A PRELIMINARY STUDY OF ENDOSCOPIC DIAGNOSIS OF GASTRIC ULCER IN EGYPTIAN DONKEYS (*EQUUS ASINUS*) PARASITISED BY *GASTEROPHILUS INTESTINALIS* (DIPTERA: OESTRIDAE)

M. ABUOWARDA¹, M. AMER², A. SHAMAA², G. SHEHAB³,
A. ABDALLAH³ & M. HASSAN²

¹Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Egypt; ²Surgery, Anaesthesiology & Radiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt; ³Pathology Department, National Animal Health Research Institute, Dokki, Giza, Egypt

Summary

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Equine gastric ulcer syndrome (EGUS) is a communal broad problem in equines but in donkeys, the disease is a subclinical disorder which leads to economic losses. *Gasterophilus intestinalis* larvae are the most predominant parasitic infestation in donkeys. The present study was planned to facilitate the early and efficient diagnosis of gastric ulceration in donkeys through gastroscopic examination. The study was conducted on 120 adult Egyptian donkeys (*Equus asinus*) from both sexes after a one-month acclimatisation period. Gastroscopic examination was used to evaluate the presence of gastric lesions. In the majority of infested donkeys, *G. intestinalis* larvae were in the non-glandular part of the stomach. The prevalence of *Gasterophilus* larvae in animals reached 96.66%. The infestation percentage among jennets (97.5%) was slightly higher than that among jacks (96.25%). After morphological identification of the 3rd instar of larvae, all donkeys were found to be infested by *G. intestinalis* larvae. The histopathological examination of the affected stomach revealed severe necrosis of the gastric mucosa. Gastroscopic examination is the main diagnostic tool for examination of gastric lesions in the donkeys, so it is necessary as a routine diagnostic tool in donkeys' examinations.

Key words: donkey, endoscope, *Gasterophilus intestinalis*, gastric ulcer

INTRODUCTION

Increased global interest in the welfare of donkeys (*Equus asinus*) is mostly due to the recent usage of these animals in the production of milk for use in infants suf-

fering from intolerance to cow's milk (Carroccio *et al.*, 2000; Muraro *et al.*, 2002) or in animal-assisted therapy and activities (Borioni *et al.*, 2012).

Renewed interest in donkeys is evidenced by many studies conducted over the past 10 years in different nations (Sgorbini *et al.*, 2013; Kim *et al.*, 2014; Veronesi *et al.*, 2014). Equine gastric ulcer syndrome (EGUS) is a common problem in horses used to define erosions and ulcers in the distal esophagus, glandular and non-glandular parts of the equine stomach and their effect on health and performance (Murray, 2002; Sanchez, 2004; Videla & Andrews, 2009; Niedźwiedź *et al.*, 2013).

Unlike horses, EGUS in donkeys is commonly a subclinical disease without clinical signs (Merritt, 2009; Videla & Andrews, 2009) and this suggests that subclinical forms of EGUS may be more common in donkeys than they used to be (Sgorbini *et al.*, 2018).

Parasites of the equine stomach causing EGUS include *Gasterophilus* and gastric nematodes (Jacobs, 1986). The stomach bot fly larvae (Calypttratae: Oestridae, *Gasterophilinae*) are considered a strong obligatory host-specific myiasis larvae (Yan *et al.*, 2019). *Gastrophilus intestinalis* is one of the *Gastrophilinae* species infesting family *Equidae* (i.e. horses, zebras, etc.) developing in the digestive tract of hosts in the Mediterranean regions, U.S.A and China (Otranto *et al.*, 2005; Colwell *et al.*, 2006; Huang *et al.*, 2016).

The warm areas in Italy and Brazil have recorded the highest prevalence rate of *Gastrophilus* species (Otranto *et al.*, 2005; Felix *et al.*, 2007), while in Egypt, it was found that 100% infestation with *G. intestinalis* larvae was in donkeys. The diagnosis of gastric ulcer caused by *G. intestinalis* larvae is based on history, clinical signs, gastroscopic examination, and response to treatment (Sgorbini *et al.*, 2018).

EGUS has numerous, often nonspecific clinical signs and not all affected

equines show signs of the disease. The most prominent clinical sign is intermittent mild colic, especially during and after eating (Videla *et al.*, 2009). EGUS is also associated with chronic diarrhoea, excessive salivation, poor appetite, weight loss, attitude changes, rough hair coat, depression, and decreased performance (Merritt, 2009; Videla *et al.*, 2009).

Gastroscopic examination has been admitted as a gold standard of diagnosis because a critical diagnosis of gastric ulceration in horses can only be achieved by visualization of the lesions in the stomach (Murray, 2002; 2009; Begg & O'Sullivan, 2003). EGUS has been rarely described in donkeys, and findings were limited to animals submitted for necropsy (Burden *et al.*, 2009; Morrow *et al.*, 2011; Al-Mokaddem, 2014).

The study was designed to facilitate the early and efficient diagnosis of gastric ulcer syndrome with parasitic cause investigating the prevalence, anatomical distribution in donkeys through gastroscopic examination.

MATERIALS AND METHODS

Animals

The study was conducted on 120 (80 jacks and 40 jennets) adult Egyptian donkeys (*Equus asinus*), 1–5 years old, weighing 100–200 kg. The experimental animals were used at the teaching hospital of Surgery, Anaesthesiology and Radiology Department, Faculty of Veterinary Medicine – Cairo University for last year undergraduate students in the period from September 2018 to June, 2019. After a one-month acclimatisation period, the donkeys were housed 2/box (4 × 4 m box stalls) and fed grass hay and pelleted feed twice daily for 45–60 days. The animals had

undergone routine general health check.

All the donkeys involved in the present study had never been vaccinated and had not received antiparasitic medications for at least one year. Animals were managed with due regard to their well-being and in compliance with the protocol VetCU1022019077 approved by Faculty of Veterinary Medicine – Cairo University Institutional Animal Care and Use Committee

Endoscopic equipment

Portascope Endoscope (PVSM3M, FL, USA) was used for gastroscopic examination. The insertion tube was 12.9 mm × 3 m (outer diameter × length) with lead lamp and microcamera technology.

Endoscopic procedure

The day before gastroscopy a clinical examination was accomplished on all the donkeys enrolled in the experiment. The animals fasted for 8–12 hours before examination and water were withheld for 2–4 h. Sedation was performed by xylazine HCl 0.5 mg/kg i.v.

All animals underwent a gastroscopic examination to evaluate the presence of gastric lesions. Gastroscopy was accomplished as described by Murray (2002) and Bain *et al.* (2004). The endoscope tube was passed through the nostrils then forward – looking to the rima glottis and into the oesophagus. The oesophagus was examined as the endoscope was carefully advanced. The stomach was distended by insufflation of air through the endoscope till the non-glandular and glandular regions of the gastric surface could be observed and advanced till the major duodenal papilla. The occurrence of *Gasterophilus* larvae in the stomach and proximal duodenum was determined during the gastroscopic examination. The findings

were recorded on digital media for subsequent revaluation and storage.

Necropsy examination

Thirty of the donkeys were euthanised for teaching and student training purposes using lethal i.v injection of thiopental sodium. The stomachs were harvested and collected intact immediately through oesophagus and duodenum ligation approximately about 10 cm proximal and distal to the stomach. The tagged stomachs were cut along the greater curvature and stretched using digital pressure to smooth out the rugae. The non-glandular and glandular regions of the gastric surface were examined and digitally photographed.

Morphological identification

Three hundred and forty-eight 3rd instar larvae collected by endoscopy from infested donkeys (3 larvae from each donkey) were identified according to the morphological keys described by Zumpt (1965).

Histopathological examination

Formalin-fixed tissue specimens of the affected stomach were routinely processed for paraffin blocks. Sections of 4–5 microns thickness were obtained and stained with haematoxylin/eosin (Bancroft & Gamble, 2008).

RESULTS

Endoscopic diagnosis and morphological identification

By general examination, all animals were considered apparently healthy. A full examination of the non-glandular mucosa was possible in all donkeys. In the majority of donkeys, a systematic examination of

Fig. 1. **A.** Endoscopic examination of the stomach revealed haemorrhagic ulceration and presence of *G. intestinalis* larvae. **B.** aggregation of *G. intestinalis* larvae. **C.** necropsy of the stomach revealed aggregation of *G. intestinalis* larvae. **D.** haemorrhagic erosion (arrows) and ulceration in the stomach. **E.** the site of *G. intestinalis* hooks after removal of the *G. intestinalis* larvae (arrows). **F, G.** larvae of *G. intestinalis* after removal from the stomach.

the glandular region was also possible to be done except for a small area where residual fluid was pooling in the ventral aspect of the fundus (Fig. 1A, B). The non-glandular portion of the stomach was the common area of attachment of *G. intestinalis* larvae.

Third instar larvae infesting all donkeys were morphologically identified as *Gasterophilus intestinalis* (Fig. 1F, G). The prevalence of larvae in all animals was 96.66% (116/120). The infestation

percentage among jennets (97.5%) was slightly higher than among jacks (96.25%) (Table 1). Twenty-nine donkeys out of the euthanised donkeys (n=30) were infested (Fig. 1C, D). The number of collected larvae in 3rd larval stage of *G. intestinalis* from them was 524.

Pathological findings

Macroscopically, the affected stomach was occluded with a large number of *G. intestinalis* larvae, attached to the gastric

Table 1. Prevalence of *Gasterophilus intestinalis* larvae in donkeys (*Equus asinus*) by endoscopic diagnosis

Donkeys	Number of examined donkeys	Infestation by <i>G. intestinalis</i> larvae	Percentage (%)
Jennets	40	39	97.50
Jacks	80	77	96.25
Total	120	116	96.66

Fig. 2. Histopathological findings of the gastric tissues. **A.** Desquamation of the tips of the lining epithelium of the gastric mucosa, hyperplasia of mucus secreting cells with appearance of cross section of the parasite (arrow) in the gastric mucosa, H&E, bar=55 µm; **B.** Severe necrosis and erosion of the gastric mucosa with oedema in the lamina propria, H&E, bar=55 µm; **C.** The bites of attachment of *G. intestinalis* larvae leaving ulcerated mucosal surface (stars), H&E, bar=55 µm. **D.** Severe desquamation of the mucosal lining epithelium (stars) of the site of helminths attachment, H&E, bar=55 µm.

Fig. 2 (cont'd). E. Hyperplasia and cystic dilatation of some gastric glands (arrows) surrounded by proliferated fibrous connective tissue, H&E, bar=55 μ m. F. Focal area of mononuclear cell infiltration (star), necrosis and oedema in the submucosal layer in response to *G. intestinalis* larvae, H&E, bar=55 μ m; G. Chronic ulceration with complete necrosis of the mucosal epithelium (black arrow) and inflammatory cell infiltration with proliferation of fibrous connective tissue (star) and thickening of the submucosal layer with proliferated capillaries (white arrows) and haemorrhage, H&E, bar=55 μ m; H. Area of ulcerated mucosal surface of the site of attachment of the *G. intestinalis* larvae (arrow); the adjacent epithelium is acanthotic and parakeratotic, H&E, bar=112 μ m.

mucosa. The bites of attachment of parasitic larvae left tiny haemorrhages and mucus secretion (Fig. 1D, E).

The microscopic examination of different sections of the infested stomach revealed various histopathological changes varying from desquamation of the tips of the lining epithelium of the gastric mucosa, and hyperplasia of mucous secreting cells with the appearance of a cross section of the parasite larvae in the gastric mucosa. Also, severe necrosis and erosion with oedema in the lamina propria were observed at the larvae attachment site. Moreover, a large portion of the gastric mucosa showed chronic ulceration with complete necrosis and sloughing of the mucosal epithelium with thickening of the

submucosal layer with proliferated fibrous connective tissue and newly formed capillaries with inflammatory cells and haemorrhage (Fig. 2).

DISCUSSION

Early and efficient *Gastrophilus intestinalis* diagnosis in donkeys by gastroscopic examination was the main aim of our study. Endoscopic gastroscopic examination is considered the main diagnostic tool of gastric ulceration in horses (Begg & O'Sullivan, 2003; Merritt, 2009), therefore, endoscopy was used for *G. intestinalis* lesions detection in the donkey's stomach. Among the donkeys enrolled in the study, 96.66% revealed *G. intestinalis*

larvae infestation. These findings are contrary to other studies that stated a 100% infestation rate in Egypt (Al-Mokaddem, 2014).

Endoscopic examination of the stomach revealed erosion and ulceration as a result of the large, hooked mouthparts (maxillae) that enable the larvae to securely attach to the lining of the stomach (Sweeney, 1990). *G. intestinalis* larvae use their flat mandibles to abrade the tissue of the stomach so haemorrhagic ulceration, chronic gastritis was also detected (Gao *et al.*, 2016).

As a consequence of aggregation of large numbers of larvae in the stomach, blockage and colic were prevalent with a consequent considerable economic loss, in line with El-Bakry & Fadly (2014). The prevalence of *G. intestinalis* larvae of 96.66% nearly agreed with that reported by Hilali *et al.* (1987) who recorded 98.3% in Egypt and 95.2% in Italy (Otranto *et al.*, 2005). Low prevalence rates were reported from Sweden (9.9%, Hoglund *et al.*, 1997), Germany (2.25%, Rehbein *et al.*, 2013), Ireland (43%, Sweeney, 1990) and Mediterranean region (28.57%, Özdal *et al.*, 2010). This difference in prevalence rate may be due to different climatic conditions and seasonal variation.

The sites of attachment of *Gasterophilus* larvae in the stomach leaves areas of haemorrhage and mucus secretion. The microscopic findings of different sections of the infested stomach revealed desquamation of the tips of the lining epithelium of the gastric mucosa, hyperplasia of mucous secreting cells with the appearance of a cross section of the parasite larvae in the gastric mucosa. These results were parallel to the study of Al-Mokaddem *et al.* (2014) who showed changes in the lamina propria of the non-glandular region in association with erosions and ulceration

including inflammatory cell infiltration and increased vasculature in as well as to findings of Sandin *et al.* (2000) and Andrews *et al.* (2002) who reported non-glandular ulcers as more common than glandular ulcers in horses. Moreover, the glandular region of the stomach showed hyperplasia and hyperactivity of gastric pits which appeared deeper, tortuous and mucus-filled. Glandular dysplasia is considered as a startling manifestation for neoplasia (Kumar *et al.*, 2007). All the donkeys in the existing study suffered from chronic gastritis in agreement with previous results showing that chronic gastritis was more common than the acute type (Rooney & Robertson, 1996). Previous studies (Rooney & Robertson, 1996; Sandin *et al.*, 2000; Sequeira *et al.*, 2001) have suggested that *Gasterophilus* spp. can cause gastric and intestinal ulceration, perforation or rupture in the gastrointestinal tract resulting in peritonitis (Van der Kolk *et al.*, 1989). Moreover, the multiple areas of the gastric mucosa at the site of parasitic larvae attachment showed severe necrosis and erosion with oedema in the lamina propria.

A large area of the gastric mucosa showed chronic ulceration with complete necrosis and sloughing of the mucosal epithelium with thickening of the submucosal layer with proliferated fibrous connective tissue and newly formed capillaries with inflammatory cells and haemorrhage, confirming the results of Al-Mokaddem *et al.* (2014) who found out that the *Gastrophilus* larvae were mainly concomitant with responsive hyperkeratosis with diffuse eosinophilic infiltration of the lamina propria and no ulcerative lesion within the glandular parts of these donkeys.

CONCLUSION

This study commends that gastric ulcers could be prevalent in apparently clinically normal donkeys and donkeys with mild clinical signs of gastric ulcers that are not in severe work. The high prevalence of gastric ulcer syndrome in donkeys without observable signs of gastrointestinal disorder as mentioned in our study is a challenge for the veterinarian.

Gastrosopic examination is important in diagnosing the gastric ulcers and their main cause, particularly the parasitic one and may not be able accurately to forecast the severity or depth of those ulcers present in the non-glandular donkey stomach. The *Gasterophilus* larvae play a major role in gastric ulceration in donkeys.

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Correspondence:

Mai Abuowarda,
Department of Parasitology,
Faculty of Veterinary Medicine,
Cairo University,
PO Box 12211, Giza, Egypt,
tel: [+2] 01063186043;
fax: [+2] (02) 35725240;
e-mail: Mai.abuowarda@cu.edu.eg;
ORCID: <https://orcid.org/0000-0003-1631-1666>