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Epidemiological Investigation of *Helicobacter pylori* as an Emerging Zoonosis in Matrouh Province, North-West Egypt: A Community Based Cross-sectional Study

Ibrahim M. Rabah[1*], Mohamed A. Nossair[2], Elsayed E. Hafez[3], Mohamed M. Elkamshishi[1], 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**
Corresponding author: Ibrahim M. Rabah
E-mail address: ibrahim.rabah@mau.edu.eg

Abstract

Helicobacter pylori is newly emerging bacteria and one of the most common infections worldwide with over one-half of the world is infected with this organism. The aim of this study was to determine the prevalence of *H. pylori* among sheep, camels, and humans in Matrouh Province, North-West Egypt using *H. pylori* stool antigen enzyme immunoassay and stool PCR. A total of 250 stool samples were collected from farm animals (sheep and Camels) and humans in Matrouh Province. Samples were examined using *H. pylori* Stool Antigen Enzyme Immunoassay test and *H. pylori* 16S rRNA PCR. Statistical analysis was applied using Chi2 and IBM SPSS Statistics 25. The overall prevalence of *H. pylori* infection in the study by HpSA and PCR were 27.6% and 24.4%, respectively. Based on the results of HpSA test, it was found that the prevalence was 12% and 26% in sheep and camels, respectively, with statistically significant association between the prevalence and locality or age of sheep. Moreover, the prevalence of *H. pylori* infection in human was 44% by HpSA test with statistically significant association between the prevalence and gender or locality being higher in males than females with greater rural prevalence than urban. On the other hand, there was a non-statistically significant differences between *H. pylori* prevalence and sex, breed, and health status of examined animals or age, residence, and occupation of enrolled individuals. Conclusively, *H. pylori* was detected in both animal and human samples is alarming in Matrouh Province. Therefore, there was an urgent need for implementing a proper control program.

KEYWORDS
Helicobacter pylori, Sheep, Camels, Humans, Prevalence, HpSA, 16S rRNA PCR, Zoonosis.

INTRODUCTION

H. pylori, a human pathogen, is one of the most common infections worldwide and a newly emerging bacteria with great public implications, affecting approximately 50% of the world's population (Fang *et al*., 2020; Horiuchi *et al*., 2021; Rasi-Bonab *et al*., 2021). In Egypt, the prevalence was reported to be 60%-80% of the adult population, where it is the most common cause of dyspepsia (Salem *et al*., 2019). The prevalence has increased worldwide to the point that more than 7 million infections are now reported annually, with approximately 4.4 billion individuals infected worldwide (Obaidat and Roess, 2019).

In 1994, the International Agency for Research on Cancer (IARC) qualified this bacterium as a class 1 risk for the development of gastric cancer (El-Shenawy *et al*., 2017) and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Pereira *et al*., 2014). Interestingly, *H. pylori* is regarded as the 2nd leading cause of worldwide cancer-associated deaths and the 4th most common type of gastric cancer, where the minimum infectious dose was 10^4 (WHO, 2019).

H. pylori is an anthroponotic type where humans are the principal reservoir as it colonizes the gastrointestinal mucosa of humans (Elhelw *et al*., 2020) and domestic animals (Jankowski *et al*., 2016). It also recovered from the gastric tissue of sheep in the absence of associated gastritis, suggesting that sheep may be a natural host for *H. pylori* (Ranjbar and Chehelgerdi, 2018). Moreover, the isolation of *H. pylori* from stool and gastric tissues of camels and sheep supporting the zoonotic hypothesis (Youssef *et al*., 2020). However, their role in the transmission of that infection to humans remains unclear (Zamani *et al*., 2017). The most commonly acknowledged hypothesis is that infection takes place through oral-oral, fecal-oral routes, iatrogenic spread through the use of unsterile endoscopes, and contaminated water and food (Momtaz *et al*., 2014; Obaidat and Roess, 2019; Seid and Demsiss, 2018). Many reports support the zoonotic transmission of *H. pylori* as a result of the isolation of *H. pylori* from gastric tissues, milk, and stool samples of farm animals, in which persons in close contact with farm animals have a higher probability of acquiring the infection, such as abattoir workers, veterinarians, and butchers (Shaaban *et al*., 2023).

H. pylori can be detected by non-invasive and invasive methods, the latter requiring endoscopy (Ganesh *et al*., 2020). The invasive methods include biopsy-based PCR, rapid urease test, histological examination, and culturing, while the non-invasive methods comprise C13- or C14-urea breath test (UBT), stool-PCR, *H. pylori* stool antigen enzyme immunoassay, and serological detection of IgG in blood (Youssef *et al*., 2020). *H. pylori* Stool Antigen test (HpSA) is preferred to invasive endoscopy due to its rapid diagnosis, inexpensive, and extensive use as a primary screening test and gold standard test for assessment of eradi-

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cation therapy according to European and Japanese guidelines (Elhariri *et al*., 2017).

Unfortunately, little information about the epidemiology of *H. pylori* in domestic farm animals in Egypt is known; there are few reports dealing with its zoonotic importance and few studies have been conducted revealing a high prevalence rate of infection in both animals and humans in Egypt (Galal *et al*., 2019). So, the current work correlated the zoonotic and public health repertoires of *H. pylori* in farm animals (sheep and camels) and humans in contact.

The main aim of this study was to determine the prevalence of *H. pylori* among sheep, camels, and humans in Matrouh Province using *H. pylori* stool antigen enzyme immunoassay and stool PCR. To the best of our knowledge, it was the first study conducted to determine the prevalence of *H. pylori* and associated risk factors in Matrouh Province, North-West Egypt.

MATERIALS AND METHODS

The study was presented in the format of a cross-sectional observational design in Matrouh Province for a period of one year starting in March 2022. Specifically, stool samples were being collected from sheep, camels, and humans in contact from almost all Matrouh localities in North-West Egypt, including EL-Hammam, EL-Dabaa, Marsa-Matrouh, El-Negaila, and Si-di-Barrani districts. Ecologically, these five districts unequivocally represented Matrouh governorate mainly for two reasons: the first, as they harbored the highest herd number of sheep and camels according to data that were obtained from the Veterinary Medicine Directorate of Matrouh, and secondly as they are geographically evenly distributed with nearly equal distances between them.

Ethical Approval and consent to participate

This study has prior approval from Institutional Animal Care and Use Committee (ALEXU-IACUC), Alexandria University, Egypt, member of ICLAS. Approval number: 0201649. Informed consent was obtained from the human participants and/or their legal guardian after informed in details about the aims of the work.

Samples

Human participants

Inclusion criteria: Patients with these criteria were included (Emara *et al*., 2017; Shaaban *et al*., 2023): 1) both male and female; 2) age range from 10 to over 60 years; 3) good mentality to understand aims, steps, and benefits of the study; and 4) agreement upon stool sampling and examination by different diagnostic tests.

A full detailed clinical assessment in the form of a questionnaire was designed for each individual to determine risk factors with specific emphasis on age, gender, place of residence, locality, and occupation (Obaidat and Roess, 2019; Salem *et al*., 2019). None of the animals had been treated with antibiotics during the last 4 weeks prior to sampling (Kubota-Aizawa *et al*., 2017) and the full history of each animal was recorded, including sex, age, breed, locality, and animal health status as described by Elhariri *et al*. (2017) and Torkan and Shahreza (2016).

Exclusion criteria: Patients were excluded from the study if they had these conditions (Hussein *et al*., 2021): 1) chronic diseases, e.g., diabetes, cirrhosis, and renal failure; 2) pregnant women; 3) prior GIT surgery; 4) prior peptic ulcer diseases other than *Helicobacter*iosis; and 5) patients who used antibiotics, proton pump inhibitors (PPIs), or probiotics within the last 4 weeks before being enrolled in the study (Abdelmalek *et al*., 2022).

Animals

A total of 150 stool samples were randomly collected from farm animals of both sexes (100 from sheep and 50 from camels) at different farms and veterinary clinics situated in Matrouh province. As well as 100 stool samples from humans attending private laboratories in Matrouh Province and from dyspeptic patients visited the Gastroenterology Department of Matrouh General Hospital and private hospitals in Matrouh province, North-West of Egypt. About 50 g of stool samples were collected, labeled, and transferred in an icebox with the minimum of delay to the laboratory or stored at -20°C (Dore *et al*., 2020; Elhariri *et al*., 2018) until being examined by ELISA and PCR 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.

Detection of H. pylori

ELISA-based detection of *H. pylori*

All samples were being tested for detection of *Helicobacter* antigens in stool using enzyme-linked immunosorbent assay (ELISA, *H. pylori* Antigen Enzyme Immunoassay test) (Chemux, Perfect, OEM supplier's, California, USA) according to the protocol specified by Marshall and Warren (1982) as described by Abdelmalek *et al*. (2022).

The *H. pylori* Antigen Enzyme Immunoassay (ELISA) test is a quantitative assay for the detection of *H. pylori* antigens in stool specimens. Purified *H. pylori* antibody is coated on the surface of microwells. An aliquot of diluted stool sample is added to wells, and the *H. pylori* antigens if present, bind to the antibody. All unbounded materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of antigen in the sample. The results (O.D. at 450nm) were read by a microwell reader (SPECTROstar Nano-BMG LABTECH, Germany) compared in parallel manner with calibrator and controls. Interpretation of the test as follows; Minimum detectable concentration = 0.5ng/ml, Negative < 15ng/ml, and Positive > 20ng/ml.

Conventional PCR assay (H. pylori 16S rRNA gene PCR-amplification)

All animal and human samples were examined for the presence of the *H. pylori* species-specific 16S rRNA gene.

DNA extraction: Extraction of DNA from stool was carried out according to the phenol-chloroform method recommended by Gaur and Reddy (2017). The extracted DNA concentration and purity were determined spectrophotometrically by measuring the A260/A280 optical density ratio by using Nanodrop (SPECTROstar Nano-BMG LABTECH, Germany) where the absorbance ratio between 260nm to 280nm is useful indication for DNA purity. Values of DNA solutions of 1.8 to 2.0 are acceptable.

Chemical and Physical Cycling conditions of PCR: The primers for 110bp product of the 16S rRNA gene represented by F: 5-CTG GAG AGA CTA AGC CCT CC-3, and R: 5-ATT ACT GAC GCT GAT

TGT GC-3 (Bolandi *et al*., 2017; Elhariri *et al*., 2018; Idowu *et al*., 2019; Youssef *et al*., 2021). The 20µl reaction mixture consisted of DNA Template (1µl), 10µMol of each primer (1µl) (Metabion, Germany), PCR Master Mix (FastGene Taq 2x Ready Mix with loading dye, Nippon Genetics, Germany) (10µl), and autoclaved d.d H2O (7µl). The physical conditions as follow: one cycle of initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, followed by a final extension step by 1 cycle at 72°C for 8 min. The amplification was carried out in an Eppendorf Thermal cycler (SureCycler 8800, Agilent Technologies, California, USA). The PCR product was separated on a 1.5% Agarose gel (Bioshop®, Canada Inc) with ethidium bromide staining (Bioshop®, Canda Inc) according to Sambrook *et al*. (1989), and a 50bp ladder was used as a DNA molecular weight marker (FastGene 50bp DNA ladder cat. no. MWI32, Nippon Genetics, Europe). A photograph was made on the Gel-Doc system (SyeGene, India).

Statistical analysis

Data were collected and statistically analyzed using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). Data were entered as variables represented as tables. The chi-square test was used to assess the association between *H. pylori* infection and various risk factors. A probability (P) value ($P < 0.05$) was considered statistically significant at a confidence interval (CI) of 95% (Norusis, 2008).

RESULTS

The obtained results, as shown in Table 1, revealed that the prevalence of *H. pylori* infection in farm animals and humans by *H. pylori* Antigen Enzyme Immunoassay Test was higher than the results of PCR, where the prevalence in sheep was 12% and 10%, respectively lower than that recorded in camels (26% and 22%, respectively). While humans represented the highest prevalences with the two tests (44% and 40%, respectively).

Based on ELISA test, the results in Table 2 illustrated that the prevalence of *H. pylori* in sheep was higher in males than females, On contrary to camels. Age-wise positivity of *H. pylori* was higher in older group of sheep, while it was higher in the smallest group of camels. Concerning the locality, the highest prevalence of *H. pylori* by ELISA was 26.67% for sheep in El-Hammam and 60% in El-Negaila and Sidi-Barrani for camels. Barki sheep represented a higher comparable prevalence than Rahmani breed. Moreover, diseased animals had a higher prevalence than apparently healthy animals.

The obtained results, as shown in Table 3 showed that prevalence of *H. pylori* by ELISA was higher in males (61.82%) than females (22.22%), was higher in the age group ≥ 60 years (60%) than age group 10 - < 20 (55%) and age group 40 - < 60 (35%). The highest prevalence of *H. pylori* by ELISA was recorded in El-Dabaa (53.33%), while the lowest prevalence was recorded in Marsa Matrouh and El-Negaila (40%). Furthermore, the prevalence was higher in rural than urban areas by 26.67%. While laboratory workers had the highest prevalence (60%), followed by health care workers (55%). The lowest prevalence was demonstrated in veterinarians and animal attendants, where the prevalence was 25% and 26.67%, respectively. There was a significant association between the prevalence of *H. pylori* in humans and gender or locality at $P < 0.05$.

Table 1. Prevalence of *H. pylori* in farm animals and humans in Matrouh Province as examined by ELISA and PCR.

Farm animals and Humans	Number of examined samples	*H. pylori* Antigen Enzyme Immunoassay test		PCR	
		Positive	%	Positive	%
Sheep	100	12	12	10	10
Camels	50	13	26	11	22
Humans	100	44	44	40	40
Total	250	69	27.6	61	24.4

Table 2. Prevalence of *H. pylori* in farm animals in Matrouh Province as examined by ELISA with priority to risk factors.

Risk factors		Sheep				Dromedary Camels			
		Number	Positive	%	X^2/P	Number	Positive	%	X^2/P
Sex	Male	40	5	12.5	0.016/	25	4	16	1.927/ 0.165NS
	Female	60	7	11.67	0.900NS	25	9	36	
Age	1- < 2 / 1- < 7	40	2	5	12.642/	20	7	35	2.183/ 0.336NS
	2- <3 / 7- < 14	40	3	7.5	0.002*	20	3	15	
	≥3 / ≥14	20	7	35		10	3	30	
Locality	Marsa Matrouh	20	3	15	9.740/	10	3	30	11.365/ 0.023*
	El-Dabaa	25	4	16	0.045*	15	4	26.67	
	El-Hammam	15	4	26.67		15	0	0	
	El-Negaila	20	1	3.85		5	3	60	
	Sidi-Barrani	20	0	0		5	3	60	
Breed	Barki	70	9	12.86	0.162/	50	13	26	---------
	Rahmani	30	3	10	0.687NS				
Health status	Apparently healthy	77	8	10.39	0.822/	41	10	24.39	0.307/ 0.580NS
	Clinically diseased	23	4	17.39	0.365NS	9	3	33.33	

** High statistically significant differences at $P \leq 0.001$; * Statistically significant differences at $P \leq 0.05$; NS = non-significant difference at $P > 0.05$

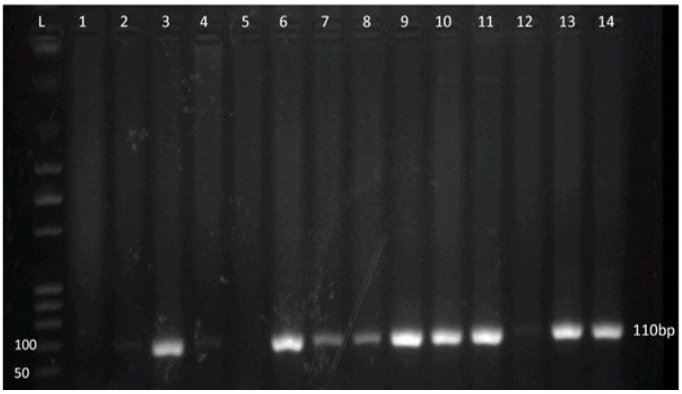

Fig. 1. Agarose gel electrophoresis of PCR products amplified using 16S rRNA gene (110bp) specific for *H. pylori* isolated from animal and human stool samples in Matrouh Province. L: 50bp molecular weight DNA ladder with a size range of 50-500bp. Lane 1 and 5: negative stool sample of camels and sheep. Lane 2, 3, and 4: positive for 16S rRNA gene specific for *H. pylori* isolated from stool samples of sheep. Lane 6, 7, 8, 9, and 10: 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 camels.

DISCUSSION

The recorded results in the current study throw the light upon the role of farm animals in Matrouh Province, North-West Egypt, in the epidemiology of *H. pylori* and their role in the transmission of that pathogen to humans in contact as a reservoir of infection highlighting the zoonotic potential of the disease in this nomadic geographic area and clarifying the need for more attention in planning a control program to reduce that infection.

The incidence of *Helicobacter*iosis in Egypt is very high and is attributed to *H. pylori* (Youssef *et al*., 2021). The problem in the diagnosis of *H. pylori* in Egypt is that there is no clear data for most infected individuals, the presence of inapparent cases, and the haphazard consumption of unhygienic fast or ready-to-eat foods (Zaki *et al*., 2016). We chose fecal samples in our study rather than other samples as it is considered the main shedding site where *H. pylori* colonizes the gastric mucosa, crosses the intestine, and then descends in feces (Jankowski *et al*., 2016). *H. pylori* antigen test is regarded as a gold standard test since the detection of bacterial DNA by PCR may be less precise due to the lower bacterial DNA count in feces as a result of bacterial DNA degradation in intestine and also due to PCR can detect the DNA from live and dead bacteria without differentiation of the active and previous infection (Okubo *et al*., 2016).

Concerning sheep, it was found in Table 1 that the prevalence of *H. pylori* in sheep was 12 and 10% by *H. pylori* Antigen Enzyme Immunoassay test and PCR, respectively. The prevalence obtained by ELISA was higher than that recorded by Shaaban *et al*. (2023) (10%) in El-Beheira, Egypt. On the contrary, it was lower than that recorded by Dore *et al*. (2020) (82% in Italy), Elhariri *et al*. (2018) (16%) in Egypt, and Momtaz *et al*. (2014) (16%) in Iran. Additionally, the result of PCR in our study was lower than that obtained by Youssef *et al*. (2021) (25%) in Ismailia, Egypt, and Saeidi and Sheikhshahrokh (2016) (29.16%) in Iran. A substantial discrepancy in the prevalence of *H. pylori* in sheep may be due to animal population, health status, susceptibility, and the hygienic measures applied in each locality. Furthermore, the lower prevalence of *H. pylori* in sheep in our study may be due to the fact that there is a lower density of microorganisms in feces as compared with those that colonize the gastric mucosa or stomach lumen. Hence, the colonization of the bacteria in the stomach of sheep as compared with other alimentary organs induces a much stronger immune response that is much more detectable by serological tests (Mishra *et al*., 2008).

The prevalence of *H. pylori* in camels was 26 and 22% by *H. pylori* Antigen Enzyme Immunoassay test and PCR, respectively. The obtained molecular result was higher than that recorded by Youssef *et al*. (2020), who found that 4 out of 32 (12.5%) Egyptian dromedary camels in Ismailia were positive, and Saeidi and Sheikhshahrokh (2016), who detected *H. pylori* in 10% of tested camels in Iran. While it is near to that obtained by Youssef *et al*. (2021) (22.22%) in Ismailia Province, Egypt.

Sex-based prevalence of *H. pylori* in sheep depending on the results of ELISA was recorded in Table 2. It was revealed that the prevalence was 12.5% and 11.67% in males and females, respectively. A Chi-squared analysis of the obtained result showed a non-significant relationship (Chi2 value = 0.016, P > 0.05) between sex and the prevalence of *H. pylori* in sheep. This result was in harmony with that obtained by Dore *et al*. (2020) in Italy, where he realized a non-significantly higher prevalence in male sheep than females (56% and 26%, respectively), Elhariri *et al*. (2018), who found a non-significantly higher prevalence in

Table 3. Prevalence of *H. pylori* in humans as examined by ELISA in relation to risk factors in Matrouh Province.

Risk factors	Variables	Samples number	Positive	%	X^2/P value
Gender	Male	55	34	61.82	35.302/0.000**
	Female	45	10	22.22	
Age (years)	10 - < 20	20	11	55	3.341/0.342NS
	20 - < 40	30	13	43.33	
	40 - < 60	40	14	35	
	≥ 60	10	6	60	
Residence	Marsa Matrouh	40	16	40	0.974/0.914 NS
	El-Dabaa	15	8	53.33	
	El-Hamam	15	7	46.67	
	El-Negaila	15	6	40	
	Sidi-Barrani	15	7	46.67	
Locality	Rural	40	24	60	6.926/0.008*
	Urban	60	20	33.33	
Occupation	Health Care Workers	20	11	55	8.956/0.062NS
	Laboratory Workers	30	18	60	
	Animal attendants	15	4	26.67	
	Farmers	15	6	40	
	Veterinarians	20	5	25	

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

male sheep than females by 22% within three Egyptian provinces (El-Giza, El-Qalyubia, and El-Fayom), and Momtaz *et al*. (2014), who observed a higher prevalence in male sheep than females in an Iranian study (56.25% and 43.75%, respectively). On the contrary, the Iranian result obtained by Saeidi and Sheikhshahrokh (2016) proved a non-significant relationship between sex and prevalence within sheep flocks, with female prevalence being higher than male (32.5% and 30.4%, respectively). On examining camels, it was revealed that the prevalence was 36% in females and 16% in males. Chi-square analysis of the obtained result showed a non-significant relationship (Chi2 value = 2.599, P > 0.05) between sex and the prevalence of *H. pylori* in camels. This result agreed with the report conducted in Egypt by Youssef *et al*. (2020), who found that the sex-related prevalence of *H. pylori* in camels in Ismailia was non-significant. While the finding of Saeidi and Sheikhshahrokh (2016) in Iran contradicted ours, as they realized a significantly higher prevalence in males than females (33% and 8%, respectively).

Age-based prevalence of *H. pylori* in sheep depending on the results of ELISA was tabulated in Table 2. It clarified that the prevalence in the age group ≥ 3 years (35%) was higher than that of the age group 2- < 3 years (7.5%) and 1- < 2 years (5%). Statistical analysis showed a significant association (Chi2 value = 12.642, P < 0.05) between age and the prevalence of *H. pylori* in sheep. This was in harmony with that noticed in the Egyptian study of Elhariri *et al*. (2018), who revealed a significantly higher prevalence in aged sheep over three years old than those less than three years old (22% and 9%, respectively) in the three Egyptian provinces (El-Giza, El-Qalyubia, and El-Fayom). This disagreed with the Italian study conducted by Dore *et al*. (2020), who observed that there was a non-significant difference between the *H. pylori* prevalence and age. Concerning camels, it was observed that the highest prevalence was observed in the age group 1- < 7 years (35%) followed by the age group ≥14 years (30%) and the age group 7- < 14 years (15%) and statistical analysis showed a non-significant association (Chi2 value = 2.183, P > 0.05) between age and the prevalence of *H. pylori* in camels. The higher prevalence rate of *H. pylori* among the age group 1- < 7 years old was supported by the Egyptian study conducted by Youssef *et al*. (2020) in Ismailia, who noticed that the young aged camels were more liable to contract that gastric infection than other older groups due to the fact that young camels with developing immunity are more susceptible to contracting that gastric pathogen during group suckling or group grazing within the same herd than older camels with sufficient immunity. On the contrary, Saeidi and Sheikhshahrokh (2016) found a significantly higher prevalence of infection in older camels than younger ones in Iran.

The prevalence of *H. pylori* in farm animals through ELISA concerning localities in Matrouh Province was recorded in Table 2, illustrating that the highest prevalence in sheep was observed in El-Hammam (26.67%) followed by El-Dabaa (16%). while, the result of camels revealed that the highest prevalence was observed in Sidi-Barrani and El-Negaila (60%); this is due to the fact that these localities harbor a large camel flock. Also, the social pattern of this area may explain the lack of awareness about the disease and its control strategy. Statistical analysis showed a significant association between the prevalence of *H. pylori* in sheep or camels and locality. These results were in harmony with those obtained by Rahimi and Kheirabadi (2012), who recorded significant differences (P < 0.05) between the four different provinces in Iran and the prevalence of *H. pylori* in farm animals including dairy cattle, buffalo, sheep, goats, and camels. Noticeably, the detection of *H. pylori* in various localities in Matrouh Province indicated the transmission and spread of that infection among farm animal flocks, supporting the zoonotic theory of the disease. Conclusively, this study indicated that *H. pylori* is widely distributed among domestic animals in the selected regions of Egypt.

The prevalence of *H. pylori* in sheep concerning breed was higher in Barki sheep than Rahmani sheep by 2.86%, with a non-significant association between prevalence and breed, similar to the Egyptian study of Elhariri *et al*. (2018), who noticed that there was no statistically significant association between *H. pylori* prevalence in sheep and various breeds in the three Egyptian Provinces (El-Giza, El-Qalyubia, and El-Fayom). The results in Table 2 showed that there was no significant difference between the prevalence of *H. pylori* in farm animals and their general health status being somewhat closely 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 result in sheep was supported by the Egyptian study of Elhariri *et al*. (2018), who detected a non-significant association between the health status of sheep in the three Egyptian governorates (El-Giza, El-Qalyubia, and El-Fayom) and *H. pylori* prevalence.

The data presented in Table 1 showed the prevalence of *H. pylori* among examined human beings through the *H. pylori* Antigen Enzyme Immunoassay test, and PCR was 44% and 40%, respectively. The recorded prevalence according to the results of ELISA (44%) was somewhat near that obtained by the Egyptian study of Elhariri *et al*. (2017) (44.4% in Giza and Cairo) and Mishra *et al*. (2008) (42.8%) in India. While it was higher than that recorded by Abdelmalek *et al*. (2022) (16.33%) in three Egyptian provinces (Giza, Menofia, and Benha), Seid and Demsiss (2018) (30.4%) in Ethiopia, Joob and Wiwanitkit (2014) (34%) in Thailand, and Hassan *et al*. (2013) (25%) in Pakistan. On the contrary, it was lower than the three Egyptian studies carried out by Shaaban *et al*. (2023) (74.8% in El-Beheira governorate), Salem *et al*. (2019) (73.7% in Menoufia governorate), and Agha *et al*. (2013) (56.7% in Mansoura governorate). Furthermore, our ELISA result was lower than the previous studies conducted by Obaidat and Roess (2019) (88.6%) in Jordan, Hamrah *et al*. (2017) (75.6%) in Afghanistan, Aje Joseph *et al*. (2016) (46.8%) in Nigeria, Hailu *et al*. (2016) (50.7%) in Ethiopia, Abebaw *et al*. (2014) (72.2%) in Ethiopia, Momtaz *et al*. (2014) (82%) in Iran, Tadesse *et al*. (2014) (83.3%) in Ethiopia, Mathewos *et al*. (2013) (65.7%) in Ethiopia, and Talaiezadeh *et al*. (2013) (53.5%) in Iran. The high prevalence rate observed in our study and other Egyptian studies carried out by Shaaban *et al*. (2023); Salem *et al*. (2019) and Agha *et al*. (2013) confirmed the role of *H. pylori* as a common and important cause of dyspepsia in Egypt.

Additionally, the PCR record was lower than the four previous Egyptian reports registered by Youssef *et al*. (2021) (44.83%) in Ismailia, Elhelw *et al*. (2020) (91.6%) in Giza, Hamza *et al*. (2018) (60%) in Cairo, and Abu-Taleb *et al*. (2018) (45.76%) in Zagazig governorate. Additionally, it was lower than that reported by Akeel *et al*. (2019) (46.52%) in Saudi Arabia, Idowu *et al*. (2019) (48.9%) in South Africa, and Wongphutorn *et al*. (2018) (72.7%) in Thailand. Furthermore, the lower prevalence of PCR than ELISA in our study contradicted what was obtained by Hussein *et al*. (2021) in Iraq, where he realized that PCR had a higher prevalence than SAT by 3.4% (70.4% and 67%, respectively).

This variation in the prevalence of *H. pylori* within humans in the current work and others may be attributed to different geographic locations, study design, sample size, different age groups, various socio-economic status, variation in occupational contact, and the type of test used regarding different sensitivities and specificities. Furthermore, the shedding of *H. pylori* in stool among the enrolled participants supports the concept that stool is the major shedding site.

The prevalence of *H. pylori* among enrolled humans, depending on the results of ELISA concerning gender, was recorded in Table 3 showed that males' prevalence (61.82%) was higher than that in females (22.22%), and statistical analysis showed a significant association (P = 0.000) between gender and the prevalence of *H. pylori*. The higher prevalence in males agreed with the two Egyptian studies carried out by Abdelmalek *et al*. (2022) in Giza, Menofia, and Benha, where he recorded the infection at 61.04% in males and 41.18% in females, and Emara *et al*. (2017), who observed that 55% of males in Zagazig governorate were infected higher than females (45%). Additionally, our finding was supported by Obaidat and Roess (2019) (91% and 86.1% in males and females in Jordan, respectively), Dutta *et al*. (2017) (the prevalnce

in males within the Indian study was significantly higher than that of females by 12.5%), Tacikowski *et al*. (2017) (40.9% and 34.9% in males and females, respectively in Poland), Hailu *et al*. (2016) (61% and 43% for both males and females in Ethiopia with statistically significant association), Tadesse *et al*. (2014) who detected through an Ethiopian study that the prevalence was higher in males than females by 3.6%, and Abebaw *et al*. (2014) (the female prevalence (68.1%) was lower than males (77.8%) in Ethiopian study),

On the contrary, it disagreed with the previous Egyptian studies conducted by Shaaban *et al*. (2023), who found that more women than men were seropositive by 14.2% in the study conducted in El-Beheira governorate; Salem *et al*. (2019) in Menoufia province, who found that female prevalence (69.6%) was higher than that of males (30.4%); and Elhariri *et al*. (2017), who realized that the prevalence in males (38.64%) was lower than that of females (50%) with non-statistical differences in the study conducted in Giza and Cairo governorates. Furthermore, our finding contradicted what obtained by Horiuchi *et al*. (2021) in Japan, Idowu *et al*. (2019) in South Africa, Wongphutorn *et al*. (2018) in Thailand (the prevalence in females was higher (56.3%) than that of males with non-significant differences), Seid and Demsiss (2018) in Ethiopia (32.3% and 28.1% for both females and males, respectively with non-significant association), Dilnessa and Amentie (2017), who found the female prevalence in Ethiopian study was higher than that of males by 23.2%, Aje Joseph *et al*. (2016) in Nigeria, where he noticed that the female prevalence (33.8%) was higher than that of males (13%) with non-statistically significant differences and Joob and Wiwanitkit (2014) in Thailand (33.8% and 34.3% for both males and females respectively with non-significant differences).

Our findings could be explained by the fact that more women are often seeking treatments for health troubles, and they are also more caring about their health than men are. Also due to the fact that men are habituated to work outdoors for a longer period of time than women, and they don't seek medical care due to much time lost at work. As a result, they are more exposed to environmental risk factors.

The age-wise prevalence of *H. pylori* in humans in Matrouh Province was illustrated in Table 3 pointed out that a Chi-square analysis of the obtained result showed a non-significant relationship (P > 0.05) between the different age groups. This was similar to what obtained by Seid and Demsiss (2018) in Ethiopia, where he found that elder people are more susceptible than young individuals with non-significant associations; Wongphutorn *et al*. (2018) in Thailand, where he proved that aged individuals over 40 years had the highest prevalence (87.1%) than the other ages with non-significant differences; and Hassan *et al*. (2013), who found that the detection rate in age group > 45 years (27.2%) was higher than that of < 45 years (23.7%) with non-significant differences in Pakistan. The highest prevalence in our study was observed in the age group over 60 years (60%) followed by the age groups 10- < 20 years (55%), 20- < 40 years (43.33%), and the age group 40- < 60 (35%). The increased prevalence in the age group > 60 years may be attributed to the fact that the older group represents the most susceptible group with insufficient immunity. This result was supported by what obtained by Shaaban *et al*. (2023), who realized that the prevalence in the age group > 50 years (87.3%) was higher than that of the age group 15 < 50 years (76.8%) in El-Beheira governorate, Egypt; Fang *et al*. (2020), who noticed that older people in Taiwan over 60 years were more susceptible to acquire the infection than younger group 20-39 years where the prevalence was 39.3% and 7.9%, respectively; Obaidat and Roess (2019) in Jordan, who detected the highest prevalence in the age group over 50 years old (93.4%) than the other age groups; Tacikowski *et al*. (2017) in Poland, who detected that older group (50-59 years old) had a higher prevalence (51.5%) than the younger groups with non-statistical significant differences; and Hailu *et al*. (2016) in Ethiopia, who observed that the prevalence in the age group ≥ 50 years (61.8%) was greater than age group below 30 years and 30-49 years (48.1% and 47.9%, respectively).

On the contrary, it disagreed with the previous results obtained by Elhariri *et al*. (2017) in Giza and Cairo governorates, Egypt, where he proved that there was a higher prevalence in the third decade (72.2%) than the age group lower than 15 years (31.4%) with statistically significant differences; Agha *et al*. (2013) in Mansoura province, Egypt, where he observed that age group 24-35 years old was significantly higher than older groups (36-45 years old and over 45 years old, where the prevalences were 20.6% and 35.3%, respectively); Dilnessa and Amentie (2017) in Ethiopia, who found that the prevalence in younger people (18-34 years = 60.7%) was higher than elder people (> 45 years = 17.9%); Dutta *et al*. (2017) in India, who proved that the prevalence within the middle age group (31-50 years) was significantly higher than that of the age groups 15-30 years and above 50 years old (48.3%, 42.6%, and 34.9%, respectively) at P = 0.002; Aje Joseph *et al*. (2016) in Nigeria, who supported the theory that infection is acquired during childhood and decreases with time as the highest prevalence was observed in the youngest age group 17-26 years old (15.1%) followed by 27-36 years old (12.2%), 37-46 years old (11%), and finally over 47 years old (8.6%); and Tadesse *et al*. (2014) in Ethiopia (the age group 25-34 years old recorded the higher prevalence of 88%).

The prevalence of *H. pylori* among examined human beings depending on the results of ELISA concerning the place of residence in Matrouh Province was illustrated in Table 3, showing that the highest prevalence was noticed in El-Dabaa (53.33%), followed by El-Hamam and Sidi-Barrani (46.67%), and finally Marsa Matrouh and El-Negaila (40% for each). Statistical analysis (Chi2 value = 0.974) showed that there was a non-significant association (P > 0.05) between the prevalence of the disease among examined human beings and their place of residence in Matrouh Province. This agreed with Talaiezadeh *et al*. (2013) in Iran, who found anon-significant association between living area and *H. pylori* prevalence within the studied population. On the contrary, the Egyptian study conducted by Galal *et al*. (2019) revealed a significant relationship between the prevalence and the place of residence, with the prevalence being higher in Lower Egypt than Upper Egypt (54.3% and 45.7%, respectively).

It was noticeable that the prevalence of the disease was 60% (24 out of 40) in those inhabiting rural areas and 33.33% (20 out of 60) in those inhabiting urban areas. There was a significant difference (P > 0.05) between the prevalence in urban and rural areas. This result in agreement with the previous Egyptian studies carried out by Shaaban *et al*. (2023), who noticed that individuals in rural places (88.5%) in El-Beheira governorate had a higher prevalence than those in urban parts (51.7%) with statistically significant association between the prevalence and locality; Galal *et al*. (2019), who also revealed the significant association between the prevalence and locality being higher in those inhabited rural areas (60.4%) than others in urban places (39.6%) in Cairo province, Salem *et al*. (2019), who found that there was a higher prevalence in farmer group (82.1%) than non-farmer group (64.9%) in Menoufia governorate, supporting the theory that *H. pylori* is a zoonotic infection and farm animals may be a source for the transmission to man especially those handling farm animals; and Agha *et al*. (2013), who found that Egyptian workers who lived in rural places had a higher prevalence (50%) than those inhabiting urban habitats (38.2%) in Mansoura governorate. Furthermore, our study was supported by the previous Ethiopian studies conducted by Seid and Demsiss (2018) and Tadesse *et al*. (2014), who observed that rural participants in Ethiopia had a higher prevalence than urban individuals by 42.4%, with statistically significant differences. The higher prevalence of *H. pylori* among rural participants in this study and similar studies may be attributed to some factors, including bad personal hygiene in rural areas, low-income levels, poor education, and poor sanitary measures. On the other hand, a higher prevalence among urban than rural residents (81.3% and 18.7%, respectively) was observed in the study conducted by Dilnessa and Amentie (2017) in Ethiopia. Additionally, Hailu *et al*. (2016) and Abebaw *et al*. (2014) realized a

non-significant association between the prevalence of infection and locality in Ethiopia.

The prevalence of *H. pylori* among examined human beings depending on the results of ELISA concerning occupation is presented in Table 3. It was shown that the highest prevalence was observed in laboratory workers (60%), followed by health care workers (55%), farmers (40%), animal attendants (26.67%), and finally veterinarians (25%). It was noticed that there was a non-significant association ($P > 0.05$) between different occupations and the prevalence of *H. pylori*. This agreed with Idowu *et al*. (2019), who realized that medical practitioners' prevalence was (58.3%) greater than that of others not engaged in medical practices (52.4%) in South Africa; Wongphutorn *et al*. (2018), who found a non-significant association between the prevalence and occupation in Thailand; and Seid and Demsiss (2018) and Dilnessa and Amentie (2017), who found that profession was statistically insignificant in getting human *Helicobacter*iosis in Ethiopia. The recorded prevalence in laboratory workers and health care workers in our study highlighted the role of direct contact with the infected animal or human and their infected specimens (infected stool samples) as a critical risk factor affecting the transmission of that disease to humans, supporting the theory of zoonotic transmission of the disease. While this result was contrary to that obtained by Salem *et al*. (2019) in Menoufia, Egypt, who found that farmers (57.1%) are more prone to acquire infection than non-farmer individuals with a statistically significant association between the prevalence and occupation; Hailu *et al*. (2016) in Ethiopia, who found pastoralists' prevalence (67.6%) was higher than that of the other occupations with a significant association between the prevalence and occupation; and Abebaw *et al*. (2014) in Ethiopia, who observed that the highest prevalence was significantly related to the farmer group. Tadesse *et al*. (2014) supported the fact of students' habituation toward fast-contaminated street foods and ready-to-eat foods, where they recorded the highest prevalence (93%), compared to the other occupational groups with a non-statistically significant association.

CONCLUSION

The HpSA test and stool PCR in our study offers a useful tool for the diagnosis of that bacterium without scarifying farm animals. Concerning humans, male more susceptible than females and elder people are more liable to contract infection, finally laboratory workers are the target category to acquire that infection.

Conclusively, this study is one of the most comprehensive surveys of the epidemiological prevalence of *H. pylori* isolated from both domestic animals and humans in contact in Matrouh Province, North-West Egypt. The diversity of the prevalence of *H. pylori* in various hosts and regions is usually related to animal, environmental, and microbial factors.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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