Original Research

Prevalence of *Helicobacter pylori* and Associated Risk Factors among Dyspeptic Patients and Dogs in Matrouh Province Regarding the Zoonotic Risks

Ibrahim M. Rabah[1*], Mohamed A. Nossair[2], Elsayed E. Hafez[3], Mohamed Morsi Elkamshishi[1] and Eman Khalifa[4]

1. Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.
2. Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.
3. Department of Plant Protection and Biomolecular Diagnosis, Arid Lands Research Institute, City of Scientific Research and Technological Applications, Borg Al Arab city, Alexandria, Egypt.
4. Department of Microbiology, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.

Correspondence*
Ibrahim M. Rabah, Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.
Email: ibrahim.rabah@mau.edu.eg.

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ABSTRACT

Helicobacter pylori is considered as one of the most threatening zoonosis all over the world with over half of the world's population was suffered from this contagious pathogen. The aim of the study was to determine the *H. pylori* prevalence rate between human and dogs in Matrouh Province through examining 200 stool samples from both hosts using ELISA based detection of *H. pylori* antigen and *16S rRNA* PCR. The results of the overall prevalence *H. pylori* in the current study by PCR and ELISA was 42% and 47.5%, respectively. Based on the ELISA results, it was revealed that the prevalence in dogs was 51% with statistically significant association between the prevalence and the age groups or dog breeds. On the other hand, no statistically significant associations were found between *H. pylori* prevalence and sex, locality, and health status of the examined dogs. Furthermore, the ELISA-based prevalence of the disease in human was 44% being higher in smokers and dyspeptic patients than non-smokers and apparently healthy individuals with statistically significant relationship between the prevalence and both smoking habits and health status. Conclusively, our findings suggested that companion animals could transmit the infection to humans.

Keywords: *Helicobacter pylori*, Dogs, Humans, Prevalence, Risk factors, ELISA, *16S rRNA* PCR

INTRODUCTION

H. pylori (formerly known as *Campylobacter pylori)* is a human pathogen that was originally isolated by **Marshall and Warren (1982)** from endoscopic biopsy specimens of human gastric mucosa **(Zamani et al., 2017)** and it is regarded as a newly emerging bacteria and one of the most common infections globally with great public implications, affecting nearly 50% of the world's population **(Rasi-Bonab et al., 2021)**. In Egypt, the prevalence was prevailed up to 80% of adult population, where it is the most causative agent of dyspepsia **(Salem et al., 2019)**. The worldwide prevalence of *H. pylori* disease has increased exceeding 80% in developing countries, even at young ages **(Ansari and Yamaoka, 2022)** where the personal hygiene is difficult to be controlled in large populations and under 40% in developed countries as a result of enhancement of personal hygiene and quality of life **(Idowu et al., 2019)**.

H. pylori, a group I carcinogen **(Amalia et al., 2023)**, is a gram-negative, microaerophilic, curved or spiral bacteria **(Alsulaimany et al., 2020)**, motile by means of multiple terminal flagella **(Elhariri et al., 2017)**, and usually found in the stomach between the gastric epithelial surface and the mucus layer, where the pH (7.4) is optimum for bacterial motility and protection against gastric acidity **(Elhelw et al., 2020)**. More than 30 *Helicobacter* spp. have been identified in humans and animals in the last two decades and are classified into two groups, gastric and enterohepatic, according to their preferred site of colonization **(Taillieu et al., 2023, Ochoa et al., 2021)**.

H. pylori was firstly discovered in the stomachs of dogs by **Bizzozero (1893)** during the second half of the 19th century as a spiral organism **(Alsulaimany et al., 2020)**. Several studies documented a high homology of DNA sequences of *H. pylori* strains isolated from household dogs and their owners **(Ashaolu et al., 2022)**, supposing that close contact with apparently healthy or diseased household dogs has been hypothesized as a risk factor for human infection **(Bolandi et al., 2017)**. Moreover, the detection of *H. pylori* antigens in the gastric biopsy and feces of domestic and pet animals reached 67-100%, indicating that *H. pylori* is considered a zoonotic infection **(Okubo et al., 2016)**. Despite

the high prevalence rate globally, the transmission pathways of *H. pylori* to humans are still unclear **(Zamani et al., 2017)**. The most proposed hypothesis is that infection can be widespread through fecal-oral, oral-oral routes **(Zhou et al., 2022)**, the use of unsterile endoscopes, and contaminated water and food **(Duan et al., 2023)**.

H. pylori can be easily detected by *H. pylori* stool antigen enzyme immunoassay (HpSA) test **(Youssef et al., 2020)**. HpSA test is widely used screening test than invasive endoscopy due to its rapid diagnosis, inexpensive, extensive use in laboratories as a gold standard test for assessment of eradication therapy according to European and Japanese guidelines **(Elhariri et al., 2017)**.

The main aim of this study was to determine the prevalence and associated risk factors of *H. pylori* among dogs and humans in Matrouh Province using HpSA test and stool PCR. To the best of our knowledge, this is the first study implemented in Matrouh Province, North-West Egypt to determine *H. pylori* prevalence rate and it's associated risk factors among dogs and human.

MATERIALS AND METHODS

Study area: The study was conducted as observational cross-sectional study in Matrouh Province for a period of one year from March 2022 till March 2023. Stool samples were being collected from dogs and humans in contact from different localities in Matrouh Province, North-West Egypt, including Marsa-Matrouh, EL-Dabaa, EL-Hammam, El-Negaila, and Sidi-Barrani districts.

Figure (1): Different localities in Matrouh Province where dogs and human stool samples were collected.

Ethical Approval: This study had prior approval from Institutional Animal Care and Use Committee (ALEXU-IACUC), Alexandria University, Egypt, member of ICLAS. Approval number: 0201649. **Sampling:** A detailed clinical questionnaire was designed for each participants to determine risk factors of the disease with special references to smoking habits and healthy status **(Salem et al., 2019)**. None of the dogs had been treated with antibiotics during the last 4 weeks prior to sampling and the full history of each animal was recorded, including sex, age, locality, breed, and animal health status **(Elhariri et al., 2017)**.

Table (1): Description of stool samples collected from dogs in Matrouh Province concerning sex, age, breed, locality, and general health status in Matrouh Province.

Risk Factor	Variables	Number
Sex	Male	60
	Female	40
Age (years)	1- <2	30
	2- <5	50
	≥5	20
Breed	Rottweiler	40
	German Shepherd	40
	Husky	20
Locality	Marsa-Matrouh	50
	EL-Dabaa	15
	EL-Hammam	15
	El-Negaila	10
	Sidi-Barrani	10
Health status	Apparently healthy	59
	Clinically diseased	41

About 100 stool samples were randomly collected from dogs of veterinary clinics and different farms situated in Matrouh province. As well, 100 stool samples were also randomly collected from dyspeptic patients attending the Gastroenterology Department of Matrouh General Hospital and from humans attending private laboratories in Matrouh Province seeking medical advice. About 100g of stool samples were collected aseptically in sterile labeled cups, and immediately transferred in an icebox to the laboratory or stored at -20 °C **(Dore et al., 2020)** until being examined by PCR and HpSA test and in the laboratory of Plant Protection and Biomolecular Diagnosis Department, Arid Lands Research Institute, City of Scientific Research and Technological Applications, Borg Al Arab City, Alexandria, Egypt.

Table (2): Description of stool samples collected from human in Matrouh Province concerning smoking habits and health status**.**

Risk Factor	Variables	Number
Smoking habits	Smoker	40
	Non- Smoker	60
Health status	GIT disturbances	65
	Apparent healthy	35

Detection of *H. pylori* using *H. pylori* Antigen Enzyme Immunoassay test (Chemux, Perfect, OEM supplier's, California, USA):

Human and dogs stool samples were being tested for detection of *H. pylori* antigens in stool using enzyme-linked immunosorbent assay (ELISA) according to the protocol described by **Abdelmalek et al. (2022)** as follows:
The HpSA test is a quantitative assay for the detection of *H. pylori* antigens in stool specimens. Purified *H. pylori* antibody was coated on the surface of microwells. The First step was placing the desired number of coated strips into the holder then dispensing 100µl of treated sample, calibrators, and controls into the appropriate wells. Secondly, tapping the

holder to remove air bubbles from the liquid. Incubation for 30 minutes at room temperature then Removal of liquid from all wells and repeated washing three times with washing buffer were necessary. Another step of dispensing 100µl of enzyme conjugate to each well followed by incubation for 30 minutes at room temperature. Then removing enzyme conjugate from all wells and repeated washing three times again with washing buffer. Finally, dispensing 100µl of TMB Chromogenic substrate to each well with 15 minutes incubation at room temperature followed by adding 100µl of stop solution to stop reaction. Reading optical density at 450nm with microwell reader. The Optical Density results (O.D. at 450nm) were observed by a microwell reader (SPECTROstar Nano-BMG LABTECH, Germany) compared in parallel manner with calibrator and controls. Interpretation of the test as follows; Positive > 20ng/ml, Negative < 15ng/ml, and Minimum detectable concentration = 0.5ng/ml.

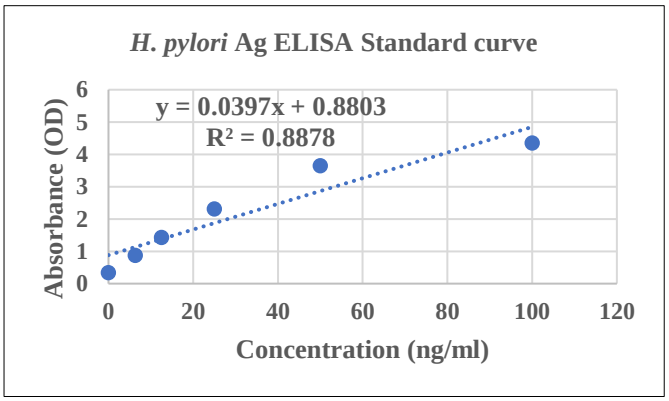

Figure (2): Calibration curve for determination of *H. pylori* antigen concentration (ng/mL) in stool samples collected from 200 clinically affected or apparently healthy cases from their optical density (OD) measured by an ELISA reader at 450nm.

PCR assay (*H. pylori 16S rRNA* PCR-amplification):

All humans and dogs stool samples were furtherly tested for the presence of the *H. pylori 16S rRNA* gene as a confirmatory analysis.

DNA extraction: DNA Extraction from stool was conducted according to the phenol-chloroform method as previously described by **Gaur and Reddy (2017)**. The extracted DNA purity and concentration were assessed spectrophotometrically by determining the A260/A280 optical density ratio through Nanodrop (SPECTROstar Nano, Germany). The values of DNA purity of 1.8:2.0 were acceptable and the absorbance ratio of 260:280nm was useful indication for DNA purity.

Preparation of diluted primer from Stock primers of 100µM solution (Metabion, Germany): Preparation of a working primer solution for each primer (forward and reverse) was done by adding 10µl of the 100µM stock primer solution (after reconstitution with the pamphilid D.D. H_2O) with 90µl autoclaved D.D. H_2O to be 100µl called working primer solution.

Chemical and thermal Cycling conditions of PCR:

Table (3): PCR primers used in the current study.

Gene	Primer	Oligonucleotide Sequence (5'-3')	Amplicon size (bp)	Reference
16S rRNA gene (specific for *H. pylori*)	F	CTG GAG AGA CTA AGC CCT CC	110	(Youssef et al., 2021)
	R	ATT ACT GAC GCT GAT TGT GC		

Table (4): The adjustment of thermal conditions of *H. pylori 16S rRNA* gene amplification.

Steps	Temperature	Duration	No. of cycles
Initial PCR denaturation step	94°C	2 min.	1 hold
Denaturation	95°C	30 sec.	30 cycles
Primer annealing	60°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	8 min.	1 hold
Cooling	Hold at 4°C till further processing		

The 20µl reaction mixture consisted of 10µMol of each primer (1µl) (Metabion, Germany), DNA Template (1µl), PCR Master Mix (ABT 2x Red Mix, Cat. No. AMP-01, Applied Biotechnology, Ismailia, Egypt) (10µl), and autoclaved d.d H2O (7µl). The reaction mixture was mixed gently by vortexing and spinning.

The amplification was carried out in an Eppendorf Thermal cycler (SureCycler 8800, Agilent Technologies, California, USA). The PCR product was applied on a 1.5% Agarose gel (Bioshop®, Canada Inc) according to **Sambrook et al. (1989)** with ethidium bromide staining (Bioshop®, Canda Inc), and a 50bp ladder was used as a DNA molecular weight marker (FastGene 50bp DNA ladder cat. no. MWI32, Nippon Genetics, Europe). The gel was run for 35-45 minutes at 100 Volt using the power supply where the negatively charged DNA migrates towards the positive anodes. After 35-45 minutes the fluorescence was visualized under UV light till the tracking dye reaches in the center of the gel using the UV-transilluminator. A photograph was made on the Gel-Doc system (SyeGene, India).

Statistical analysis:

Data were statistically analyzed using IBM SPSS Statistics 25 and chi^2 test to assess the relationship between prevalence of *H. pylori* and different risk factors. A probability *P* value at $P < 0.05$ was considered statistically significant **(Norusis, 2008)**.

RESULTS

As shown in **Table (5),** the results revealed that the prevalence of *H. pylori* in dogs and humans by HpSA test was higher than PCR results, Moreover, the prevalence in dogs by the two tests (51% and 44%, respectively) was higher than that recorded in humans (44% and 40%, respectively).

Table (5): Prevalence of *H. pylori* in dogs and humans in Matrouh Province as examined by HpSA test and PCR.

Dogs and Human	Number of examined samples	*H. pylori* Antigen Enzyme Immunoassay test		PCR	
		Positive	%	Positive	%
Dogs	100	51	51.0	44	**44.0**
Humans	100	44	44.0	40	**40.0**
Total	**200**	**95**	**47.5**	**84**	**42.0**

The prevalence of *H. pylori* in dogs based on HpSA test as clarified in **Table (6)** showed that it was lower in females than males. Age-based detection of *H. pylori* was higher in aged dogs. Moreover, the highest prevalence of *H. pylori* by HpSA test was recorded in Marsa Matrouh that contains the largest dog farms and pet shops. Rottweiler dog represented a higher comparable prevalence than German shepherd and Huskies. Finally, apparently healthy dogs had a lower prevalence than diseased dogs.

Table (6): Prevalence of *H. pylori* in dogs in Matrouh Province as examined by HpSA test concerning risk factors.

Risk factors		Dogs		
		No.	Positive	%
Sex	Male	60	34	56.67
	Female	40	17	42.50
	X^2/P	1.927/0.165NS		
Age	1- < 2	30	0	0.0
	2- < 5	50	36	72.0
	≥ 5	20	15	75.0
	X^2/P	44.658/0.000**		
Locality	Marsa Matrouh	50	30	60.0
	El-Dabaa	15	6	40.0
	El-Hammam	15	6	40.0
	El-Negaila	10	4	40.0
	Sidi-Barrani	10	5	50.0
	X^2/P	3.561/0.469 NS		
Breed	Rottweiler	40	24	60.0
	German Shepherd	40	22	55.0
	Husky	20	5	25.0
	X^2/P	6.963/ 0.031*		
Health status	Apparently healthy	59	33	55.93
	Clinically diseased	41	18	43.90
	X^2/P	1.401/0.237NS		

** High statistically significant differences at P ≤ 0.001
* Statistically significant differences at P ≤ 0.05
NS = non-significant difference at P > 0.05

The obtained results, as presented in **Table (7),** revealed that *H. pylori* prevalence in human by HpSA test was higher in patients with GIT disturbances (56.92%) than apparently healthy individuals (20%) and was higher in smokers (57.50%) than non-smokers (35%) with statistically significant association between the prevalence of *H. pylori* in humans and both risk factors.

Table (7): Prevalence of *H. pylori* in human in Matrouh Province as examined by HpSA test with special reference to smoking habits and health status.

Risk factors	Variables	Samples number	Positive	%
Health status	GIT disturbances	65	37	56.92
	Apparently healthy	35	7	20.00
	X^2/P	12.587/ 0.000**		
Smoking habits	Smoker	40	23	57.50
	Non- smoker	60	21	35.00
	X^2/P	4.931/ 0.026*		

** High statistically significant differences at P ≤ 0.001
* Statistically significant differences at P ≤ 0.05

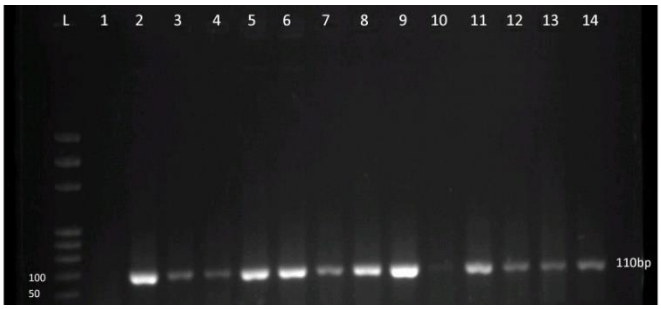

Figure (3): Amplified PCR products on agarose gel electrophoresis using *16S rRNA* gene specific for *H. pylori* isolated from dogs and human stool samples in Matrouh Province.

L: 50bp molecular weight DNA ladder with a size range of 50-500bp. **Lane 1 and 10**: negative stool sample of human and dog, respectively. **Lane 2: 9**: positive for *16S rRNA* gene specific for *H. pylori* isolated from stool samples of humans. **Lane 11, 12, 13, and 14:** Positive for *16S rRNA* gene specific for *H. pylori* isolated from stool samples of dogs.

DISCUSSION

The HpSA test and stool PCR in our study offered a useful tool for the diagnosis of that bacterium without scarifying companion animals. The high prevalence of *H. pylori* in companion animals proved their role in the transmission of that pathogen to humans in contact as a reservoir of infection. So, appropriate control and preventive methods should be implemented to circumvent the future potential for economic losses and the public health hazard of *H. pylori*.

The prevalence rates of *H. pylori* in dogs were 51 and 44% by HpSA test, and PCR, respectively. The result obtained by stool antigen test (51%), somehow higher than that obtained by the two Egyptian studies conducted by **Shaaban et al. (2023)** (24% in El-Beheira), and **Elhariri et al. (2017)** (37.2%) in Giza and Cairo, while **Hong et al. (2015)** didn't detect any *H. pylori* in stool samples of dogs in Korea. On the other hand, it was lower than that recorded by **Dore et al. (2020)** (100%) in Italy and **Okubo et al. (2016)** (94.7%) in

Brazil. Furthermore, the prevalence by PCR in our study was higher than that recorded by **Ochoa et al. (2021)** with a prevalence rate of 12.3% in Chile, **Kubota-Aizawa et al. (2017)** with a prevalence rate of 34.7% in Japan, **Jankowski et al. (2016)** with a prevalence rate of 23.3% in Poland, and the two Iranian findings of 5.41% and 8.66% that were represented by **Torkan and Shahreza (2016)**, and **Bolandi et al. (2017)**, respectively.

On the other side, the current molecular result was lower than the three reports in Egypt registered by **Youssef et al. (2021)** of 57.14% in Ismailia, **Hamza et al. (2018)** of 76.6% in Cairo, and **Elhelw et al. (2020)** of 62.5 in Giza. Additionally, the prevalence determined by PCR in dogs is also lower than that obtained by **Guerra Segundo et al. (2021)** of 74.2% in Brazil. Epidemiological evidence of *H. pylori* in dogs may shed light on the dangerous role played by dogs in the continuous shedding of that disease to other livestock as well as humans in Matrouh Province, so strict control measures must be followed to avoid risks attributed to the rearing and management of dogs with domestic animals.

It was found in **Table (6)** that 34 out of 60 males tested positive (56.67%), while 17 out of 40 females tested positive (42.5%). Chi-square analysis of the obtained result showed a non-significant relationship (Chi^2 value = 1.927, P > 0.05) between sex and the prevalence of *H. pylori* in dog. This result agreed with the Egyptian study carried out in Giza and Cairo governorates by **Elhariri et al. (2017)**, who noticed that the prevalence in males (40%, 22 out of 55) was higher than that of females (33.3%, 13 out of 39), with non-significant differences, and **Bolandi et al. (2017)**, who realized that male prevalence was significantly higher than that of females (12.3% and 5.88%, respectively) in Iran. While **Okubo et al. (2016)** found that all tested Brazilian dogs, either male or female, were evaluated as positive for *H. pylori*.

The prevalence in **Table (6)** was higher in the age group ≥5 years (75%) than the age group 2- < 5 years (72%), while it is free in the age group 1- < 2 years. Statistical analysis showed a significant relationship between age and the prevalence of *H. pylori*. This finding was supported by the Japanese case report of **Kubota-Aizawa et al. (2021)**, who noticed that the two 11-years-old Pomeranian dogs were positive for *Helicobacter* genus-specific and *H. pylori*, confirming that aged dogs have a higher chance of contracting that infection. Our finding contradicted the Brazilian observation of **Okubo et al. (2016)**, who realized that all dogs of all ages were positive for *Helicobacter* spp. without any considerations. Additionally, it disagreed with the Italian study of **Dore et al. (2020)** and the Egyptian study of **Elhariri et al. (2017)**, who observed that there was a non-significant relationship between age and infection rate in dogs in Giza and Cairo governorates. The higher rate of infection in the older dogs in our study may be due to their advanced age, as the organism may remain latent or chronic for an unspecified period before manifesting any clinical disease. Alternatively, the older animals have a greater chance of becoming infected and of coming into contact with other animals. On the other hand, younger animals tend to be more resistant to *H. pylori* infections; however, latent infections can occur in these animals **(Kubota-Aizawa et al., 2021)**.

The highest prevalence of *H. pylori* in our study was observed in Marsa Matrouh (60%) followed by Sidi-Barrani (50%) where Marsa Matrouh is considered as urban locality having more pet shops and pet breeding places than the other localities. Statistical analysis showed a a non-significant association between the prevalence of *H. pylori* in dogs and locality. The results recorded by **Elhariri et al. (2017)** revealed a significant association between *H. pylori* prevalence in dogs and different localities in Cairo and Giza governorates, Egypt, that contradicts what we obtained in our study. Noticeably, the detection of *H. pylori* in various localities in Matrouh Province indicated the transmission and spread of that infection among different farms, supporting the zoonotic theory of the disease. Conclusively, this study indicated that *H. pylori* is widely distributed among companion animals in the selected regions of Egypt.

Rottweiler dogs represented the highest prevalence rate compared to other dog species, where it was 60%, followed by German shepherds (55%), and finally huskies (25%), which had the lowest prevalence due to the hypothesis mentioned by **Bolandi et al. (2017)**, who stated that household dogs had a lower prevalence than other outdoor or stray dogs as they usually feed on cooked house foods that are free from such pathogens. That finding was contrary to that obtained by **Elhariri et al. (2017)**, who noticed that the prevalence in domestic housed dogs was higher than that of stray dogs, with significant differences between the prevalence and dog breeds in Egypt (Giza and Cairo governorates), and **Abdel-Raouf et al. (2014)**, who found that *H. pylori* prevalence in domestic dogs was higher than that of stray dogs in Mansoura Governorate, Egypt (45.3% and 29.4%, respectively).

There were no significant differences between the prevalence of *H. pylori* in dogs and their general health status as shown in **Table (6),** being somewhat similar between both apparently healthy and clinically diseased animals, supposing that both apparently healthy (latent host) and diseased animals play an imperative role in the transmission of that infection to humans. The study on dogs conducted by **Ekman et al. (2013)** in Sweden supported our finding that there was no significant association between *H. pylori* prevalence in tested dogs and histopathological changes in their gastric or duodenal mucosa. On the other hand, the result in dogs in our study was contrary to that obtained by **Bolandi et al. (2017)** in Iran, who observed significant differences between the health status of dogs and disease prevalence being higher in diseased than healthy dogs (8.57% and 5.55%, respectively). Additionally, the findings of **Kubota-Aizawa et al. (2017)** and **Abdi et al. (2014)** contradicted our findings where the former found that Japanese dogs infected with *Helicobacter* spp. had a severe degree of gastritis compared to dogs negative for

Helicobacter spp. at P = 0.044, and the latter detected a significantly higher prevalence in Iranian dogs with gastric atrophy or fibrosis than other dogs with healthy gastric mucosa through histological examination. Furthermore, the study in Portugal conducted by **Amorim et al. (2015)** on 69 dogs revealed a significant association between *Helicobacter* spp. prevalence and the gastric clinical outcomes at $p < 0.05$, where one case represented a normal gastric mucosa and negative *Helicobacter* spp., two dogs represented a positive *Helicobacter* spp. with normal gastric mucosa (2.9%), while the remaining (95.7% or 66/69) showed gastritis with positive *Helicobacter* spp. isolates. Concerning the higher prevalence within apparently healthy dogs than clinically diseased ones in our study, the Iranian study of **Torkan and Shahreza (2016)** contradicts our finding, where they found that the prevalence in diseased dogs with gastric ulcers was higher than in other apparently healthy dogs (6.36% and 4.61%, respectively).

The role of healthy dogs as carriers of infection was supported by **Ochoa et al. (2021)**, who noticed that 15.4% of studied dogs in Chile carried *H. pylori* without any gastric disorders. Since a considerable percentage of the examined dogs in our study were infected with *H. pylori* without demonstrating any clinical signs, it seems that dogs may be a natural carrier host for that pathogen.

The high prevalence of *H. pylori* within several studies was 67%: 86% within clinically healthy dogs and 61%: 100% within diseased dogs suffering from chronic vomiting, confirming the theory that most dogs suffering from gastric ulcers and chronic vomiting may act as a natural reservoir of that infection and shed that bacteria to humans through the fecal-oral route or oral-oral route **(Elhelw et al., 2020)**. The presence of *H. pylori* in the stool samples of dogs with gastritis in our study meant that they harbored this pathogen in their gastric mucosa, confirming the zoonotic perspective of *H. pylori* transmission through stool, mainly by the fecal oral route due to the typical canine behavior of anal licking. The oral-oral route hypothesis was confirmed by the case report stated by **Kubota-Aizawa et al. (2021)**, who stated that the woman in the study had the habit of feeding their dogs the masticated food and letting them lick her mouth, aiding in supposing that this may be a transmission route between the woman and their Pomeranian dogs. Additionally, **Kubota-Aizawa et al. (2021)** proved the zoonotic hypothesis of *H. pylori* by studying the case report of a 53-year-old dog owner who contracted *H. pylori* from his own two 11-year-old Pomeranian dogs, where biopsy samples were collected and analyzed by *Helicobacter* genus and species-specific PCR, revealing that all samples from the dogs and their owner were positive for *Helicobacter* genus-specific and *H. pylori* species-specific PCR, while negative for *H. felis, H. heilmannii,* and *H bizzozeronii*.

About 56.92% (37 out of 65 patients) of the examined human samples collected from patients complaining of GIT disturbances were found to be positive for *H. pylori* as illustrated in table **(7)**. On the other hand, only 20% (7 out of 35 individuals) of the examined samples of apparently healthy individuals were found to be positive for *H. pylori*. Statistical analysis showed a significant association (P = 0.000) between health status and the prevalence of the disease. This result was supported by the previous Egyptian studies carried out by **Galal et al. (2019)** in Cairo governorate, who found a significant association between the prevalence and clinical presentation of participants; **Salem et al. (2019)** in Menoufia, who found that those with previous peptic ulcer history was higher in prevalence than others with no PU by 14.2% with statistical significant differences; **Haggag et al. (2016)** in El-Behira, who noticed that the prevalence was most common in individuals suffered mainly from gastrointestinal disorders (100%) than apparently healthy individuals (64%) with highly statistically differences between the prevalence and health status, **Abdel-Raouf et al. (2014)** in Mansoura governorate, who proved that the prevalence in apparently healthy individuals was lower to that of diseased (23.9% and 64.3%, respectively); and **Agha et al. (2013)** in Mansoura governorate, who found a significantly higher prevalence among patients with epigastric pain symptoms (43.3%) than unexposed individuals (20%). Furthermore, our study was in harmony with that obtained by **Fang et al. (2020)** in Taiwan, who observed a higher prevalence in symptomatic individuals than others who were non-symptomatic (25.6% and 19.1%, respectively), and **Dilnessa and Amentie (2017)**, who observed that the prevalence is higher in dyspeptic patients (58.2%) than non-dyspeptic individuals (39.1%) in Ethiopia. On the contrary, our result counteracted what was obtained by **Hailu et al. (2016)** in Ethiopia, who found that patients with previous GIT disturbances (48.5%) were more resistant than others with non-previous medical GIT history (53.6%) with a non-significant association; **Hassan et al. (2013)** in Pakistan; and **Talaiezadeh et al. (2013)** in Iran, who found a non-significant association between detection rate and individuals with dyspeptic symptoms.

The prevalence of *H. pylori* by ELISA was higher in smokers (57.50%) than non-smokers (35%) as presented in **table (7).** Chi2 value of 4.931 that was significant at $P < 0.05$ revealed a significant association between smoking habits and the prevalence of the disease. The higher prevalence in the smoker group in our study may be attributed to the fact that smoking increases the risk of gastric ulcers and gastric mucosal damage, increasing the risk of acquiring infection. This finding was supported by **Idowu et al. (2019)** in South Africa, who observed that the prevalence in smokers (56.9%) was higher than that observed in non-smokers (50.3%); and **Dutta et al. (2017)** in India, who proved a statistically significant association between *H. pylori* prevalence and smoking habits. On the contrary, our result contradicted the previous Egyptian studies carried out by **Salem et al. (2019)** in Menoufia governorate, who observed that non-smokers had a higher prevalence than smokers (75% and 25%, respectively) with a non-significant association; **Emara et al. (2017)** in Zagazig, who found the stool *H. pylori* negative group among participants included more smokers than the *H. pylori* positive group; and **Agha et al. (2013)** in Mansoura,

who found a non- significantly higher prevalence in non-smokers (61.8%) than smokers (38.2%).

Additionally, our finding contradicted what detected by **Seid and Demsiss (2018)** in Ethiopia, who found that the prevalence in cigarette smoker was lower than that of non-smokers (22.2% and 30.9%, respectively) with non-significant association; **Hamrah et al. (2017)**, who noticed that there was no statistically significant difference between the prevalence and smoking habits in Afghanistan, being higher in non-smoker than current smokers (54.8% and 39.1%, respectively); **Dilnessa and Amentie (2017)** who found a non-significant higher prevalence in cigarette non-smoker group in Ethiopia; and **Hailu et al. (2016)** in Ethiopia, who realized that the prevalence was 45.5% and 50% for current smoker and never smoker groups, respectively.

CONCLUSION

The recorded results in the current study throw the light upon the role of dogs in Matrouh Province in the epidemiology of *H. pylori* and the transmission of *H. pylori* to human. It was clear that there was a relationship between the recorded higher comparable prevalence of *H. pylori* in dogs and humans in contact in Matrouh province, highlighting the zoonotic potential of the disease in this nomadic geographic area. Furthermore, the shedding of *H. pylori* in stool among the enrolled participants supports the concept that stool is the major shedding site.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the planning of the work, experimental design, measurement of parameters, and writing of the manuscript. IMR, MAN, EEH, and EK designed the experiments. IMR and EEH conducting the molecular part. IMR, MAN, and EK measured the parameters. IMR and MME statistically analyzed the data. IMR, MAN, EEH and EK wrote the manuscript. All authors read, reviewed, and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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