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MOLECULAR AND SEROLOGICAL DETECTION OF HELICOBACTER PYLORI IN COW'S MILK AND ITS IMPACT ON HUMAN HEALTH

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ABSTRACT

Helicobacter pylori is a widespread human pathogen causing serious illnesses, such as gastroduodenal ulcers and gastric cancer. It has been hypothesized that the infection route by *H. pylori* involves multiple pathways, including food-borne transmission. The organism has been detected in several food types and dairy products. The study aimed to detect the presence of *H. Pylori* in raw cow's milk sold in small markets in different localities in Egypt. The detection of *H. Pylori* in milk samples based on the molecular detection of the phosphoglucosamine Maltese gene (*glmM*) using PCR beside serologically surveillance for persons consuming such milk.The results shown that 11% of raw cow's milk samples were positive for the presence of *glmM* gene of *H. Pylori* and 59% of the examining human stool samples were positive using *H. pylori* antigen test antibody while the serum samples were positive in 50% of the samples using *H. pylori* antibody detection ELISA. The human results go parallel to the investigations on milk, which proposing that milk may be playing a role in transmission of *H. pylori*.

Keywords: *H. pylori*, *glmM* gene, cow's milk, human's stool, human's serum

INTRODUCTION

Helicobacter pylori is considered as a major pathogen involved in the pathogenesis of human chronic gastritis, Peptic and duodenal ulcer diseases [1, 2, and 3]. It has acquired the shape of an epidemic emerging pathogen as a principle

cause of gastric carcinoma [4]. Approximately 50% of the world populations are known to be infected by *H. pylori* with higher incidence rate in developing countries than in developed countries. Several risk factors associated with the occurring of infection, which may include, poor social and economic development, low education level, poor hygiene practices, absence of sanitary drinking water and food handled inappropriately [5].

The transmission routs of *H. pylori* remain unclear. The existence of animal reservoirs has been hypothesized as a source of dissemination of this pathogen. It was isolated from the gastric mucosa of calves, pigs, horses and sheep's gastric tissue and milk, which may support that animals may act as reservoirs and spreader of the organism [6]. There are many researches refers to the infection takes place through fecal–oral route and contaminated water, which may play an important role in transmission of the organism to human [7, 8]. *H. pylori* was detected in drinking water, which could survive for about 2 years at 4°C without decrease in its count [9,10].

On the other hand, *H.pylori* could survive in some foodstuffs, such as fresh fruit and vegetables, fresh poultry or fish, fresh meats, and some ready-to-eat foods, yoghurt, chicken meat, tofu [11,12].The epidemiological studies were recorded its presence in milk and considered it as a possible transmission vehicle for human [3, 13].

Based on the fact, that *H.pylori* can revert from normal helical bacillary cultivated form to coccoid viable none cultivated form under the food adverse conditions which still infective to human [14], therefore culture is incapable to detect such forms of *H. pylori* while the PCR is a more efficient to detect such disease.

In order to control the high percentage of *H.pylori* human infections we need to do more studies on the transmission pathways. Considering the cow's milk as an important source of such infection, the hypothesis needs to be confirmed.

The present study was carried out for the molecular detection of *H. pylori* in cow's raw milk sold in small markets in some Egyptian governorates as well as serum and stool samples collected from persons whom suspected to be buying milk from the same markets.

MATERIALS AND METHODS

Samples:

A total of 100raw cow's milk samples were collected from Giza (*n*=35), El-Kalyobia (*n*= 35) and El-Fayoum (*n*= 30)governorates for detection of *H.pylori* by PCR, 100 human's serum samples (40, 32 and 28 samples from the 3 governorates, respectively), and 100stool samples(38, 33

And 29 samples, respectively.) were collected for serological detection of H. pylori

Detection of *H. pylori* from milk using PCR method:

One ml of each milk sample was used for extraction of DNA by a DNA isolation kit (GF-1, Vivantis, Malaysia) for cells and tissues according to the manufacturer's instructions with some modification and its concentration was assessed by optic densitometry. Extracted genomic DNA was amplified according to Rahimi and Kheirabadi [3] for the *glmM* gene (294 bp) using the following specific primers:

HP-F: 5-GAATAAGCTTTTAGGGGTGTTAGGGG-3 and HP-R: 5-GCTTACTTTCTAACACTAACGCGC-3. PCR reactions were performed in a final volume of 50 µL containing 25 µL Green Master mix (Sigma), 10 µL genomic DNA as a template, 13µL free ionized water and 1 µL of each primer. PCR was performed using a thermal cycler (MJ, Research, Inc, Germany) under the following conditions : an initial denaturation was done for 10 min at 94℃, 35 cycles for 1 min at 94℃, 1min at 55℃, 1 min at 72℃, and a final extension at 72℃ for 10 min. The PCR products were electrophoresed through 1.2% agarose gels (Fermentas Co., Germany) containing ethidium bromide and 100 BP DNA ladder (Fermentas Co.) to detect the molecular weight of the observed bands under an ultraviolet (UV)

Detection of *H. pylori* from stool.

Fecal samples from 100 milk consumer were also examined for the presence of *H. pylori* antigen by the antigen detection ELISA kit [Abon Biopharm (Hangzbou)]

Detection of *H. pylori* from human's serum

Serum samples from 100 milk consumers were serologically examined by for the presence of *H.pylori* antibody using the antibodies detection ELISA kit[AbonBiopharm (Hangzbou)].

RESULTS

The results of detection of *H. pylori* by PCR revealed that 11% of the examined raw cow's milk samples were positive. In particular, *H. pylori glmM* gene was detected in 3 cow milk samples from Giza (8.6%), 5 cow's milk samples from El-Kalyobia (14.3%) and 3 cow's milk samples from El-Fayoum (13.3%) as shown in Table 1 and Fig. 1.

The serological studies of *H.pylori* antigen in human stool samples were 59 out of 100 (59%) and in serum samples were 50out 100 (50%), Table 2 and Fig. 2.

Table 1: Occurrence of *H. pylori* in cows' milk samples using Polymerase Chain Reaction

Locality	No. of milk samples	No. of positive samples
Giza	35	3 (8.6%)
El-Kalyobia	35	5 (14.3%)
El-Fayoum	30	3 (13.3%)
Total	100	11 (11%)

Table 2: Occurrence of *H. pylori* among humans' stool and serum samples:

Type of samples	Stool samples		Serum sample	
Locality	Total No.	No. of positive	Total No.	No. of positive
Giza	38	18 (47.4%)	40	14 (35%)
El-Kalyobia	33	22 (66.7%)	32	20 (62.5%)
El-Fayoum	29	19 (65.5%)	28	16 (57.1%)
Total	100	59 (59%)	100	50 (50%)

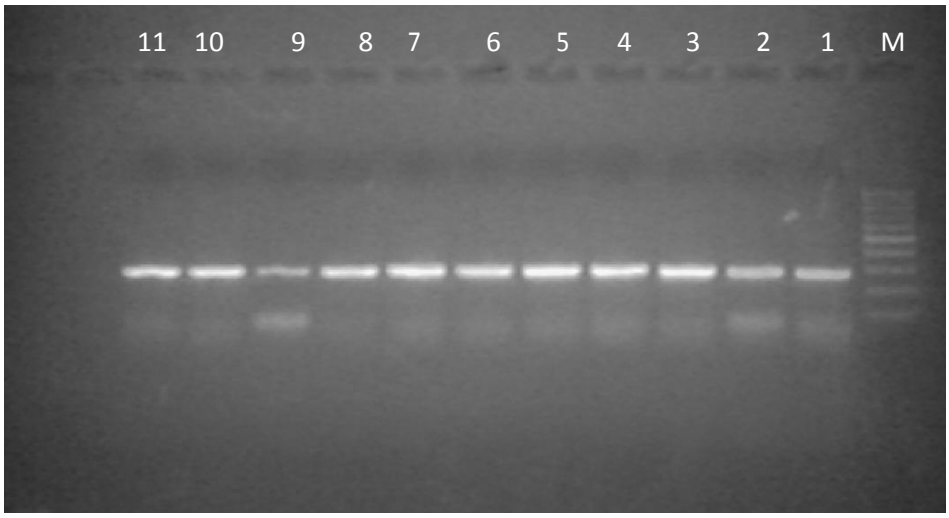

Fig. 1: Agarose gel electrophoresis of the PCR amplified products (294 bp) for the *glmM* gene from cow's milk samples. Lanes 1–3: raw cow milk samples from Giza, lanes 4–8: raw cow milk samples from El-Kalyobia, lanes 9-11: raw cow milk samples from El-Fayoum and lane M: molecular size marker (100 bp).

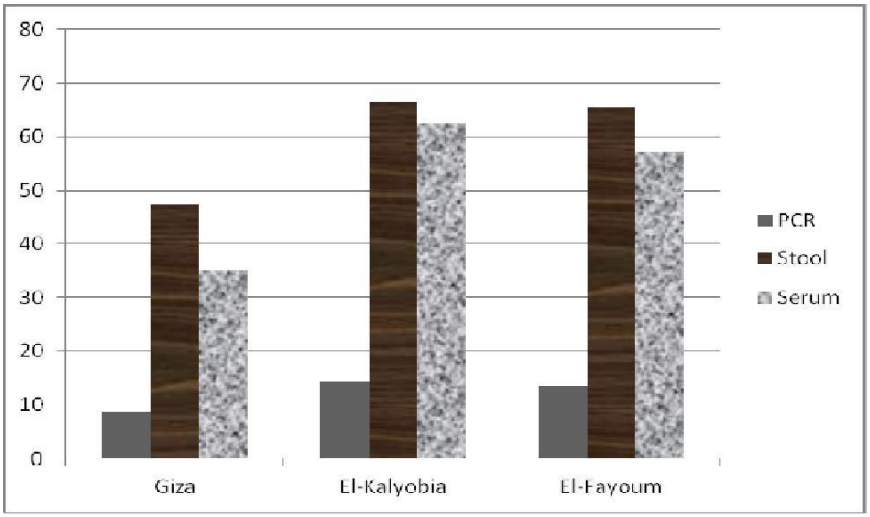

Fig. 2: Percentage of positive PCR milk sample of *H. pylori*, human stool and serum samples in different Egypt localities

DISCUSSION

The transmission way of *H. pylori* still remains unknown, but the possibility of zoonotic transmission of the pathogen has been suggested in several studies and many other studies have addressed the role of food in the transmission of *H. pylori* [7,15, 16] as well as some evidence of *H. Pylori* transmission through milk [3, 13]. In the current study, we investigated the role of milk in transmitting that pathogen to the human consumer.

H. pylori is a fastidious microorganism and requires complex growth media, so the

PCR assay is very sensitive and accurate, in this work we specifically targets *glmM* gene which is essential for the growth of *H. pylori* and shown to be sensitive and specific for recognition of it. During this investigation, the *glmM* gene of *H. pylori* was detected in 11% raw cow milk. These results were nearly similar to those in a study conducted in Iran by Rahimi et al., [3] who reported that the *H. pylori glmM* gene was detected in 12.5% Iranian milk samples, including 19 cows (14.1%), 11 sheep (12.2%), 9 goats (8.7%), 2 camels (3.6%) and 15 buffalo (23.4%) milk samples.The bacteria can invade cow udder when it is sprawled on the ground from cow's feces and soil [17] and this confirmed by Sasaki et al., [18] who detected the *H.pylori* in 50% of cow's faeces and 38% of soil samples.

The detection of *H. pylori* in diluted bulk milk highlighting the high incidence of milk contamination with H. Pylori which reflecting a bad hygienic measures applied during production of such milk.

H. pylori infection in human can be detected by a variety of methods. The simplest, least expensive method is serological testing.The stool assay was a reliable and easy-to-use tool for diagnosis of *H pylori* infection. The test was accurate, even shortly after treatment [19]. In this study, the antigen test detected *H. pylori* in samples of the milk consumers were 59%, while antibody test detected *H. pylori* in the serum samples of the same humans were 50%.

The high prevalence of *H. pylori* detected in human may consider the consumption of raw milk would be a potential risk of *H. pylori* infection since the direct sale of unpasteurized milk and dairy products from producers to the consumer is more common.

CONCLUSION

The poor hygienic management during milking, chilling, and storage could be a sources for *H. pylori* infection in human. Therefore, emphasis on good hygiene can be an exceptional way for reducing the load of *H. Pylori* in milk and subsequently to human.

REFERENCES

[1] Hatakeyama M and Brzozow T, Pathogenesis of *Helicobacter pylori* infection. Helicobacter 11, 2006, 14–20.

[2] Das JC and Paul N, Epidemiology and pathophysiology of *Helicobacter pylori* infection in children. Indian J Pediatr, 74, 2007, 287–290.

[3] Rahimi E and Kheirabadi EK, Detection of *Helicobacter pylori* in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne Pathog. Dis.9(5), 2012, 453–456.

[4] Ghosh P and Bodhankar SL, Determination of risk factors and

transmission pathways of *Helicobacter pylori* in asymptomatic subjects in Western India using polymerase chain reaction. Asian Pacific Journal of Tropical Disease, 2012, 12-17.

[5] Van Duynhoven YT and de Jonge R,Transmission of Helicobacter pylori: Arole for food? Bull World Health Organ, 79, 2001, 455– 459.

[6]Dore MP, Sepulveda AR, El-Zimaty H,Yamaoka Y, Osato MS, Mototsugu K, Nieddu AM, Realdi G and Graham DY, Isolation of Helicobacter pylori from milk sheep–implications for transmission to humans. Am J Gastroenterol 96, 2001, 1396– 1401.

[7] Vale FF and Vitor JM, Transmission pathway of Helicobacter pylori: Does food play a role in rural and urban areas? Int J Food Microbiol 138, 2010, 1–12.

[8] Fujimura S, Kato S, and Kawamura T, *Helicobacter pylori* in Japanese river water and its prevalence in Japanese children. Lett Appl Microbiol 38, 2004, 517-21.

[9] Piqueres P, Moreno, Y, Alonso JL and Ferrus MA, A combination of direct viable count and fluorescent in situ hybridization for estimating Helicobacter pylori cell viability. Res Microbiol 4, 2006, 345–349.

[10] Shahamat M, Mai U, Paszkokolva C, Kessel M, and Colwell RR, Use of Autoradiography to Assess Viability of *Helicobacter Pylori* in Water. Applied and Environmental Microbiology 59 (4), 1993, 1231–1235.

[11] Fan, XG, Chua A, Li TG and Zeng QS, Survival of *Helicobacter pylori* in milk and tap water. Journal of Gastroenterology and Hepatology 13, 1998, 1096–1098.

[12] Poms RE and Tatini SR, Survival of *Helicobacter pylori* in ready-to eat foods at 4 degrees C. International Journal of Food Microbiology 63 (3), 2001, 281– 286.

[13] Fujimura S, Kawamura T, Kato S, Tateno H, Watanabe A, Detection of Helicobacter pylori in cow's milk. Lett Appl Microbiol 35 (6), 2002, 504–507.

[14] Buck A, Oliver JD. Survival of spinach-associated Helicobacter pylori in the viable but non culturable state. Food Control 2010; 21:1150–1154.

[15] Meng X, Zhang H, Law J, Tsang, R, Tsang T, Detection of Helicobacter Pylori from Food Sources by a Novel

Multiplex PCR Assay. J Food Saf 28 (4), 2008, 609–619.

[16] Vale FF, Vítor JM. Transmission pathway of Helicobacter pylori: does food play a role in rural and urban areas? Int J Food Microbiol 2010; 138 (1-2): 1-12.

[17] Plastridge WN, Bovine mastitis: a review. Journal of Dairy Science 41, 1958, 1141–1181.

[18] Sasaki K, Tajiri Y, Sata M, Fujii Y, Matsubara F, Zhao M, Shimizu S, Toyonaga A and Tanikawa K, Helicobacter pylori in the Natural Environment. Scandinavian Journal of Infectious Diseases 31 (3), 1999, 275-279.

[19] Vaira MD, Peter MD, Axon AT, Deltenre M, Hirschl AM, Gasbarrini G, O'Morain C, Garcia JM, Quina M, Tytgat GN, Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. The Lancet 354. 9172, 1999, 30-33.