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 phosphoglucomamine Maltese gene (*glmM*) using PCR beside serologically surveillance for persons consuming such milk. The results showed 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 routes 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 100 raw 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 100 stool 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'-GAATAAGCTTTAGGGTGTAGGGG-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, 13 μL 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 °C, 35 cycles for 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, and a final extension at 72 °C 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) lamp.

**Detection of H. pylori from stool.**

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

**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 [Abon Biopharm (Hangzou)].

**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 50 out of 100 (50%), Table 2 and Fig. 2.

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of milk samples</th>
<th>No. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giza</td>
<td>35</td>
<td>3 (8.6%)</td>
</tr>
<tr>
<td>El-Kalyobia</td>
<td>35</td>
<td>5 (14.3%)</td>
</tr>
<tr>
<td>El-Fayoum</td>
<td>30</td>
<td>3 (13.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>11 (11%)</td>
</tr>
</tbody>
</table>

Table 1: Occurrence of H. pylori in cows' milk samples using Polymerase Chain Reaction
Table 2: Occurrence of *H. pylori* among humans’ stool and serum samples:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Total No.</th>
<th>No. of positive</th>
<th>Total No.</th>
<th>No. of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giza</td>
<td>38</td>
<td>18 (47.4%)</td>
<td>40</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>El-Kalyobia</td>
<td>33</td>
<td>22 (66.7%)</td>
<td>32</td>
<td>20 (62.5%)</td>
</tr>
<tr>
<td>El-Fayoum</td>
<td>29</td>
<td>19 (65.5%)</td>
<td>28</td>
<td>16 (57.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>59 (59%)</td>
<td>100</td>
<td>50 (50%)</td>
</tr>
</tbody>
</table>

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 source 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**


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


