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Epidemiological Study on H. pylori in Cattle and Its Milk with Special Reference to its Zoonotic Importance

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Abstract

This study was carried out to investigate some epidemiological aspects on the occurrence of Helicobacter pylori in cattle, milk and humans at Dakahlia province, Egypt during the period from February 2014 to June 2015. A total of 304 samples including 117 rectal swabs (53 cows and 64 buffaloes) and 85 milk samples (36 cows and 49 buffaloes) sand 102 humans stools were collected and subjected to bacteriological examination by culturing on Columbia Blood Agar (CBA) and biochemically identified. The results showed that the overall occurrence of *H. pylori* were 21.7% in collected samples. The occurrence in cattle faeces was 18.8% (11.9% in cows and 6.9% in buffaloes). However, the isolation rate from cattle milk was 28.2% (10.5% in cows and 17.7% in buffaloes). Moreover, the frequency distribution of *H. pylori* from human stool was 19.6%. Concerning animal breed, native breed of cattle and their milk showed higher occurrence (5.2% in cow's faeces, 11.9% in buffalo's faeces, 7% of cow's milk and 17.7% of buffalo's milk). Regarding animal age, the occurrence of *H. pylori* was increased with increasing age. On the other hand, the frequency distribution of *H. pylori* was more prevalent in the samples (faeces and milk) collected from Mansoura center. In relation to human samples, with respect to gender, males showed higher isolation rate (11.7%) than females (7.8%).Whereas, frequency of *H. pylori* in adults (4.9%) was higher than young (1.96%).On the other hand, *H. pylori* was more frequently isolated from patients (8.8%) with gastrointestinal disorders. Moreover, the occurrence of *H. pylori* was higher in human samples collected from persons of occupations related to animals such as veterinarians (6.8%), dairy workers, and farmers (3.92% each) than others. It could be concluded that *H. pylori* could be isolated from cattle, milk and humans with recognizable percentages, suggesting its zoonotic significance and role played by cattle especially buffaloes and its milk as potential reservoir and source of human infection. The zoonotic significance for *H. pylori* as well as the recommended preventive measures which should be taken to avoid the risk of contamination of milk and human infection were fully discussed.

Keywords: Epidemiology; Zoonosis; *H. pylori*

Introduction

The Helicobacter genus consists of a group of microaerophilic, none sporulating, Gram-negative rods that colonize on the mucus layer covering the epithelial surface of the gastrointestinal tract of humans and a variety of animal species. There are currently 6 validated Helicobacter species isolated from gastric tissue and 16 validated entero hepatic species. Some Helicobacter species may be commonly (*H. aurati*) or occasionally (*H. bilis and H. muridarum*) isolated from both gastric and entero hepatic sites [1].

Although *H. pylori* is present in the stomachs of about half of world's population, the routes of transmission are still unclear and non-human reservoirs have not been identified. The prevalence of *H. pylori* infection increases with age and is inversely related to socio-economic and hygiene status, suggesting person-to person transmission. Several studies have shown high prevalence of antibodies against *H. pylori* in abattoir workers, such as veterinarians, butchers and slaughterers, suggesting that *H. pylori* might be transmitted from animals to man. Dogs and sheep have also been implicated in the transmission of Helicobacter infection [2].

From the zoonotic point of view and public health importance of H. pylori, recently recovery of *H. pylori* from cows by Dore et al. [3] supported this idea. Milk (especially raw milk) and its products are indicated as an important vehicle for transmission of pathogenic microorganisms, where, milk is considered as cultural and growth media for such organism [4].

Little information about the epidemiology of *H. pylori* in dairy animals in Egypt are known , also the reports dealing with its zoonotic importance and role of cattle and its milk which act as reservoir and source of human infection in Egypt are scarce or absent. So, this work was conducted to carry out some epidemiological studies on the occurrence of *H. pylori* in cattle and their milk. Also, the epidemiological aspects of *H. pylori* in man were investigated.

Materials and Methods

A total of 304 cattle, milk and human samples were collected from Mansoura and Aga centers, at Dakahlia province, Egypt. From cattle, 117 faecal samples including (53 cows faeces, 64 buffaloes faeces) of different ages and breeds were taken directly from the rectum of animals using sterile swabs, The swabs then directly immersed in tubes containing Tryptone soya broth and immediately transported to the laboratory in ice box under complete aseptic conditions. The detailed data concerning locality, age, sex, breed, housing, health status, number of parturition, stage of lactation, water supply, feeding pattern and hygienic disposal of animal wastes. From raw milk, 85 milk samples consisting of (36 cows and 49 buffaloes) were

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collected in sterile cups. From human stool specimens, 102 represented (45 apparently healthy, 35 with gastrointestinal disorders, and 22 diarrheal stool specimens) were obtained .All samples were collected in sterile cups and transported to laboratory in ice box under complete aseptic conditions. The data including age, gender, health status, socio-economic level, hygienic practices, occupations and previous suffering from dyspepsia. All collected samples were cultured within six hours from collection.

Isolation and Identification of *H. pylori*

All collected animal rectal swabs were directly streaked on CBA supplemented with antibiotic (Vancomycin, Trimethoprim and Cefsulodin). A loopful from each collected milk sample was plated on CBA. A sterile swab was taken from each human stool sample and directly cultured on CBA. All cultured plates were incubated under microaerophilic conditions (5% O_2, 15% Co_2 and 80% N_2) using generating kits (Campygen TM 2.5 L) (Oxoid CN0025A) for creation of microaerophilic atmosphere at 37°C for one week (5-7 days) After the incubation, the suspected growing colonies (tiny, small and translucent) were picked up and inoculated on CBA slope, incubated microaerophilically at 37°C for 5-7 days for further identifications [5].

The tests used for identification of *H. pylori* isolates were Gram staining, oxidase, catalase, urease, TSI, growth at 1% glycine and resistance to Nalidixic acid and cephalothin [6].

Results and Discussion

Helicobacter pylori, is well established as a major cause of gastritis, peptic ulcer, duodenal ulcer and chronic gastritis. It is also implicated in the development of gastric cancer. Consequently, the detection of *H. pylori* infection has become important, due to fastidious and slow growing nature of *H. pylori,* great care was needed in the collection, transport and culture [7]. However, the origin and transmission of this bacterium has not been obviously explained. One of the suggested modes of transmission is cattle milk consumed by human beings [8].

H. pylori is one of the most common bacterial infectious agents inhabits the stomach of more than half of world's population. The presence of *H. pylori* antigens in faeces of cows which contaminates the cow's milk and might be a transmission route of *H. pylori* infection to man. Moreover, faecal contamination to milk due to improper hygienic practice during production and management processing could transfer *H. pylori* to milk consumers. From the zoonotic point of view, it was utmost of importance to study the role of cattle and its milk as reservoir and source of human infection with *H. pylori* .According to available data and literatures, there was no previous studies in Egypt dealing with zoonotic importance of H. pylori, moreover, the articles investigated and discussed the epidemiology of *H. pylori* in cattle in Egypt are scarce or absent.

In the present study, the frequency distribution of *H. pylori* from Holstein cows was 1.7% (Table 1). In a previous study carried out on 92 lactating Holstein cows in Shahrekord, Iran found that the prevalence of *H. pylori* antigens in faeces of cows were 10.8 %. The recorded results in Table 1 showed that the native or Baladi breeds of cows had higher frequency distribution of *H. pylori* (5.2%) While the buffaloes native breed were showed a percentage of (11.9%). From the results recorded in Table 1, it could be notice that the examined native breeds of cows and buffaloes showed higher isolation rates of *H. pylori* than the examined Holstein breeds. This might be suggested that either native or Baladi breeds are more susceptible to exposed to carry *H. pylori* in their gut and shedding in their faeces or the native breeds might be reared under low level of hygienic practices (Table 2).

The frequency distribution of *H. pylori* in animal faecal samples in relation to age was illustrated in Table 2. There were various frequency distribution of H.pylori with different age group in cattle (from 0-6 months, from 6-12 month, from 12-18 month, from 18-24 month and over 2 years), the frequency distribution of *H. pylori* were 1.7%, 0.85%, 0.85%, 0.85%, and 2.65%, respectively, whereas their respective frequency distribution of *H. pylori* in buffaloes were 0.85%, 0.85%, 1.7%, 1.7% and 6.8% (Table 3).

It was obvious from recorded results in Table 2 that the highest Incidence (6.8% and 2.65%) of *H. pylori* was in buffaloes and cows over than 2 years, respectively. The most examined over than 2 years .Females are kept for lactation and breeding for old ages which they were more exposed to *H. pylori* infection. However, the present results showed that small or young ages were with low frequency rates (1.7%) in cows and (0.85%) in buffaloes. This explained that the age may be considered as a risk factor for *H. pylori* infection. This result suggesting the old age of cows and buffaloes are more exposed to harbor *H. pylori* infection (Table 4).

The results showed that the examination of the faecal samples collected from various localities showed variable frequency distribution rates from one area to another (Table 3). In cows the samples collected from Aga district, Nawasa Al-Bahr village, Mitishna village, Mansoura district, Awish Al-Hagar village were showed occurrence of 0.85%, 1.7%, 0.85%, 2.65% and 0.85%, respectively, while the respective occurrence frequency distribution in buffaloes were 1.7%, 0.85%, 2.6%, 5.9% and 0.85%. The recorded results revealed that higher frequencies (5.9% and 2.65%) were found in buffaloes and cows in Mansoura district (Table 5).

This might be due to the examined farms in Mansoura district were mixed herds of cows and buffaloes of low hygienic conditions in farms, which facilitate the contact between buffaloes and cows and give the opportunities to harbor the *H. pylori* microorganism.

In the present study, the frequency distribution of *H. pylori* from Holstein cow's milk was 3.5 %, while from the native breed or Baladi breed was 7%. On the other hand the frequency distribution of buffalo's milk from the examined native breed (which is the only buffalo breed in Egypt) was 17.7% which was higher than Holstein cows and native breed cows. Lower frequency distribution from raw samples from Holstein cows in different geographic areas of Japan was previously detected by Fujimura et al. [4] who found that 72.2% were positive for *H. pylori* from raw milk samples and 55% commercial pasteurized milk.

It was found that the frequency distribution of *H. pylori* in cow's milk from different localities of Aga district, Nawasa Al-Bahr village,

	Breed of the animal	Number of examined samples	Number of Positive Samples	Percentage of Positive samples
Cows	Holstein	12	2	1.7
	Native breed cow (Baladi)	41	6	5.2
	Total	53	8	6.9
Buffaloes	Native breed buffaloes	64	14	11.9
Total		117	22	18.8

Table 1: Frequency distribution of *H. Pylori* in animal faecal samples in relation to breed.

	Age of the animal	Number of examined samples	Number of Positive Samples	Percentage of Positive samples
Cows	0-6 month	4	2	1.7
	6-12 month	7	1	0.85
	12-18 month	2	1	0.85
	18-24month	4	1	0.85
	Over 2 years	36	3	2.65
	Total	53	8	6.9
Buffaloes	0-6 month	2	1	0.85
	6-12 month	4	1	0.85
	12-18 month	6	2	1.7
	18-24month	3	2	1.7
	Over 2 years	49	8	6.8
	Total	64	14	11.9
Total		117	22	18.8

Table 2: Frequency distribution of *H. Pylori* in animal faecal samples in relation to age.

		Locality of collected animal faecal samples	Number of examined samples	Number of Positive Samples	Percentage of Positive samples
Cows	Aga Center	Aga district	8	1	0.85
		Nawasa Al-Bahr village	14	2	1.7
		Mitishna village	9	1	0.85
	Mansoura Center	Mansoura district	12	3	2.65
		Awish Al-Hagar village	10	1	0.85
		Total	53	8	6.9
Buffaloes	Aga Center	Aga district	13	2	1.7
		Nawasa Al-Bahr village	6	1	0.85
		Mitishna village	21	3	2.6
	Mansoura Center	Mansoura district	16	7	5.9
		Awish Al-Hagar village	8	1	0.85
		Total	64	14	11.9
	Total		117	22	18.8

Table 3: Frequency distribution of H. pylori in animal faecal samples in relation to locality.

	Breed of the animal	Number of examined samples	Number of Positive Samples	Percentage of Positive samples
Cows	Holstein	13	3	3.5
	Native breed cow(Baladi)	23	6	7
		36	9	10.5
Buffaloes	Native breed buffalo	49	15	17.7
Total		85	24	28.2

Table 4: Frequency distribution of H. pylori in raw milk samples in relation to breed.

Mitishna village, Mansoura district, Awish Al-Hagar village were 2.3%, 3.55%, 1.2%, 4.7% and 2.3%, respectively, while their respective frequency distribution of buffaloes milk were 3.55%, 3.55%, 1.2%, 8.2% and 1.2%. The highest frequency distribution of *H. pylori* (8.2% and 4.7%) was from cow's milk and buffalo milk in relation to locality was found in Mansoura district. The lowest frequency distribution was found in Mitishna village which was 1.2% in both cows and buffaloes (Table 5).

		Locality of collected raw milk samples	Number of examined samples	Number of Positive Samples	Percentage of Positive samples
Cows	Aga Center	Aga district	7	2	2.3
		Nawasa Al-Bahr village	7	3	3.55
		Mitishna village	2	1	1.2
	Mansoura Center	Mansoura district	13	4	4.7
		Awish Al-Hagar village	9	2	2.3
		Cows	36	9	10.5
Buffaloes	Aga Center	Aga district	11	3	3.55
		Nawasa Al-Bahr village	7	3	3.55
		Mitishna village	2	1	1.2
	Mansoura Center	Mansoura district	20	7	8.2
		Awish Al-Hagar District	9	1	1.2
		Buffaloes	49	15	17.7
Total			85	24	28.2

Table 5: Frequency distribution of H. pylori in raw milk Samples in relation to locality

The overall isolation rate of *H. pylori* from human stool samples was 19.6%. The isolation rates were 11.7 % and 7.8% in males and females, respectively. In a study higher isolation rates (48.27%) were recorded of patients with hydatid liver diseases were positive, of which were 32.7% and 15.5% were in males and females, respectively. in another study carried out by Uemura et al. [9] who detected *H. pylori* in 445 non ulcer dyspeptic patients with the percentage of 46.2% in males and 53.7% in females, moreover in patients with duodenal ulcers, the isolation rates in males were 72% and 28% in females. In patients with gastric ulcer, the isolation rates of *H. pylori* were76% and 24%in males and females, respectively. Furthermore, in patients with gastric polyps, the results were 36.6% and 64.4% in males and females. The higher isolation rates were also previously reported by Rasheed et al. [10] who found the overall percentage of *H. pylori* was 74.4%, moreover the incidence of *H. pylori* was 73.5% in males and 75.4% in females. The isolation rate of *H. pylori* from females (7.8%) was almost similar to the isolation rate of a study previously reported by Ahmed et al. [11] who found the isolation rates of the examined females percentage was 7.4%, while their isolation in males was 28%. The recorded results revealed that *H. pylori* was slightly higher in examined males than females, this could illustrate that males which are more susceptible than females to carrying and infection with *H. pylori* (Tables 6 and 7).

This conviction was fully supported by the idea of Klein [12] who stated that males which are more susceptible than females to infections caused by bacteria, viral, fungi and parasites due to males generally exhibit reduced immune responses fully compared to females. These differences are usually attributed to socio-ecological, physiological (hormonal in origin), and occupational (referred to animal contact), so, the females less susceptible to infection than males, not only because of the androgenic hormones which reduce the immunity but also sex steroid hormones affect disease resistant genes and behavior which make males more susceptible to infection.

Regarding frequency of *H. pylori* from human stool with regard to age, there were various frequency distribution of *H. pylori* with different age groups in humans, the frequency distribution of *H. pylori* in human were 2.95%, 1.96%, 3.92%, 3.92%, 1.96 % and 4.9%, respectively. The age

Gender of human	Number of examined samples	Number of positive samples	Percentage of Positive samples
Male	59	12	11.7
Female	43	8	7.8
Total	102	20	19.6

Table 6: Isolation rate of H. Pylori from Human stool concerning to gender.

Age of human	Number of examined samples	Number of positive samples	Percentage of Positive samples
1-10 years	15	3	2.95
10-20 years	17	2	1.96
20-30 years	22	4	3.92
30-40 years	20	4	3.92
40-50 years	15	2	1.96
50-60 years	13	5	4.9
Total	102	20	19.6

Table 7: Frequency of *H. pylori* from human stool with regard to age.

groups varied (From 1-10 years, from 10-20years, from 20-30, from 30-40, from 40-50, from 50-60). The highest rate (4.9%)was found in the age group from 50-60 years old, while the lowest frequency distribution rate (1.96%) was found in 2 age groups from 10-20 years and from 40-50 years .In a study performed by Windsor et al. [13] recorded that the males under age group less than 10 years showed highest prevalence (11%), whereas, females aged from 11 to 20 years showed that the highest prevalence (14%) in rural community. In urban community, the males aged from 11to 20 years was highest (9%), however in females, the highest prevalence was in age group from 31-40 years with (13%). Incidence rate of *H. pylori* detected in adults in the present study agreed with the previous reports of Malaty et al. [14] who carried out a study on a total of 413 person (161 adults and 252 children) adult age range from 20 to 75 years and the children from 1 to 19 years. The overall seropositivity rate of *H. pylori* was 75% among adults and 22% among children. Higher frequency of isolation of *H. pylori* in young age was also recorded in another study carried out by Naficy et al.[15] who reported that 42 % children aged from 6-17 months were positive for *H.pylori* infection.

It was obvious that adults showed higher prevalence of *H. pylori* than young. This indicated that the prevalence increases with increasing the age, this conviction was previously supported by Rasheed et al. [10] who mentioned that the prevalence of *H. pylori* in human population increased with increasing age and presence of household animals and size of family and members of family.

Concerning results of bacteriological examinations of human stool samples for *H. pylori* in relation to healthy state, the highest occurrence (8.8%) had been showed in the patients with gastrointestinal disorders, the lowest percentage (4.9%) was found in the diarrheal patients, while the apparently healthy persons showed a percentage of (5.8 %) (Table 8).

From the achieved results, it was obvious that *H. pylori* isolated was more frequently isolated from patients with gastrointestinal disorders; this conviction was fully supported by the results of Javed et al. [16] who reported that *H. pylori* isolated was more frequently isolated from patients with gastrointestinal disorders. *H.pylori* had been isolated from patients with upper gastrointestinal symptoms of peptic disease, in patients with gastritis and peptic ulcer [17] (Table 8).

Healthy state of human	Number of Examined samples	Number of positive samples	Percentage of Positive samples
			%
Apparently healthy	45	6	5.8
Patients with gastrointestinal disorders	35	9	8.8
Diarrheal stool samples	22	5	4.9
Total	102	20	19.6

Table 8: Results of bacteriological examination of human stool samples for *H. pylori* in relation to healthy state.

Source of samples	Occupation	Number of examined samples	Number of positive samples	Percentage of Positive samples
Human stool	Children less than 10 years	17	2	1.96
	Farmers	15	4	3.92
	Veterinarians	22	7	6.8
	Officers	15	1	0.98
	Housewives	13	2	1.96
	Dairy workers	20	4	3.92
	Total	102	20	19.6

Table 9: The occurrence of H. pylori in human stool samples with respect to occupation.

The occurrences of *H. pylori* in human stool samples with respect to occupation in Table 9 are illustrated. The occurrence of *H. pylori* in children, farmers, veterinarians, officers, Housewives and dairy workers were 1.96%, 3.92%, 6.8 %, 0.98%, 1.96% and 3.92%, respectively. The highest occurrence 6.8% was found in veterinarians and the lowest occurrence 0.98% were found in officers the dairy worker group showed occurrence of 3.92%. It could be concluded that *H. pylori* could be isolated from cattle faeces, their milk and humans in the examined area reflecting the important role of cattle especially buffaloes as new potential zoonotic reservoir. So, the recommended measures to reduce and avoid the risk of *H. pylori* are standard hygienic practices in animal management, feeding, hygienic disposal of animal wastes, preparation of silage, and periodical cleaning and disinfection must be applied to reduce *H. pylori* carriages in cattle. Avoid faecal contamination for milk. All raw milk and its products must be efficiently heat treated to avoid risk of *H. pylori* for milk consumers. Gastroenterology hospitals must be provided with a rapid urease test kits, all patients with peptic ulcers disease and other gastric disease must be monitored for detection of *H. pylori* by rapid tests then confirming with culturing or PCR assays. The positive *H. pylori* cases must be treated to avoid development of gastric cancers. Persons had occupations related to animals such as veterinarian, dairy worker and farmers must be healthy educated to avoid the risk of *H. pylori* from cattle by application strictly personal hygienic practices. Further research in needed to explain the role of other domestic animals such as horses, sheep, goats and pet animals as reservoir for *H. pylori*. Also, the effect of ecology, seasons and other risk factors on isolation rates of *H. pylori* must be studied in different geographic areas in Egypt to obtain clear picture on the epidemiology of *H. pylori* in animals and man.

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