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Novel detection of *Helicobacter* species in gastric problems in Equine in Egypt

Dina A.E. AbuElghait[1], Kh. F. Mohamed [2], Eldessouky Sheta[3], Naglaa A. AbdElkader[3] and Ahmed Samir[2]

[1]Ph.D Microbiology, Faculty of Veterinary medicine, Cairo University
[2]Department of Microbiology, Faculty of Veterinary medicine, Cairo University
[3]Department of Surgery, Anaesthiology and Radiology, Faculty of Veterinary medicine, Cairo University
Corresponding Author: Khaled Farouk El Amry

ABSTRACT

Helicobacter pylori is one of the most common human pathogens worldwide with serious clinical out-comes. Equine Gastric ulceration (EGUS) is the most common gastric disease that affects between 53 to 90% of adult horses, although several risk factors for the development of gastric ulcers have been widely studied, investigation of microbiological factors has been limited, therefore in our study, fifty faecal samples were collected from fifty diseased horses and application of PCR assay for detection of Helicobacter specific gene 16S rRNA, twelve samples were positive among the examined samples resulting in (24%) positivity, young foals show higher prevalence than adult horses. The occurrence of Helicobacter in horse may act as a potential zoonotic risk hazard thus, further studies are needed to investigate the zoonotic pathway to the human contacts.

Keywords: Novel detection of Helicobacter, species in gastric problems, Equine in Egypt
Correspondence:
Khaled Farouk El Amry
Microbiology, Faculty of Veterinary Medicine, Cairo University

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic, spiral-shaped and flagellated bacterium infecting about half the world's population whose main reservoir is the human stomach. In Africa its prevalence reaches as high as 80% as the infection is acquired during childhood (**Antonietta et al., 2018, Stella et al., 2019**).

H. pylori infection is the main cause of chronic gastritis and peptic ulcer disease and has a determinant pathogenic role in the development of distal gastric adenocarcinoma and gastric mucosa associated lymphoid tissue (MALT) lymphoma, it can also contribute to gastric carcinogenesis by stimulating gastric cell proliferation without counterbalancing with adequate apoptosis **(Blaser et al., 1999, Lehours et al., 2005, Romano et al., 2006).**

Beyond its role in several gastroduodenal disorders, *H. pylori* has been involved in many extra-gastroduodenal manifestations, like idiopathic thrombocytopenic purpura, cardiovascular diseases, chronic liver diseases, iron-deficiency anaemia, and diabetes mellitus **(Ribaldone et al.,2016**, **De Korwin et al., 2017)**.

The number of species in the genus *Helicobacter* has rapidly expanded during the past decade, at least 24 formally named helicobacters have been identified and an additional 35or more novel helicobacters await formal naming**. (Fox, 2002)** Moreover**,** genus *Helicobacter* have been described in a wide variety of animal hosts, including wild birds, chickens, dogs, cats, cattle, sheep, swine, rodents, non-human primates, cheetahs, ferrets, rabbits, dolphins and whales **(Whary and Fox, 2004,Van den Bulck et al., 2005).**

Recently,in **2015, Abdel-Moein et al.,** suggested an evidence that tilapia fish could be considered a potential zoonotic reservoir for *H.pylori* not only a mechanical vector; therefore, playing an important role in the epidemiology of such pathogen through the possible fecal shedding taking in consideration that *H. pylori* can survive for many days in water.

Interestingly, *Helicobacter* species also have been isolated from poultry birds (*H. pullorum*) **(Javed et al.,2017)**, wild house mice (*H. rodentium, H. typhlonius , H. hepaticus , H. bilis , and H. muridarum*) **(Wasim et al.,2012),**hamster (*H. aurati, H. cholecystus , H. cinaedi, H. mesocricetorum*) **(Mark and James 2006)**,and domestic ferrets (*H. mustelae*) **(James and Robert,2001).** Some enterohepatic helicobacters (*H. canis*, *H. pullorum*, *H. cinaedi*, *H. fennelliae*, *H. canadensis*, *H. winghamensis*, *H. westmeadi*, and *H. rappini*) have been isolated from diarrhoeic and/or bacteraemic humans and some of the species may also have zoonotic potential. **(Solnick et al., 2001, Erdman et al., 2001, Shomer et al., 2001).**

Equine gastric ulcer syndrome (EGUS) is the most common gastric disease that affects between 53 to 90% of adult horses and has been associated with colic, weight loss, and decreased performance **(McClure et al., 1999)**, and it is a multifactorial syndrome caused by exposure of the stomach to inorganic compounds, feed, management stress and non-steroidal anti-inflammatory drugs such as corticosteroids which all act synergistically increasing acid production which in turn leads to gastric ulcers **(Andrews et al., 2005).**

Another cause of EGUS is the presence of species of *Helicobacter* in the stomachs of horses, but the relevance of this genus on EGUS has not been demonstrated **(Morales et al., 2010).**

A new enterohepatic *Helicobacter* species *H. equorum*, was isolated from fecal samples of clinically healthy horses in Belgium as well as *H. equorum* DNA was found in the feces of foals less than 1 month old and also foals 1 to 6 months old **(Moyeart et al.,2007b, Moyeart et al.,2009)**

There are few studies have reported the presence of *Helicobacter*-specific DNA in the stomach of horses and

one new species of *Helicobacter* named *H. equorum* was isolated from the faeces of two asymptomatic horses **(Moyaert et al.,2007a)**.

Aim of the study: Up to our knowledge, there are no studies have been published on *Helicobacter* species in equine in Egypt; therefore, our aim was to detect *Helicobacter* spp. in gastritis cases in horses in Egypt and study of public health burden of such pathogen.

MATERIALS AND METHODS

Samples

This study was carried out on fifty diseased horses (30 foals and 20 adults) suffering from various degrees of abdominal colicky pain and samples were taken during episodes of colic. These horses were selected from the clinic of the Faculty of Veterinary Medicine, Cairo University and also from different farms for breeding Arabian horses in Cairo. Fecal samples were collected directly from rectum from each horse in clean, dry, waterproof containers containing no detergents, preservatives or transport media, and then transferred directly in ice box to the laboratory.

Polymerase chain reaction (PCR)

DNA extraction

DNA was extracted from 200 mg of each fecal sample using QIAamp DNA Stool Mini Kit (Qiagen, Germany) relying on the kit instructions DNA extractions were frozen at-20^{0}C until further analysis.

Primers and PCR amplification condition

According to **(Moyaert et al., 2008)** the PCR reaction was conducted using *Helicobacter* species specific primer for 16S rRNA gene.

Primer name and direction	Nucleotide sequence (5" to 3")	Amplicon (bp)
16S rRNA for *Helicobacter* spp Forward Reverse	 5-GGCTATGACGGGTATCCGGC-3 5-GCCGTGCAGCACCTGTTTTC-3	 764

All PCR reactions were performed in a 25 µl volume, the reaction mixture contained 12.5 µl Master Mix (Takara, Japan), 10 pmol/µl of each forward and reverse primers, 5.5 µl nuclease free water, and 5 µl of extracted DNA under the conditions for amplification as following: one initial denaturation cycle at 95°C for 5 min; 45 cycles of 30second denaturation at 95°C, 30 second annealing at 65°C, and30 second elongation at 72°C; and a final extension cycle of 10 min at 72°C. All PCR products were subjected to electrophoresis in an agarose gel and visualized **(Lee et al., 2012)**. Bands were scored as positive when they had the diagnostic size of 764bp. *H. pylori* (ATCC #51110) used as a positive controls and DNase/RNase-free water as negative controls.

RESULTS

Out of the 50 examined horses, 12 (24%) were positive for *Helicobacter* species and all were detected in foal's samples (table.1and fig.1).

The results were obtained by PCR after using 16S rRNA gene (Fig.2)

Table 1

Occurrence of *Helicobacter* spp. in the examined fecal samples by PCR

Horses	Number of horses	Number of positive
Adult horses	20	0
Foals	30	12
Total	50	12

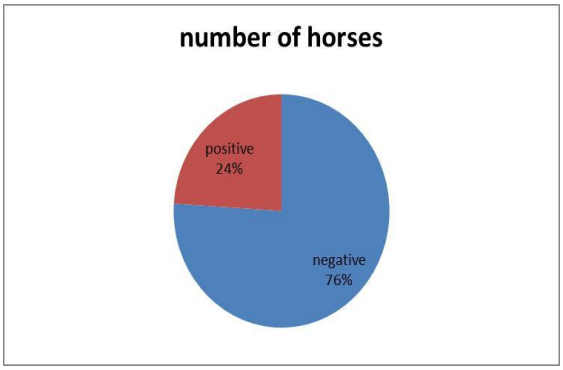

Fig .1. Rate of positive and negative PCR results.

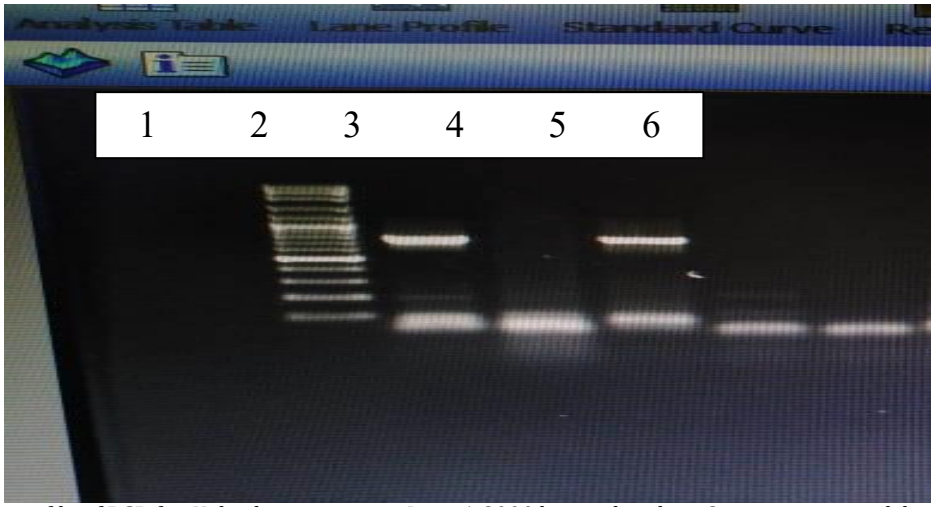

Fig.2. Electrophoretic profile of PCR for *Helicobacter* species. Lane 1:3000 bp marker, lane 2: positive control, lane 3: negative control, lane 4: positive sample show specific band at764 bp, lanes 5, 6: negative samples.

DISCUSSION

To the best of our knowledge, our study is one of the first studies which describe the identification of *Helicobacter* gene material from live horses in Egypt. There was one study detecting *H.pylori* in fish **(Abdel-Moein et al.,2015)** and another one in ruminants **(Aya et al.,2016).**

Infection with *Helicobacter pylori* is of primary importance in the etiology of gastric ulceration in man **(Marshall and Warren, 1984)**.That discovery increased interest to other spiral bacteria that had been seen in many animal species most of these bacteria belong to the genus *Helicobacter* **(O'Rourke et al., 2001)**.In the present study *Helicobacter* spp. was detected in 12 from 50 examined horses (24%) by PCR, the animals were suffering from gastrointestinal diseases. This confirms the colonization of *Helicobacter* spp. in horse's gastric mucosa **(Murray,1997, Scott et al., 2001, Belli et al., 2003).**

Gastric ulcers are common in horses resulting in decreased performance and economic losses to the industry and caused by many factors, infection with *Helicobacter* species is one of them **(Andrews et al., 2005)** but its clinicopathologic effect on gastric mucosa of horse remain dispute.

All the examined specimens were picked up from diseased cases not healthy ones, thus, we could detect positive results in such rate and this may be owed to the changes in the gastric microbial community in diseased cases rather than healthy ones that increase the prevalence of *Helicobacter* spp. DNA (**Moyaert et al., 2007b**, **Costa et al., 2012, Steelman et al., 2012**).

Although the isolation and bacterial culture is the gold standard method, it is limited due to the difficulty of culture of such pathogen **(Morales et al., 2010, Hee-Jin et al., 2016).)**.Therefore, PCR technique was used in the current study to detect it through detection of 16S rRNA specific gene for *Helicobacter* species. Moreover, the use of PCR has an optimal specificity of 100% when compared to culture for the detection of *Helicobacter* in clinical samples **(Hepburn, 2004, Contreras et al., 2007).**

Interestingly, the results in the current study demonstrated that the prevalence of *Helicobacter* spp. was higher in foals than adults (table 1).This may be owed to the active and mature immunity in adults which can overcome the infection, thus they acquired the pathogen and persists lifelong in most infected subjects and so harbour low and subdetectable levels of helicobacters in their gut which shed in low numbers and may be missed **(Perez- Perez et al., 2004).**

Moreover, **Oxley and McKay (2004)** reported the intermittent faecal shedding of enterohepatic helicobacters in adult equine faeces may be also a cause of missing the organism in faeces.

Additionally, in this age category, foals tend to become more independent from their dam, start to inspect their environment and to take up feed and water. Drinking water has indeed been suspected as a possible mode of transmission of *Helicobacter* spp. **(Azevedo et al., 2008).** Also oral–oral contact between mare and foal and faecal–oral exposures might explain the high *Helicobacter* infection rate in young foals **(Moyaert et al., 2009)**. Finaly, there is a possibility that adult horses are thought to be resistant to *R. equi* infection due to the development of a mature immune system **(Takai et al., 1986)** and a similar mechanism could be suspected.

Our finding may spotlight the potential zoonotic pathway for *Helicobacter* among horses and humans as it can affect equines and this has been reported in many previous articles **(Moyaert et al., 2007c Moyaert et al., 2009**, **Bezdekova et al., 2009, Young-Min et al., 2015)** as they could find *H.equorum* DNA in either privately owned, riding school or hospitalized horses. On the other hand, *H.equorum* like bacterium was reported in Japan **(Funato et al., 2011)** in a human with X-linked agammaglobulinemia who had refractory chronic pleurisy indicating that the pathogen can infect humans and act as possible zoonotic pathogen.

Helicobacters other than *H. pylori* such as *H.equorum* have been associated with gastritis, gastric ulcers and gastric mucosa-associated lymphoid tissue lymphoma in humans furthermore, some species display carcinogenic potential in animals and harbor numerous virulence genes and may cause diseases not only in animals but also in humans **(Irena et al., 2017).**

CONCLUSION

It is noteworthy that our findings may have useful public health implications, as the occurrence of *Helicobacter* in horse may make such species a direct source for human infection.

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