy Gastric *Helicobacter* species and enterohepatic *Helicobacter* species. Dimola & Caruso (1999) reported the presence of rod-shaped organisms with morphology similar to that commonly reported for *Helicobacter pylori* in the stomach of 15 horses. However, after this single preliminary report, horses have never been confirmed as hosts for *Helicobacter*-like organisms (Moyaert, et al., 2007). A new enterohepatic *Helicobacter* species, *Helicobacter equorum*, was isolated from fecal samples of two clinically healthy horses (Videla and Andrews, 2009). Also, *Helicobacter equorum* DNA was found in the feces of two of seven (28.6%) foals less than 1 month of age and 40 of 59 (67.8%) foals 1 to 6 months of age (Moyaert, et al., 2009). Furthermore, *Helicobacter*-like DNA was detected in the stomach of 10 Thoroughbred horses in Venezuela (Contreras, et al., 2007). In this study, *Helicobacter*-like DNA was detected in two of seven horses with gastric ulcers, three of five horses with gastritis, five of six horses with both pathologies, and one horse with normal gastric mucosa (Contreras, et al., 2007; Videla and Andrews, 2009). Furthermore, 10 of 11 of the horses infected with *Helicobacter* had either gastric ulcers or gastritis or both pathologies. However, 39% of the horses in that study did not have gastric lesions, so multiple causes are likely (Hepburn, 2004;

Videla and Andrews, 2009). There are no reports in the literature of *Helicobacter* in horses in Spain. The aim of this study was to detect the presence histopathological of *Helicobacter* (HLO: *Helicobacter* Like Organism) in gastric mucosa of Spanish horses.

MATERIALS AND METHODS

Were studied 12 horses with clinical signs for equine gastric ulcers syndrome. Race Spanish Purebred 5 and 8 crossbreeding of Spanish Purebred. They were referred for necropsy (Protocol by necropsy for horses by Aluja and Constantino, 2002) with previous history of colic, in the Department of Anatomy and Comparative Anatomic Pathology, College of Veterinary Medicine. University of Cordoba, Spain. In relation to sex: 8 males and 4 females. The ages were classified in a foal of 4 months and 11 horses of 4-12year old. All horses were necropsies. The gastric mucosa was evaluated for classified Merrit, 2003: Grade 0 Epithelium is intact throughout; no hyperemia, no hyperkeratosis (yellowish color, sloughing). Grade 1 Mucosa is intact but there are areas of hyperemia and/or hyperkeratosis. Grade 2 Small, single or multi-focal erosion or ulcer. Grade 3 Large, single or multi-focal ulcers, or extensive erosions and sloughing. Grade 4 Extensive ulcers, with areas of deep submucosal penetration. Samples of gastric tissue were collected. The tissue samples fixed in formalin were processed by conventional histological techniques (dehydration, inclusion in paraffin, microtome slicing and routine staining with Hematoxylin-eosin)

and examined by histopathology (Banks, 1996). Additionally, the special staining procedures of Warthin-Starry were also carried out (Morales, et al., 2010). Bacteriology culture from stomach performed.

RESULTS

Of the 12 horses studied, 8% Grade 0 Epithelium is intact throughout; no hyperemia, no hyperkeratosis (yellowish color, sloughing). Grade 1 Mucosa is

Table 1. Result of macroscopic lesions, histology and special staining.

Category	Number Equine	Histology	Warthin Starry
Grade 0	1	Normal	0
Grade 1	4	Gastritis Acute	3
Grade 2	5	Gastritis Chronic	3
Grade 3	1	Erosion & Ulcer	1
Grade 4	1	Ulcer	1
Total	12	12	8

Figure 1. Stomach with Equine Gastric ulcer syndrome Grade 3. Large, single or multi-focal ulcers, or extensive erosions.

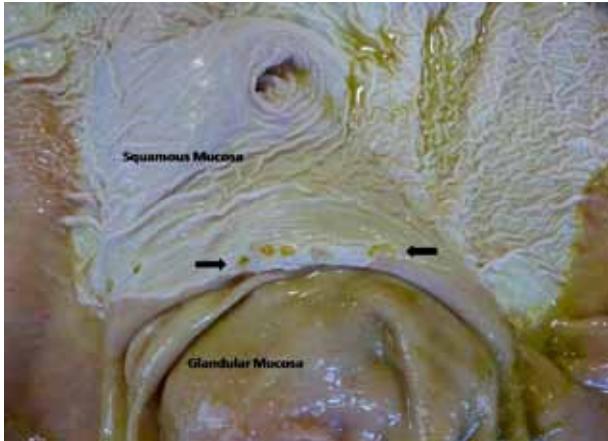


Figure 2. Stomach with Equine Gastric ulcer syndrome Grade 4. Extensive ulcers, with areas of deep submucosal penetration.



Figure 3. Gastric mucosa with Equine Gastric ulcer syndrome Grade 4. Erosion with infiltrated lymphocytic and damage of lamina propria (H&E 4X).

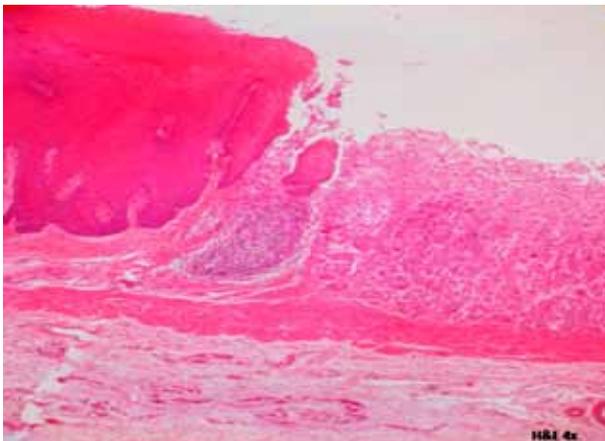


Figure 4. Gastric mucosa of equine with Equine Gastric ulcer syndrome Grade 4. Erosion and ulcer with severe damage of lamina propria and infiltrated lymphocytic (arrows)(H&E 10X).

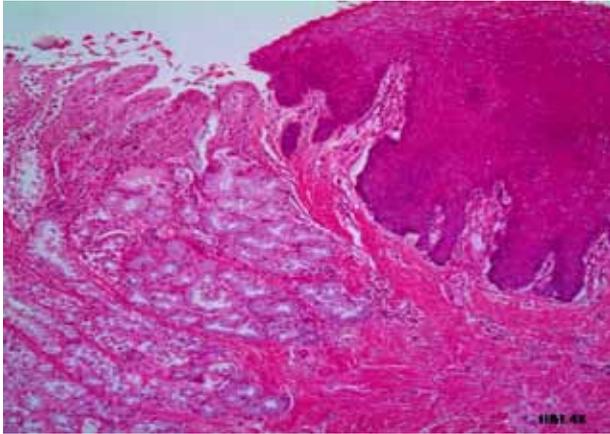
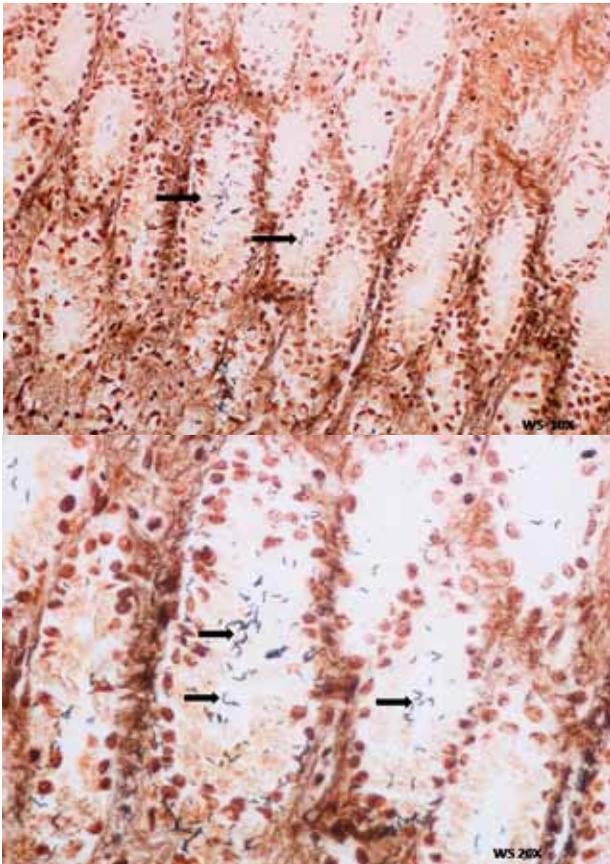


Figure 5. Gastric mucosa of equine with Equine Gastric ulcer syndrome Grade 4. Special staining Warthin-Starry showed spiral-shaped bacteria intra-glandular (arrows)(Warthin-Starry method 20X).



intact but there are areas of hyperemia and/or hyperkeratosis 33%. Grade 2 Small, single or multi-focal erosions or ulcer s 42%. Grade 3 Large, single or multi-focal ulcers, or extensive erosions and sloughing 8%. Grade 4 Extensive ulcers, with areas of deep submucosal penetration 8%. The histologic slices revealed severed damage on the gastric mucosa a loss of continuity of the gastric mucosa, with corium exposure and subcorionic edema with parakeratotic hyperkeratosis together with a mixed lymphoplasmocytic mononuclear infiltrate. Specifically 8% with epithelium is intact throughout; no hyperemia, no hyperkeratosis. Mucosa is intact but there are areas of hyperemia and/or hyperkeratosis 33%. Small, single or multi-focal erosions or ulcers 42% infiltrated of lymphocytes in lamina propria. Large, single or multi-focal ulcers, or extensive erosions and sloughing 8%, infiltrated of lymphocytes in lamina propria. Extensive ulcers, with areas of deep submucosal penetration infiltrated of lymphocytes in lamina propria with exposition of corium (8%). Fifty-eight horses had gastric ulcerations around the margo plicatus, gastritis and presented both types of lesions squamous region fifty-eight and forty glandular regions. Using the Warthin-Starry special stain, spiral-shaped bacteria in the gastric mucosa glandular were found in 8/12 (67%), Grade 1: 75%, Grade 2: 60%, Grade 3: 100%, Grade 4: 100%. There was no bacterial growth in either case.

DISCUSSION

A high prevalence (92%) of ulcers and gastritis was found in Spanish horses during our study and gastrointestinal disorders at the time of their euthanasia. This agrees with other earlier studies reporting the occurrence of gastric ulcers in 80 to 90% of Thoroughbred racehorses. The greatest proportion of such lesions appear on the squamous mucosa region close to the margo plicatus, with fewer lesions on the glandular mucosa portion (Murray, et al., 1994; Hepburn, 2004; Contreras, et al., 2007; Morales, et al., 2007; Videla and Andrews, 2009);,

similarly with our study. Were detected a presence of species of *Helicobacter* in the stomachs of horses, but the clinic relevance of this genus on EGUS has not yet been demonstrated (Moyaert, et al., 2009b). In Thoroughbred horses we detected high presence of *Helicobacter* Like Organisms in the gastric mucosa of Thoroughbred horse's treatment with phenylbutazone and damage gastric (Morales, et al., 2011). In horses of Slaughterhouses of Chile was reported 62.5% of prevalence of *Helicobacter* spp. in gastric ulcers (Cardona, et al., 2009). Only two studies have reported the presence of *Helicobacter*-specific DNA in the squamous and glandular mucosae of horses (Contreras, et al., 2007; Moyaert, et al., 2007) and one new species of *Helicobacter* named *H. equorum* was isolated from the faeces the two asymptomatic horses (Moyaert, et al., 2007). In adult horses, the prevalence of *H. equorum* seems to be quite low, but these animals can be found as part of the intestinal microflora (Moyaert., 2009 b). In ponies aged 2-6 months has been detected *H. equorum* in 67.8% from fecal samples (Moyaert, et al., 2009 a). While in adult horses has been detected in between 0.8-0.9% (Moyaert, et al., 2009 b). Apparently there is some association between the presence of *Helicobacter equorum* and presentation of clinical disease or intestinal lesions (Moyaert., 2009). Gastricus *Helicobacter* spp. It has been associated with lesions in the glandular stomach of the horse (Husted, et al., 2010). However, in our study we observed the presence of bacteria, inflammatory response of the gastric mucosa and erosive and ulcerative lesions, suggesting that the bacterium induces gastric mucosal irritation and are therefore compatible with equine gastric ulcer syndrome. These results are consistent with those reported in other studies where the prevalence of EGUS is 80-90% of Thoroughbred horses in race (Murray, et al., 1994, Morales, et al., 2006, Videla and Andrews, 2009). The special stains showed the presence of bacteria with morphology of spiral type, and short bacilli cocco-bacillus; morphology could be appreciated in greater

detail in the special Warthin-Starry staining. The 67% percent (8/12) of horses infected with *Helicobacter* in this study showed gastric mucosal lesions associated with gastritis, ulcers and / or the two conditions without apparent association with the degree and location of the lesion in their stomachs. In horses the diagnosis of EGUS and *Helicobacter* sp. can be done as follows: study for serological and immunological, test evaluation of sucrose. The urease test that can behave in case of a positive and negative gastric where an enterohepatic species. The isolation and bacterial culture is the ideal method but it is limited by bacteria difficult to grow, the methods of molecular biology, specifically chain reaction polymerase chain reaction (PCR), immunohistochemical study and study by macroscopic and histopathological staining special silver salts described in this paper. To conclude, we detected histopathological the presence of *Helicobacter* Like Organisms in the gastric mucosa with Equine Gastric Ulcer Syndrome of Spain horses. The *Helicobacter* presence could be an important risk factor of EGUS. Further studies concerning the role of *Helicobacter* species in cases with and without EGUS in Spain horses are needed. Furthermore, the cultures and subsequent bacterial identifications are still a requisite to establish the effects and pathogenesis of *Helicobacter* spp. on horse's gastric mucosa.

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