The aim of this study was to describe the effects of Nonsteroidal Anti-inflammatory drug on the prevalence of Helicobacter Like Organisms in the gastric mucosa of Thoroughbreds horses. We studied 54 Thoroughbred horses in the national race track “La Rinconada” Caracas-Venezuela. All equine were treated by seven days with phenylbutazone at an intravenous dose of 4.4 mg/kg. All horses presented Equine gastric ulcer syndrome acute superficial gastritis (25/54), chronic gastritis with erosion focal (16/54), chronic gastritis with erosion focal and ulcers (14/54) in the gastric in both regions mucosa squamous region and glandular regions (fundus). Helicobacter Like Organisms infection in the stomach was confirmed by Warthin-Starry (38/54). Gastric mucosa revealed numerous spiral-shaped bacteria morphologically resembling Helicobacter Like Organisms in squamous regions, margo plicatus (20/38) and numerous spiral-shaped bacteria in fundic glands (18/54). In conclusion, we detected a high presence of Helicobacter Like Organisms in the gastric mucosa of Thoroughbred horse’s treatment with phenylbutazone.

Key words: Thoroughbred; horses; equine; HLO; Helicobacter; phenylbutazone; EGUS.
squamous and the glandular mucosae of Thoroughbred horses. The aim of this study was describe effects of Nonsteroidal Anti-inflammatory drug on prevalence of Helicobacter Like Organisms in gastric mucosa of Thoroughbreds horses.

Material & methods

Animals: Were studied 54 Thoroughbred horses (30 female and 24 male), between 2-5 years old, in training in the national race Track “La Rinconada” Caracas-Venezuela.

Clinical signs: were lameness, acute abdominal pain recurrent and weight loss syndrome.

Therapeutically: All equine were treated by seven days with phenylbutazone at an intravenous dose of 4.4 mg/kg.

Necropsy and histology: All equine were euthanized and study by necropsy. Samples of tissue were collected from the gastric mucosa, bowel kidneys and liver. The tissue samples fixed in formalin were processed by conventional histological techniques (dehydration, inclusion in paraffin, microtome slicing and routine staining with Hematoxylin-eosin). Additionally, the special staining procedure of Warthin-Starry, Toluidin blue and Giemsa was also carried out.

Results

Necropsy: revealed weight loss, loss fatty subcutaneous, xantomatosis of subcutaneous tissue (20/54). Equine gastric ulcer syndrome severed in all horses, specifically acute superficial gastritis (25/54), chronic gastritis with erosion focal (16/54) (Figure 1a), chronic gastritis with erosion focal and ulcers (14/54) (Figure: 2a & 2b) in the gastric in both regions mucosa squamous region and glandular regions (fundus). Colitis chronic and focal hemorrhage (16/54).

Liver was swollen, friable with fibrosis chronic (36/54). Multi-focal necrotic areas were present in the other lobes (45/50). Renal cortical and papillary necrosis, acute tubular necrosis (54/54).

Histology: The histologic slices revealed a loss of continuity of the gastric mucosa in 29/54 horses (Figures: 1b, 2b & 3b), with corium exposure and subcorionic edema with parakeratotic hyperkeratosis together with a mixed lymphoplasmocytic mononuclear infiltrate. With regard to ulcer distribution, both regions of the stomachs showed similar patterns of lesions. Included large numbers of lymphoid nodules throughout all regions of the gastric mucosa and were most numerous in the fundus and body. A mild, diffuse lymphocytic infiltrate with small numbers of plasma cells and eosinophils was also present in the subglandular region of all portions of the gastric mucosa. To determine now NSAIDs affect Helicobacter Like Organisms infection-induce of chronic inflammatory activity in the gastric mucosa were quantified by manual counting per field. The mononuclear cell score showed increasing in the presence of HLO infection. The neutrophil score was low. Chronic colitis lymphoplasmocytic (12/54). Liver with periarterial necrosis with a prominent acinar pattern and fatty degeneration severed (45/50). Centre-acinus necrosis and bililary cluster (15/54). Necrosis and vacuolar (glycogen) degeneration islets of langerhans, fibrosis and chronic (12/54). Fibro-cortical and medullary necrosis, acute tubular necrosis, generation vacuolar and glycogen nephrosis (54/54), glomerulonephritis membranous (26/54).

Special staining: Helicobacter Like Organisms infection in the stomach was confirmed by Warthin-Starry (39/54) (Figures: 1c, 2c & 3c), Toluidin blue (35/54) and Giemsa staining (36/54). Gastric mucosa revealed numerous spiral-shaped bacteria morphologically resembling Helicobacter Like Organisms in squamous regions, margo plicatus (20/38) and numerous spiral-shaped bacteria in fundic glands (18/54).

Discussion

Background Nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the most widely prescribed drugs worldwide. They have now probably overtaken H. pylori as the commonest cause of gastrointestinal injury in Western countries. The use of NSAIDs is common in horses presenting with acute abdominal pain, lamenesses and other pain. Typically, these horses are given either phenylbutazone or flunixin meglumine intravenously to control pain during a colic episode. Phenylbutazone and flunixin meglumine have been found to induce gastric ulcers in horses, but usually at higher-than-recommended doses. Several factors may predispose towards phenylbutazone. The histologic slices revealed a loss of continuity of the gastric mucosa in 29/54 horses (Figures: 1b, 2b & 3b), with corium exposure and subcorionic edema with parakeratotic hyperkeratosis together with a mixed lymphoplasmocytic mononuclear infiltrate. With regard to ulcer distribution, both regions of the stomachs showed similar patterns of lesions. Included large numbers of lymphoid nodules throughout all regions of the gastric mucosa and were most numerous in the fundus and body. A mild, diffuse lymphocytic infiltrate with small numbers of plasma cells and eosinophils was also present in the subglandular region of all portions of the gastric mucosa. To determine now NSAIDs affect Helicobacter Like Organisms infection-induce of chronic inflammatory activity in the gastric mucosa were quantified by manual counting per field. The mononuclear cell score showed increasing in the presence of HLO infection. The neutrophil score was low. Chronic colitis lymphoplasmocytic (12/54). Liver with periarterial necrosis with a prominent acinar pattern and fatty degeneration severed (45/50). Centre-acinus necrosis and bililary cluster (15/54). Necrosis and vacuolar (glycogen) degeneration islets of langerhans, fibrosis and chronic (12/54). Fibro-cortical and medullary necrosis, acute tubular necrosis, generation vacuolar and glycogen nephrosis (54/54), glomerulonephritis membranous (26/54).

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zone toxicity in the horse, including breed and age, but high dosage is considered to be particularly important. NSAID are thought to cause more severe ulcers in the glandular mucosa because of their effect on prostaglandin inhibition. Prostaglandins in inhibition results in decreased mucosal blood flow, decreased mucus production, and increased HCl secretion. Although prostaglandins are also important in the regulation of acid production and sodium transport, it may be their effect on mucosal blood flow that is the most important. Gastric mucosal ischemia may lead to a hypoxia-induced cellular acidosis and release of oxygen-free radicals, phospholipase, and proteases, which may damage the cell membrane leading to necrosis. Although NSAIDs are commonly used, they have the potential to exacerbate EGUS in horses with colic. Helicobacter spp (other than Helicobacter pylori) have been isolated from humans and a variety of animals suffering from gastric ulcers and gastritis. Furthermore, Helicobacter-like DNA was detected in the stomach of 10 Thoroughbred horses in Venezuela. 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. Furthermore, 10 of 11 of the horses infected with Helicobacter had either gastric ulcers or gastritis or both pathologies. Bacterial colonization of the gastric ulcers in horses of the stomach may delay ulcer healing. Helicobacter pylori (H. pylori) infection and nonsteroidal anti-inflammatory drugs (NSAIDs) use are considered to be the most important risk factors having influence on the onset of bleeding gastroduodenal lesions. The majority of the examined cases were associated with both H. pylori infection and NSAIDs use. A statistically significant difference among the studied groups of patients was proven. The majority of bleeding gastroduodenal lesions was associated with the coexistence of H. pylori infection and NSAIDs use, while their independent influences were statistically less important.

These results are humans and are similar to those observed in our study in horses. A high prevalence (70%) of ulcers and gastritis with Helicobacter Like Organisms and phenylbutazone was found in Thoroughbred racehorses during our study even though none of them had a previous record of 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 and Helicobacter in horses 61%. In a clinical report on the interaction between NSAIDs and H. pylori, Hawkey et al. showed that H. pylori eradication in long-term users of NSAIDs, with past or current peptic ulcer or troublesome dyspepsia, led to a impaired healing of the gastric ulcers, implying that under certain circumstances some patients with H. pylori are less prone to NSAID-induced ulceration than noninfected patients. This may be due to the opposing effects of H. pylori and NSAIDs on PG synthesis in the gastric mucosa. However, other studies have shown that the eradication of H. pylori prior to NSAID therapy reduces the occurrence of peptic ulcers, and the level of apoptosis in gastric mucosa, and that NSAID users infected with H. pylori carry a greater risk of peptic ulcer than noninfected NSAID users. It was also reported that H. pylori infection may reduce the adaptation threshold so that the eradication of H. pylori restored the ability of the mucosal mucosa to adapt to aspirin. In the equine large intestine, endogenous prostaglandins had a variable effect on contractility, depending on the location in the colon and orientation of the smooth muscle. The administration of NSAID inhibited contractility, with flunixin meglumine generally inducing the most profound inhibition relative to the other NSAID evaluated in substance P-stimulated smooth muscle of the equine intestine. The results of this study indicate that prolonged use of NSAID may potentially predispose horses to development of gastrointestinal tract stasis and subsequent impaction. With preexisting chronic gastric ulcers, H. pylori infection may attenuated significantly the aspirin-induced inhibition of ulcer healing and accompanying fall in the gastric blood flow at the ulcer margin of these ulcers, suggesting negative interaction between aspirin and H. pylori on ulcerogenesis. Accumulating evidence in humans and animals shows that both aspirin and H. pylori upregulate the expression of cyclooxygenase (COX) 2 both at mRNA and protein levels at the ulcer margin and failed to influence significantly that of COX-1. It was, therefore, proposed that H. pylori may in fact, antagonize, aspirin-induced delay of ulcer healing due to suppression of acetylation by the enhancement in PGE2 possibly derived from COX-2 expression and activity and to the overexpression of growth factors such as TGF alpha and VEGF. Lesions in liver and kidney toxicity suggest NSAIDs in all horses studied. The lesions of the colon were not significant, which disagree with reports in the literature.

**Conclusion**

In conclusion, we detected high presence of Helicobacter Like Organisms in the gastric mucosa of Thoroughbred horses under treatment with phenylbutazone. In reviewing current knowledge of the clinical pharmacology of this important group of drugs, it is hoped to provide the clinician with a rational, scientific basis for their safe and effective use in equine practice.

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