Abstract

Introduction: Helicobacter pylori (H. pylori) is one of the most common bacterial infections among humans worldwide. Although many records imply its interfamilial acquisition, the role of animals remains poorly understood. This study was undertaken to investigate the seroprevalence of H. pylori in animals and their human contacts in Cairo and Giza governorates, Egypt.

Methodology: Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to detect IgG antibodies to H. pylori in dogs, cattle, and humans.

Results: Seropositive dogs (35/94; 37.2%), cattle (24/80; 30%) and humans (40/90; 44.4%) were found. Seroprevalence in animals significantly varied in different areas of sample collection, but there was no association with sex or age. Human seropositivity rates were associated with increasing age; moreover, seropositive dog owners (51.7%; 15/29), had seropositive dogs. However, infection was not associated with subject’s sex, occupation, or history of animal contact.

Conclusions: Our findings indicate H. pylori is widely distributed in cattle and dogs and their human contacts in Cairo and Giza, Egypt. Further studies to determine infection in other occupational groups are needed. This study provides baseline information on the seroprevalence of H. pylori, which may be required to begin prevention control programs in our area.

Key words: dogs; cattle; human; Egypt; ELISA; Helicobacter pylori.


(Received 23 August 2016 – Accepted 13 January 2017)

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Introduction

Helicobacter pylori (H. pylori) is one of the most common bacterial infections among humans, and has worldwide distribution. The causative agent is a helical, microaerophilic, flagellated Gram-negative bacterium that inhabits and adapts to the acidic human gastric mucosa [1]. The organism colonizes about 50% of the world population’s upper gastrointestinal tracts [2], which makes the infection a public health concern worldwide [3]. Although infected individuals usually never encounter clinical symptoms except chronic gastritis, acute infections can cause acute gastritis with abdominal pain or nausea [4,5]. The ability of the organism to hydrolyze urea leads to gastric ulcers and increases the risk of developing duodenal and stomach cancer to the level that the World Health Organization (WHO) classifies as a class I carcinogen, where the infection is found in 80%–90% of patients with gastric ulcers [6,7].

The prevalence rate of H. pylori varies greatly by the geographic area, age, and socioeconomic status. Infection appears to be more common in developing than in developed countries [8,9]. Modes of infection are yet not clearly understood. Although spread can occur through the environment or via reservoirs or vectors, little is known about the main route of dissemination from an infected individual [10]. Transmission from person to person, through gastrointestinal, oral-oral, fecal-oral routes, and through exposure to contaminated food or water is highly controversial; furthermore, close and intense human contact with animals has been identified as a risk factor [11-13]. The possibility of zoonotic transmission from animals has been previously suggested [14]. Moreover, detection of anti-H. pylori antibodies in abattoir workers in France and in northern Sardinian shepherds, at higher levels than their siblings inhabiting the same house [15,16], demonstrates the zoonotic importance of the organism.
However, the exact role of animals as a reservoir of infection remains unclear.

Most data about the rates of *H. pylori* infection in different geographical and demographic populations comes from seroprevalence studies [17]. Serological tests using enzyme-linked immunosorbent assay (ELISA) are preferred as a noninvasive alternative method to endoscopy and biopsy for rapid diagnosis of *H. pylori*. Thus, serological tests have been used extensively in screening humans in clinics and for epidemiologic studies [18]. Unfortunately, in Egypt, very little epidemiological data are available about the situation of *H. pylori* infection in humans and animals. To provide further information, the present study was undertaken to address the occurrence of *H. pylori* infection in apparently healthy humans and animals, including their owners.

**Methodology**

**Ethics statement**

Protocols for the collection of samples were reviewed and approved by the Scientific Research Committee and Bioethics Board of Suez Canal University, Faculty of Veterinary Medicine, Ismailia, Egypt (No. 2016086).

**Sample collection**

Whole blood was collected from convenience samples of apparently healthy cattle and dogs. Cattle were randomly sampled from farms in El-Badrasheen and Mazghona and Gameet Ahmed Orabi of Giza and Cairo governorates of Egypt, respectively. Domestic dogs were those mostly kept indoor and admitted with their owners to small animal veterinary clinics in El-Haram and Heliopolis and El-Maadi for other purposes. Stray dogs were those had been captured roaming in rural and suburban areas in Giza and Cairo governorates. Human whole blood samples (n = 90) were collected from consenting apparently healthy people (n = 61) attending the Giza and Cairo hospitals for routine health examinations and from owners of some of the sampled dogs (n = 29). Demographic data on age and sex were also obtained.

Following collection, samples were transported on ice box to Cairo University, Faculty of Veterinary Medicine, where whole blood samples were centrifuged, and aliquots of sera were separated and stored at -20°C for ELISA.

**Serological assay**

The canine, bovine, and human *H. pylori* IgG (Hp-IgG) indirect ELISA kits (MyBioSource, San Diego, USA) were used according to the manufacturer’s instructions to detect IgG antibodies against *H. pylori* in sera of dogs, cattle, and humans, respectively.

The sample optical densities (OD) were measured using a microplate reader (CLINDIAG, Orange, USA) at 450 nm, and the sample-to-negative ratio was determined. As recommended by the manufacturer, for human kits, samples were considered to be ELISA positive if the OD sample/OD negative ≥ 2.1, while if OD sample/OD negative less than 2.1, the sample was considered as negative. For canine and bovine *H. pylori* IgG ELISA kits, the cut-off was calculated based on the following formula: average of negative control + 0.15. Samples exceeding the calculated cut-off value were considered positive.

**Statistical analysis**

PASW Statistics, SPSS version 18.0 software (SPSS Inc., Armonk, USA) was used to analyze the data. Chi-squared ($\chi^2$) and Fisher’s exact tests were performed to analyze *H. pylori* antibody positivity between various groups. Differences were considered statistically significant if the P value was < 0.05.

**Results**

**Serologic detection of anti-** *H. pylori** antibodies in dogs**

Overall, a total of 264 whole blood samples from dogs (n = 94), cattle (n = 80), and people (n = 90) were tested. Generally, there was no statistically significant difference in the seroprevalence in dogs (37.2%; 35/94), cattle (30%; 24/80), and humans (p = 0.151). A

<table>
<thead>
<tr>
<th>Table 1. Seroprevalences of IgG antibodies to <em>H. pylori</em> by ELISA in dogs from Cairo and Giza governorates.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source of sample collection</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Stray dogs</td>
</tr>
<tr>
<td>El-Maadi</td>
</tr>
<tr>
<td>Heliopolis</td>
</tr>
<tr>
<td>El-Haram</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
relatively large percentage of dogs were found to have antibodies to *H. pylori* in the ELISA test (37.2%; 35/94) (Table 1). Prevalence significantly varied by the area of sample collection, with the highest found in dogs from El-Haram (75%; 18/24), El-Maadi (29.2%; 7/24), and Heliopolis (17.9%; 5/28) (p < 0.05). There was no significant difference in seropositivity in male (22/55) and female dogs (13/39) (p > 0.05) (Table 1).

**Serologic detection of anti-*H. pylori* antibodies in cattle**

Cattle from three different farms were examined: one in Cairo (Gameet Ahmed Orabi) and two in Giza governorates (Badrashen and Mazghona), in addition to sporadic cases bred by individual farmers (Table 2). Thirty percent of the total examined cattle were seropositive (24/80). Prevalence significantly varied by the area of sample collection, with the highest found in Badrashen in Giza governorates (52%, 13/25) and Gameet Ahmed Orabi in Cairo governorate (30%, 6/20); the lowest was in Mazghona in Giza governorate (14.3%, 5/35) (p < 0.05). Seropositivity was not associated with age or type of animal production (p > 0.05) (Table 2).

**Serologic detection of anti-*H. pylori* antibodies in humans**

A total of 44.4% (40/90) of humans were found to have antibodies to *H. pylori*. There was no statistically significant difference in the seropositivity in men (17/44) and women (23/46), while there was significant difference among different age groups (p < 0.05) (Table 3, Figure 1). Although 15 seropositive dog owners had seropositive dogs, there was no statistically significant difference in seropositivity in dog owners (51.7%; 15/29) and people with no history of animal contact (41%; 25/61) (p = 0.338) (Table 4).

**Discussion**

This study was undertaken as an attempt to evaluate the distribution and possible zoonotic relationship of *H. pylori* infection in Giza and Cairo governorates, Egypt. Overall, the differences of seroprevalence in the examined dogs, cattle, and humans was not statistically significant (p = 0.151). Seroprevalence in dogs was 37.2% (Table 1). Generally, serodiagnosis in dogs represents a big challenge, since they might be infected with several *Helicobacter* species [19-21].

Thirty percent of the examined cattle were seropositive (Table 2), whereas lower seroprevalence

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**Table 2. Seroprevalences of IgG antibodies to *H. pylori* by ELISA in cattle from Cairo and Giza governorates.**

<table>
<thead>
<tr>
<th>Source of sample collection</th>
<th>Purpose of animal</th>
<th>Cairo</th>
<th>Giza</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gameet Ahmed Orabi</td>
<td>Badrashin</td>
</tr>
<tr>
<td>Farms</td>
<td>Meat production</td>
<td>Adults</td>
<td>7 (4, 57.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calves and heifers</td>
<td>13 (2, 15.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>20 (6, 30%)</td>
</tr>
<tr>
<td></td>
<td>Milk production</td>
<td>Adults</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calves and heifers</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>---</td>
</tr>
<tr>
<td>Sporadic cases</td>
<td>Adults</td>
<td>---</td>
<td>10 (4, 40%)</td>
</tr>
<tr>
<td></td>
<td>Calves and heifers</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>---</td>
<td>10 (4, 40%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20 (6, 30%)</td>
<td>25 (13, 52%)</td>
</tr>
</tbody>
</table>
was reported in other studies [22,23]. Previous detection of *H. pylori* from bovine feces [23,24] and/or milk [23,25] and seroconversion in farm workers [26,27], however, might suggest the probable role of this animal species in transmission of infection to humans in case of inappropriate farm management practices.

In our investigation, the overall seroprevalence in humans was relatively high (44.4%) (Table 3). This was somehow higher than results shown in previous studies from other countries: United Kingdom (27.6%) [28], Australia 15.4% [29], and United State (36.3%) [30], while it was lower than reports in Uganda (87%) [31], China (62%) [32], and neighboring Araban countries, mainly Libya (94%) [33] and Sudan (65%) [34]. The diversity of crowding, socioeconomic status, and environmental and hygiene factors may play an important role in increased rates of *H. pylori* infection in developing countries.

Results showed an association between seroprevalence and increasing age of the examined subjects (Table 3, Figure 1). Similarly, this was reported in other studies [29,35]. Individuals in the third decade of life were more likely to have a higher seroprevalence than those under 20 years of age. This may be because Egyptian youth in such age groups like to have food away from home during their outside activities in trips or camps; thus, they might be infected from consumption of contaminated food or water sources. Generally, it is estimated that colonization of *H. pylori* in gastric mucosa is associated with old age, male sex, and low socioeconomic status [11,12].

The controversial finding is that around fifty percent (51.7%; 15/29) of the dog owners who reacted on ELISA had seropositive dogs (Table 4). Although there was no significant difference in the seropositivity between dog owners and the other group, contact with dogs was identified as a risk factor for acquiring *H. pylori* infection in other studies [36,37]. Similar to our findings, in other seroepidemiological studies, the exact relationship between pet ownership and human seropositivity cannot be clearly established [38,39].

It is worth mentioning that an association had been reported between animal contact and seropositivity in abattoir workers and veterinarians working in abattoirs and meat processing plants in New Zealand [40,41]. However, the cross-reactivity with antibodies to other gastrointestinal organisms which might be acquired from slaughtered animals was not included in those studies. Thus, these findings are questionable, and it was suggested that this higher prevalence was due to cross-reactivity to *Campylobacter jejuni* [14]. In another study, high seroprevalence was reported in shepherds from northern Sardinia in comparison to their siblings inhabiting the same house who had no contact with sheep. Authors claimed that contact with sheep and sheepdogs was a risk factor for infection [16]. However, other authors failed to isolate *H. pylori* and/or to detect its antibodies due to natural infection from stray and pet cats [42,43] and pigs in abattoirs [44]. These questionable data suggest doubtful zoonotic transmission of such agent, and whether animals are true reservoir hosts for *H. pylori* or not is still not obvious. It seems that infection might be contracted from a common source (*e.g.*, drinking water, consumption of raw vegetables) or might suggest that *H. pylori* infection could be an anthroponosis

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### Table 3: Seropositivity of IgG antibodies to *H. pylori* by ELISA among humans of different age groups.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
<th>Total examined (positive, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 15</td>
<td>20</td>
<td>4 (20%)</td>
<td></td>
<td>15</td>
<td>7 (46.7%)</td>
<td></td>
<td></td>
<td>35 (11, 31.4%)</td>
</tr>
<tr>
<td>16–30</td>
<td>8</td>
<td>7 (87.5%)</td>
<td></td>
<td>10</td>
<td>6 (60%)</td>
<td></td>
<td></td>
<td>18 (13, 72.2%)</td>
</tr>
<tr>
<td>31–45</td>
<td>12</td>
<td>5 (41.7%)</td>
<td></td>
<td>8</td>
<td>4 (50%)</td>
<td></td>
<td></td>
<td>20 (9, 45%)</td>
</tr>
<tr>
<td>More than 45</td>
<td>4</td>
<td>1 (25%)</td>
<td></td>
<td>13</td>
<td>6 (46.2%)</td>
<td></td>
<td></td>
<td>17 (7, 41.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>17 (38.6%)</td>
<td></td>
<td>46</td>
<td>23 (50%)</td>
<td></td>
<td></td>
<td>90 (40, 44.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation of human subjects</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
<th>Total (positive, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy</td>
<td>28</td>
<td>11 (39.3%)</td>
<td></td>
<td>33</td>
<td>14 (42.4%)</td>
<td></td>
<td></td>
<td>61 (25, 41%)</td>
</tr>
<tr>
<td>Dog owners</td>
<td>16</td>
<td>6 (37.5%)</td>
<td></td>
<td>13</td>
<td>9 (69.2%)</td>
<td></td>
<td></td>
<td>29 (15, 51.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>17 (38.6%)</td>
<td></td>
<td>46</td>
<td>23 (50%)</td>
<td></td>
<td></td>
<td>90 (40, 44.4%)</td>
</tr>
</tbody>
</table>

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(infections maintained mainly in humans that may be transmitted to animals) [42].

It was very difficult in this investigation to implicate or rule out infection of Helicobacter pylori to animal contact alone since combinations of other factors, including hygienic conditions, environmental factors, and socioeconomic status, contribute to the spread of the disease.

Conclusions

Helicobacter pylori is common in humans and animals in Cairo and Giza governorates, Egypt. Epidemiology of H. pylori is complex, and the zoonotic risk has not been clearly identified in this study. Further investigations with greater numbers of samples are essential to study the mechanism of disease transmission and potential risks for acquisition of infection. The present results provide a background and baseline data that may be required for commencing Helicobacter control programs in the studied area.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References


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Conflict of interests: No conflict of interests is declared.