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Detection of Gastric Helicobacter like Organisms (GHLO) in Pet Animals by culture, ELISA and rapid urease test

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SUMMARY

A total number of 50 stool, 50 saliva and 50 serum samples were randomly collected from 50 diseased dogs and cats of different breeds hosted in pet animals hospital in port-Said governorate. In addition 23 stomach samples were collected from domestic cats recently euthanized. Samples were prepared and plated on Brain heart infusion agar containing 5% horse serum and Dent supplement antibiotic and selective media Colombia agar with 7% sheep blood and Dent supplement antibiotic. Each type of specimens were subjected to isolation by culture and identification by biochemical activity and ELISA. The results demonstrate that among 50 studied dogs, GHLO isolates from stool and saliva samples were found to be 13 (26%) and 18 (36%) respectively and 31 out of 50 (62 %) were +ve by ELISA. Also the results demonstrate that among 50 studied cats, GHLO isolates from stool and saliva samples were found to be 10 (20%) and 11 (22%) respectively and 25 out of 50 (50 %) were +ve by ELISA. Selective 23 studied cat's stomachs, the results revealed that GHLO isolates were found to be 30.4% were +ve using culture and 47.8% and 52.2% were +ve using rapid urease test (RUT) and ELISA, respectively

INTRODUCTION

Helicobacters spp. are spiral-shaped or curved Gram-negative bacteria, with numerous flagella, microaerophilic, and high urease activity, that inhabit the gastric mucosa. Several species have been identified *Helicobacter heilmanii, Helicobacter bizzozeronii, Helicobacter felis, Helicobacter salmonis, Flexispira rappini* and *Helicobacter billis* described in dogs and cats are morphologically

distinct from *H. pylori* with their more tightly coiled body shape and larger size and cannot be distinguished when they are examined by light microscopy, they are collectively referred to as "gastiric *Helicobacter*-like organisms (GHLO)" (Buczolitis *et al.*, 2003 and Simpson, 2006). The total prevalence of gastric Helicobacter-like organisms (GHLOs) has been reported as 40% and 100% in healthy and sick cats and 61% and 100% in healthy and sick dogs respectively (De Majo *et al.*, 1998; Ettinger and Feldman, 2005 and Simpson, 2006). The present study aimed to isolate GHLO from cats and dogs from their feces, saliva and stomach in port-Said city, using culture characters, biochemical activities, and diagnosis of GHLO by ELISA.

MATERIALS AND METHODS

A total number of 50 stool, 50 saliva and 50 serum samples were randomly collected from 50 diseased dogs and cats of different breeds hosted in pet animals hospital in port-Said governorate. As well as 23 stomach samples were collected from domestic cats recently euthanized in port-Said governorate. Two biopsy specimens were taken from fundus region of the stomach of cats. One mucosal sample for rapid urease test and the second for isolation and identification. Stool and saliva samples were collected in sterile plastic bags. The stool was diluted to a 20% w/v solution in phosphate-buffered saline (PBS) and the suspension sieved through a 250 µm sterile strainer before plating onto selective media and saliva samples were collected by cotton swabs and treated with 1ml sterile physiological saline solution. All samples were plated on Brain heart infusion agar containing 5% horse serum and Dent supplement antibiotic and selective media Colombia agar with 7% sheep blood and Dent supplement antibiotic (Parsonnet *et al.*, 1999). Plates were incubated microaerobically at 37°C for 3-7 days in a jar with 10%, CO_2, 5 %O_2, and humidity obtained by gas generating system (Hachem *et al.*, 1995). Blood was collected aseptically by vein puncture and the serum was separated by standard laboratory techniques and stored at -20°C until further analysis by ELISA (Sigma Co.). Plates coated by sonicated gastiric *Helicobacter*-like organisms antigen according to Norgaard *et al.*, (1995).

Microscopical and biochemical identification:

Film from the growing suspected colonies were stained by Gram stain and studying their culture characters and biochemical activity, oxidase test, urease test, catalase test and nitrate reduction test as recommended by Handt, *et al.*, (1994).

RESULTS

Among 50 studied dogs, GHLO isolates from stool and saliva were found to be 13 (26%) and 18 (36%) respectively. 31 out of 50 serum samples (62 %) were +ve by ELISA. Among 50 studied cats, GHLO isolates from stool and saliva were found to be 10 (20%) and 11 (22%) respectively, 25 out of 50 serum samples (50 %) were +ve by ELISA (Table 1) Within 23 studied cat's stomachs, GHLO isolates were found to be 30.4 % using culture, 11 out of 23 (47.8%) and 12 out of 23 (52.2%) were +ve using RUT and ELISA respectively (Table 2)

Table (1): Frequency of GHLO among the examined dogs and cats samples using culture and ELISA.

Host	Number of Cases	GHLO isolates from stool	GHLO isolates from saliva	ELISA
Dogs	50	13 (26%)	18 (36%)	31 (62 %)
Cats	50	10 (20%)	11 (22%)	25 (50 %)

GHLO: gastiric *Helicobacter*-like organisms

Table (2): Incidence of GHLO among 23 studied cats stomach biopsies using culture, RUT and ELISA.

Cats breed	GHLO isolates	RUT	ELISA
Egyptian Mau(23)	**7(30.4%)**	**11 (47.8%)**	**12(52.2%)**

GHLO: gastiric *Helicobacter*-like organisms

DISCUSSION

Spiral bacteria other than *H. pylori* found in stomach of human (causing gastritis), animals, dogs and cats have been considered a potential reservoir of zoonosis (Wiinberg *et al.*, 2005). The isolation of *H. pylori* and its incrimination as a cause of gastric ulcers in human, led to an increased attention to similar organisms in animals *Helicobacter* organisms were observed in biopsies from the stomachs of dogs, cats, pigs and other carnivores (Eaton *et al.*, 1996 and Hanninen *et al.*, 1996). Still culturing of *Helicobacter* organisms are

difficult, time consuming, expensive, and the sensitivity of this method is dependent on bacterial density, transport conditions, culture medium, incubation conditions, and the skill of laboratory. Not all of the *Helicobacter* spp. are culturable and sensitivity of this technique is low. The prevalence of GHLO in dogs and cats also depends on the techniques employed to detect *Helicobacter* spp. In research studies using several diagnostic tests including a test of high sensitivity, such as ELISA, rapid urease test, impression smears and polymerase chain reaction (PCR) (Strauss-Ayali and Simpson 1999; Krogfelt *et al.*, 2005 and Shabestari *et al.*, 2007).

In the present study, gastric *Helicobacter*-like organisms (GHLO) isolates from feces and saliva of dogs were 26%, and 36% respectively. ELISA results showed that, 62% of dogs were positive. Nearly similar results were obtained in other studies (Happonen 1999 and Shabestari *et al.*, 2007). Higher prevalence rates of gastric *Helicobacters* in dogs were reported (Strauss-Ayali and Simpson 1999; Ettinger and Feldman, 2005; Kolodzieyski *et al.*, 2008 and Shabestari *et al.*, 2008). High prevalence rates are suspected to appear in animals living in colonies (Eaton *et al.*, 1996), other studies showed lower prevalence rates (Otto *et al* 1994). This discrepancy in results may be due to difference in method and samples employed, culture of canine gastric *Helicobacters* is difficult, and even if isolation succeeds, the high frequency of mixed infections in dogs may cause misinterpretation if only one species is identified (Mendall, 1997). Clinical importance of GHLO's in dogs and cats are not clearly proved, although they were present in animals with clinical signs of gastritis (Hwang *et al.*, 2002a).

In the present study, GHLO isolates from stool samples and GHLO isolates from saliva samples of cats were 20% and 22%, respectively. ELISA results showed that, 50% of cats were positive. Higher prevalence rate of GHLO were reported in other studies (Akhtardanesh *et al.*, 2006; Ghil *et al.*, 2009 and Tabrizi *et al.*, 2010). This depends on the study, diagnostic tools employed, sampling method included complete collection of oral secretions vs. the application of oral swabs, selection of the animal population and geographical considerations (Tabrizi *et al.*, 2010)). Positive detection rates were higher in domestic cats feces samples than in saliva samples (46.5% versus 26.6%) and this may suggest that under natural circumstances fecal-oral transmission is more likely than oral-oral transmission among cats (De Schryver *et al.*, 2006). Although cats are popular pets with a significant amount of human contact, there is limited data available regarding the status of

non pylori *Helicobacters* in the oral cavity of these animals, as one of the possible sources of transmission (Tabrizi *et al.*, 2010). Debongnie (1994) indicated that animals may act as natural reservoirs for transmission to humans. The frequent detection of gastric *Helicobacter*-like organisms (GHLOs) in the cats led to the hypothesis that feline *Helicobacter* spp. may be transmitted to humans, but the relationship between these organisms and gastric disease has never been resolved (Eaton *et al.*, 1996; De Groote *et al.*, 2005 and Robić *et al.*, 2007). The significance of oral route as a probable means of *Helicobacter* transmission to humans is, however, supported by other studies (Ghil *et al.*, 2009 and Tabrizi *et al.*, 2010).

The obtained data showed that, among 23 studied cats necropsies, GHLO isolates 7 out of 23 (30.4%) were +ve using culture and 11 out of 23 (47.8%) were +ve using RUT and ELISA results showed that, 52.2% of were positive. In other studies, higher prevalence of *Helicobacter* spp. infection of cats were determined by urease test (Hwang *et al.*, 2002b and Tabrizi *et al.*, 2010) and this agree with other previous studies (Akhtardanesh *et al.*, 2006 and Agharid 2008).

In the present study, GHLO isolates were found to be 30.4 % among cats necropsies using culture. This result disagree with other studies as detection of *Helicobacter* spp. in gastric biopsies was found to be high, ranging from (Akhtardanesh *et al.*, 2006; Takemura *et al.*, 2009 and Tabrizi *et al.*, 2010). However, lower prevalence rates have also been reported (Otto *et al.*, 1994). Clearly, the method used for sampling and primary isolation is critical. In clinical practice, a sample of mucosal tissue obtained by endoscopy from the corpus and/or fundus should therefore be adequate for the detection of *Helicobacter* spp. provided that the sample is of good quality (Willard *et al.*, 2008). The prerequisites in obtaining bacterial growth were the long incubation period, high atmospheric humidity, and use of moist, freshly prepared media, each of which contributed to the high level of contaminants encountered. Fasting the animals prior to biopsy was found to be important in order to decrease the number of contaminating organisms, particularly since *Helicobacter* growth was often first seen on the plate in a relatively small area, making subculture difficult. In addition, contamination of the primary plates of the cat biopsies was more severe than comparable investigations of canine samples. Careful handling of samples and a close attention to methodological detail played an essential role in the results obtained (Eaton *et al.*, 1996). Zoonotic transmission of gastric spiral organisms from domestic dogs and cats has been postulated, because bacteria with morphologies similar to those of both *H.*

felis and other GHLO have been observed in the stomachs of humans with gastritis (Lavelle *et al.*, 1994).

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اكتشاف ميكروب الجاستيرك هليكوباكتر (GHLO) في الحيوانات الأليفة بواسطة الزرع والاليزا و اختبارااليوريز

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تم جمع 50عينه من البراز واللعاب و السيرم من 50 كلب مريض و50 قطه مريضه من مستشفى الحيوانات الاليفه ببورسعيد وكذلك 23 عينه من النسيج المعدى من القطط بعد قتلهم قتلا رحيما. تم تجهيز العينات وزرعها على المستنبتات المناسبه.

لقد اظهرت النتائج عزل ميكروب الجاستيرك هليكوباكترفى عينات اللعاب و البراز بين الكلاب 26% و36% على التوالى. لقد اظهرت النتائج عزل ميكروب الجاستيرك هليكوباكتر فى عينات البرازواللعاب للقطط بنسبة 20%و22%على التوالى وأظهرت النتائج أن 50% من عينات السيرم ايجابية باستخدام اختبار الإليزا. لقد اظهرت النتائج عزل ميكروب الجاستيرك هليكوباكترفى عينات النسيج المعدى للقطط بنسبة 30.4% باستخدام المستنبتات المناسبة و 47.8% من عينات النسيج المعدى و52.2% من عينات السيرم ايجابية لميكروبات الجاستيرك هليكوباكتر باستخدام اختبار اليوريزو اختبارالإليزا على التوالى