

Original Article

Investigating the status of *Gasterophilus* infections in donkeys around Urmia using the equine endoscopic method

Masoud Ahmadnejad¹, Ghader Jalilzadeh-Amin^{2*}

- 1- Department of Clinical Science, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
- 2- Department of Internal Medicine, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

*Corresponding author: g.jalilzadeh@gmail.com

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Summary

Donkey breeding in the intensive system has been expanding in recent years due to the nutritional benefits of donkey milk. One of the common problems of horses, including donkeys, in intensive systems, is Gasterophilosis, which is often associated with equine gastric ulcer syndrome (EGUS). L3 larvae of *Gasterophilus intestinalis* can cause gastric ulcers in donkeys by attaching to margo plicatus. There are also reports of human infection with *Gasterophilus* spp. The aim of this study was to investigate the presence of Gasterophilosis and related EGUS in intensive system donkeys. This evaluation was done on 12 donkeys that were bought from different places around Urmia and were kept in Urmia University Veterinary Hospital. Animals were kept in sanitary stables and received alfalfa-based rations. Gastrointestinal endoscopy from throat to pylorus was performed to check for parasitic infections and related ulcers. The animals appeared perfectly healthy during the clinical examination. No parasitic infections such as *Gasterophilus* adhesions were observed in the upper gastrointestinal tract up to the gastric pylorus. Gastrosocopy did not confirm any ulcers above grade 0. After the first endoscopy, ivermectin was prescribed, and three months later, the endoscopy was repeated, and again, no *Gasterophilus* infection was observed. Breeding donkeys in an intensive system by itself cannot cause Gasterophilosis and EGUS, although dietary adjustment can be helpful in preventing the disease.

Keywords: Donkey, Gasterophilosis, Gastrosocopy, EGUS, Antiparasitic

Introduction

Since ancient times, donkeys (*Equus asinus*) have been used to carry goods and people, and now they are used for these purposes in some inaccessible or undeveloped areas. The similarity of donkey milk to breast milk and its low allergenic properties suggest it administrated to feed human infants and patients with cow's milk protein allergy (CMPA). Besides, the recent evidence demonstrated that this

milk has anti-inflammatory, anti-tumor, antioxidant, antimicrobial, and anti-diabetic activities and manages the immune system and digestive flora (Derdak et al., 2020). The published data showed that of the 110 million equine population in developing countries, approximately 40 million are donkeys. Importantly, because of the special economic and geographical conditions in the rural areas of these countries, donkeys are

commonly used as an essential means of transportation (Khamesipour et al., 2021).

Equine gastric ulcer syndrome (EGUS) is frequently determined by one or more ulcers in the glandular or/and non-glandular stomach of the equine. Ulcers can progress to the lower esophagus or upper duodenum (Andrews et al., 1999). A previous study indicated that 51% of the examined donkeys had EGUS, and all of them were infected with *Gasterophilus* spp., particularly around *margo plicatus* (Sgorbini et al., 2018). In donkeys, EGUS is frequently caused by underlying diseases like hyperlipidemia or renal disease. It is believed that donkeys that eat much food in one or two meals have a higher risk of developing EGUS (Sprayberry and Robinson, 2014). Moreover, the sick donkey foals and orphans have a high risk for EGUS developing. The affected foals present colic symptoms similar to horses, like bruxism and after feeding colic (Blikslager et al., 2017). EGUS diagnosis is usually based on history, clinical findings, endoscopic examination, and response to treatment (Reese and Andrews, 2009). In this regard, *Gasterophilus* spp. can also be a risk factor for EGUS (Morales Briceño et al., 2015).

Gasterophilus fly (Diptera: Oestridae) larvae are the common cause of gastrointestinal myiasis in equines (Zumpt, 1965). Six species of *Gasterophilus*, including *G. nasalis*, *G. intestinalis*, *G. haemorrhoidalis*, *G. pecorum*, *G. nigricornis*, and *G. inermis*, that are called equine stomach bots, can find in horses, mules, and donkeys (Constable et al., 2017). Adult flies lay their eggs on the animal's hair in summer and the larvae develop their life cycle from the mouth to the stomach. During this maturation, various stages of the larva attach to the digestive system such as the tongue, hard palate, cheeks, epiglottis, pharynx, esophagus, and various parts of the stomach, which are different for each species (Li et al., 2019). *Gasterophilus* spp. can lead to equine gastric ulcer syndrome (EGUS), difficulty swallowing, anemia, volvulus, intestinal obstruction, rectal prolapse, diarrhea, and indigestion (Otranto et al., 2005). Of note, *Gasterophilus* species have also

been reported in rural horses and donkeys in Iran (Rezazadeh and Gharehaghajlou, 2020; Tavassoli and Bakht, 2012).

The genus *Gasterophilus* includes eight species that infect horses, and human cases with creeping dermal and oral or ocular infections have been reported (Rezaei, 2022). In this regard, there are evidence of human infection with horse fly larvae in close contact. Migration of first-stage larvae is associated with skin and eye myiasis in humans. Larvae burrowing under the skin may leave a visible tortuous path with severe itching and burrowing larvae behind the lip or inner cheek causes discomfort (Sellon and Long, 2013).

Because of the growing importance of donkey breeding in intensive systems, and the incidence of EGUS in equines and the effect of parasitic infections in gastrointestinal ulcers, we conducted the current study.

Material and methods

Animals

The present study was performed on 12 donkeys kept for educational and research purposes in the Veterinary Hospital of Urmia University, Urmia, West Azerbaijan Province, Iran. The animals were bought from the surrounding areas of Urmia, which were between 3 to 7 years old and were both genders. The donkeys weighed between 120 to 170 kg and had body scores of three to four. All animals received alfalfa-based diets to gather with free access to water. During the current study, no working or sports activities or starvation were imposed on the donkeys.

Physical examination

The animals were examined for general health before any diagnostic procedure. Oral and particularly tongue examinations were performed for the probable lesions due to invasion of *Gasterophilus* larvae.

Endoscopy

About 12 hours of fasting and 4 hours of water deprivation before gastric endoscopy were

considered for each donkey. Sedation was performed using a combination of acepromazine (0.03 mg/kg, NEUROTRANQ®, Alfasan, Woerden, Holland) and xylazine (1 mg/kg, XYLAZINE®, Alfasan, Woerden, Holland) (Matthews and van Loon, 2019). Gastroscopy was performed on the squamous and glandular mucosa of the gastric to the pylorus by equine video endoscope (STORZ®, RP100, Tuttlingen, Germany). For a more accurate view, the food remaining on the gastric mucosa was washed with distilled water, and greater curvature, less curvature, dorsal fundus, and pylorus were evaluated. Horse gastric ulcer scoring provided by Andrew et al. was used to assess the presence and degree of EGUS. In this method, grades 0, 1, 2, and 3 represent complete health, single or multiple small ulcers, single or multiple large ulcers, and

extensive or in-depth ulcers, respectively (Andrews et al., 1999). After the first endoscopy the donkeys received an oral dose of ivermectin (0.2 mg/kg, IVERGEN®, Laluk, Tehran, Iran) (Gokbulut et al., 2005). Three months after taking the antiparasitic drug, the endoscopy was repeated in the same way.

Results

The animals appeared completely healthy on clinical examination before endoscopy. Appetite, body temperature, GI sounds, and alertness were all in the normal range. Examination of the mouth and tongue showed no apparent signs of the larval burrow.

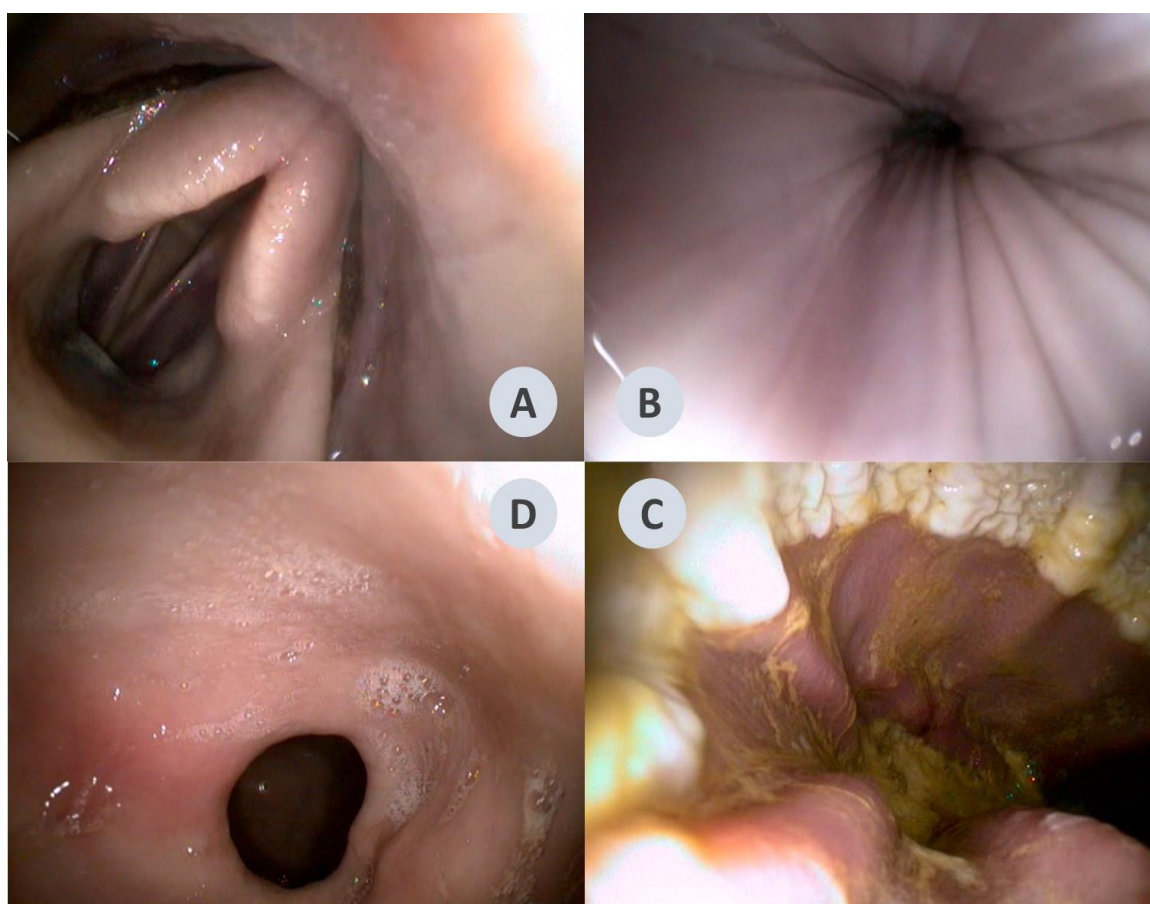


Fig. 1-A: pharynx; B: esophagus; C: a comprehensive view of the around Margo Plicatus; D: pylorus, without any ulcer or *Gasterophilus* species adhesion.

Examination of the pharyngeal and esophageal structures showed no signs of adhesions or lesions left from larval attachment. Gastroscopy of non-glandular and glandular gastric mucosa, margo plicatus, and pylorus showed no ulcers above 0 degrees. Only in one case was local hyperemia observed in the gastric mucosa's non-glandular part, which is considered in the 0-grade classification. Endoscopic examinations did not show any attached *G. intestinalis* or other *Gasterophilus* larvae to the gastric mucosa. Complete intact gastric mucosa indicates that there has been no recent parasitic adhesion to the mucosa (Figure 1). There was no sign of third-stage *Gasterophilus* larvae in fecal monitoring. In the next endoscopy, which was performed three months after consuming ivermectin, no *Gasterophilus* infection or EGUS was seen.

Discussion

There were no signs of EGUS or gasterophilic infections in the donkeys we studied. Previous studies have confirmed that donkeys are infected with *Gasterophilus* species, and may have EGUS. A study of a large population of British donkeys found that 42% of them had gastric ulcers and 0.4% had *Gasterophilus* species infection (Morrow et al., 2011). A study in Nigeria found that 52.2% of donkeys were infected with *Gasterophilus* species, which 30.4% were related to *G. intestinalis* and 21.7% to *G. nasalis*. (Onyishi et al., 2022). Parasitic infections, such as *Gasterophilus* species have been reported in 3 of 10 donkeys who underwent gastroscopy (Morales Briceño et al., 2015). Of the 39 donkeys studied in Italy, 17 belonged to educational and research departments, and 22 belonged to two private farms. These donkeys were kept in sand paddocks, fed meadow hay, and not receive any vaccine or antiparasite. Twenty of these donkeys had EGUS, and all 39 had *Gasterophilus* species. Regardless of the presence or absence of EGUS, endoscopic examinations of all donkeys infected with *G. intestinalis* showed

larvae attached close to Margo plicatus and no parasites were found in the proximal duodenum. In this study, although the donkeys were kept in the veterinary school, they had parasitic infections that could be due to the lack of use of antiparasitic drugs (Sgorbini et al., 2018). Macrocytic lactones (such as ivermectin) are the recommended drug to prevent Equine *Gasterophilus* infections (Constable et al., 2017). Oral ivermectin has also been used in donkeys at a dose of 0.2 mg/kg (Binev et al., 2005; Gokbulut et al., 2005). Recently, a study was performed on combining nano chitosan with ivermectin, which creates synergy by reducing side effects (AbdElKader et al., 2021). In the present study, a single oral dose of ivermectin (0.2 mg/kg) was used, and the results were very satisfactory.

Keeping donkeys outdoors and feeding them pasture forage can also increase the risk of Gasterophilosis. Previous researchers have also attributed changes in the prevalence of *Gasterophilus* spp. in equines to climate changes (Attia et al., 2018). Most of the *Gasterophilus* species reported cases were from areas with a Mediterranean climate (Attia et al., 2018). In previous studies, donkeys had the highest parasitic infection among equine, and the equine in the northwestern region had the most helminth infestation (Khamesipour et al., 2021). However, the present study was performed on donkeys kept in northwestern Iran, and none of the animals had *Gasterophilus* species on endoscopic examination. Although researchers attribute most of the equine gastrointestinal parasites to pasture, they acknowledge that some species, such as *G. intestinalis*, have little to do with feed due to their life cycle (Khamesipour et al., 2021). The necropsies of nine dead horses in a herd revealed many L3 *G. pecorum* larvae attached to the esophagus and gastric mucosa. All the horses in this herd were fed only from the pasture (Moshaverinia et al., 2016). *G. pecorum* is the only species of *Gasterophilus* that lays its eggs on pasture plants (Constable et al., 2017). Therefore,

the relationship between pasture and *Gasterophilus* species infection is only in *G. pecorum*. The non-use of pasture by the animals participating in this study can be useful in preventing *Gasterophilus* species infections.

The change in keeping donkeys in an intensive system has caused the feeding shift from pasture to grain food. The type of diet consumed is one of the critical risk factors for the development of EGUS (Reese and Andrews, 2009). Cereal grains increase the risk of EGUS in donkeys. Gastric ulcers can cause colic in donkeys (Sprayberry and Robinson, 2014). Our results suggest that receiving alfalfa-based, grain-free diets may help prevent EGUS in donkeys.

Conclusion

The results of our investigations on donkeys around Urmia showed that the occurrence of *Gasterophilus* infections in these animals and related zoonotic concerns had been controlled. Farming in clean stables, the use of alfalfa-based diets, regular feeding times, and antiparasitic drugs are some of the success factors in maintaining the health of donkeys in intensive systems against EGUS and *Gasterophilus* species infestations. However, more studies are needed on the risk factors for EGUS and *Gasterophilus* in donkeys.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals including the protocol approved by the Animal Research Ethics Committee of the University of Urmia were followed.

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