Natural maternal transmission of *H. pylori* in Mongolian gerbils

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**Abstract**

**Aim:** To investigate maternal *H. pylori* infection status to determine the potential of maternal transmission.

**Methods:** In the present study, we examined these issues in an experimental murine model, which is a Mongolian gerbil model that has been reported as an optimal laboratory animal model to study *H. pylori*. Pregnant Mongolian gerbils, infected experimentally with *H. pylori*, were divided into as four groups. Following the experimental design, the stomachs of the mother and litters were isolated and assessed for transmission of *H. pylori* at the prenatal period, parturition day, 1 wk and 3 wk old, respectively. Bacterial culture and polymerase chain reaction (PCR) were used to examine the presence of transmitted *H. pylori*.

**Results:** All litters showed no transmission of *H. pylori* during pregnancy and parturition day. However, they revealed 33.3% and 69.6% at 1-wk and 3-wk of age respectively by PCR.

**Conclusion:** These results suggested that vertical infection during the prenatal period or delivery procedure is unlikely as a route of mother-to-child *H. pylori* infection. It may be that *H. pylori* is acquired through breast-feeding, contaminated saliva and fecal-oral transmission during co-habitation.

**Key words:** *H. pylori*; Vertical; Maternal; Transmission

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**INTRODUCTION**

*H. pylori* is a gram-negative, spiral-shaped, microaerophilic bacterium that infects the human gastric mucosa[1]. Chronic infection is thought to be associated with chronic active gastritis, peptic ulcer and gastric malignancies, such as mucosa-associated B cell lymphoma and adenocarcinoma[2-4]. In particular, this organism has been categorized as a class I carcinogen by the World Health Organization[5] and previous studies have confirmed that long-term infection with *H. pylori* induces adenocarcinoma in Mongolian gerbils[6-8]. In-depth knowledge of the transmission patterns may constitute important information for future intervention strategies. In the absence of consistent and verified environmental reservoirs, a predominantly person-to-person transmission has been postulated. *H. pylori* infection is associated with poor living conditions, and possible transmission routes are fecal-oral, oral-oral, or gastro-oral, but firm evidence is lacking[9-11]. Young children are particularly vulnerable to infection by transmission of *H. pylori* from their infected parents, especially infected mothers[12-15], and it is generally believed that such transmission is influenced by socio-economic status[16-17]. However, little is known about how and when maternal transmission occurs during the perinatal period, especially whether this occurs before or after parturition. In the present study, we examined these issues in an experimental murine model, which is a Mongolian gerbil model that has been reported as an optimal laboratory animal model to study *H. pylori* in vivo[18].

The present study was designed to examine the incidence of vertical transmission of *H. pylori* from their infected mother during the perinatal period in an experimental murine model.

**MATERIALS AND METHODS**

**Experimental design**

The experimental scheme of this study was summarized in Figure 1. Pregnant Mongolian gerbils were infected experimentally with *H. pylori*. The stomachs of the litters were isolated and assessed for transmission of *H. pylori* during pregnancy and at parturition day, 1 wk and 3 wk after delivery respectively. Their mother was also evaluated for the infectious status of *H. pylori*. To determine the vertical transmission of *H. pylori*, bacterial culture assay and polymerase chain reaction (PCR) were conducted with each sample.

**Animals**

Specific pathogen-free (SPF) 3-mo-old male and female...
Mongolian gerbils (*Meriones unguiculatus*) were obtained from the SPF Animal Facilities of College of Medicine, Seoul National University, South Korea. All animals were kept in the inspecting facility of Wonkwang University (Iksan, South Korea) for 1 wk before experimentation to allow acclimation. Thereafter, they were kept in an isolated SPB barrier room with regulated temperature (23°C ± 1°C), humidity (50% ± 5%) and light/dark cycle (12/12 h). The animals were fed a sterilized pellet diet by 2 M rad radiation (Purina, Korea) and sterilized water *ad libitum*. All studies were performed in accordance with the Guide for Animal Experimentation by Wonkwang University and approved by the Institutional Animal Care and Use Committee of Wonkwang University (Iksan, South Korea). All efforts were made to minimize pain or discomfort of animals used.

**Preparation of *H pylori* & inoculation**

*H pylori* (ATCC 43504; American Type Culture Collection, USA) was incubated in a brain-heart infusion broth containing 10% fetal bovine serum at 37°C overnight under a microaerophilic atmosphere and allowed to grow to a density of 2.0 × 10^9^ colony-forming units (CFU) per 1 mL of culture broth. Animals were inoculated twice at 3-d intervals by oral administration of 1.0 × 10^9^ CFU of *H pylori* suspended in 0.5 mL of broth. The animals were fed a sterilized pellet diet by 2 M rad radiation (Purina, Korea) and sterilized water *ad libitum*. The stomachs of the mother and fetuses were isolated and submitted to examine *H pylori* infection. Three mothers were suspended in 0.5 mL of broth. The challenged animals were fed a sterilized pellet diet by 2 M rad radiation (Purina, Korea) and sterilized water *ad libitum*. The stomachs of the mother and fetuses were isolated and submitted to examine *H pylori* infection.

**Isolation of *H pylori***

Aliquots of homogenate were cultured on M-BHM pylori agar medium plates and the plates were incubated under the previous described condition. To confirm *H pylori* infection, the remainder of the homogenate was used for the following PCR procedure.

**Polymerase Chain Reaction**

Bacterial DNAs were extracted from the above homogenate by bead beater-phenol extraction method. Each sample homogenate was suspended in 200 μL of Tris-EDTA-NaCl buffer [10 mmol/L Tris-HCl, 1 mmol/L EDTA, and 100 mmol/L NaCl (pH 8.0)]. A bacterial suspension was placed in a 2.0-mL screw-cap microcentrifuge tube filled with 100 μL of glass beads (diameter, 0.1 mm; Biospec Products, USA) and 100 μL of phenol-chloroform-isomyl alcohol (25:24:1) (Sigma Chemical Co., USA). The tube was oscillated on a Mini-Bead Beater (Biospec Products) for 1 min and was centrifuged (12000 × g, 5 min) to separate the phases. The aqueous phase was subsequently transferred into another clean tube; 10 μL of 3 mol/L sodium acetate and 250 μL of ice-cold absolute ethanol were added. To precipitate the DNA, the mixture was kept at -20°C for 10 min. The harvested DNA pellets were dissolved in 60 μL of Tris-EDTA buffer [10 mmol/L Tris-HCl and 1 mmol/L EDTA (pH 8.0)] and were used as a template DNA for PCR. A set of primers (HF, 5’-ACTTTAAACGCATGAA; and HR, 5’-ATATTTTGACCTTCTGGGGT-3’) was used to detect specific nucleic acid of *H pylori*. The template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube (Maxime PCR Premix; iNtron Biotechnology, Korea) containing 1 U of Taq DNA polymerase, 250 μmol/L each deoxynucleoside triphosphate, 50 mmol/L Tris-HCl (pH 8.3), 40 mmol/L KCl, 1.5 mmol/L MgCl₂, and the gel loading dye. The volume was adjusted with distilled water to 20 μL. The

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*Figure 1* Scheme of experimental vertical transmission.

*Figure 2* The mother and her litters were housed in one cage per family. For 12 h before their sacrifice, they were housed in a grated cage and deprived diet.
reaction mixture was subjected to 30 amplification cycles (5 min at 95°C, 30 s at 94°C, 30 s at 52°C, 45 s at 72°C, and 5 min at 75°C) followed by a 5-min extension at 72°C (model 9600 Thermocycler; Perkin-Elmer Cetus, USA). The PCR products were electrophoresed on a 1.2% agarose gel.

**RESULTS**

While culture of the bacterium is one of the gold standards in the diagnosis of *H pylori* infection, in the present study, we also used the PCR with the culture method to detect *H pylori*. The culture method was not considered to be ideal for determination of *H pylori* transmission because only small amounts of bacteria were suspected to colonize the stomach and the detection limit of the quantitative culture assay was 1 × 10^2 CFU/g feces and higher than those of other assays[20]. Vertical transmission was examined at 2 wk after pregnancy and at parturition day (corresponding to the transplacental or intrauterine transmission during prenatal period and the delivery transmission during birth canal passage, respectively). Each stage group was composed of three pregnant females and their litters.

As the results of assessment performed at 2 wk after pregnancy, the mothers revealed *H pylori* infected status by culture assay and PCR (Table 1). However, their fetuses were not infected with *H pylori* (Figure 3). For the evaluation of delivery transmission during birth canal passage, all litters showed no transmission of *H pylori* (Figure 4) although the mothers were identified *H pylori* infected by culture assay and PCR (Table 1). However, their fetuses were not infected with *H pylori* (Table 1). *H pylori* was not detected in any litters and their mothers of the negative control group (Table 1).

Maternal transmission was examined at 1 wk and 3 wk postpartum (corresponding to the transitional milk and weaning stage, respectively). Each stage group was composed of three families. At 1 wk and 3 wk postpartum, the mothers revealed *H pylori* infected status by culture assay and PCR and some of their litters were infected with *H pylori* (Figure 5). The frequency of maternal transmission was increased during the nursing period. The transmission rate at 3 wk postpartum was significantly higher than at 1 wk postpartum (Table 1). *H pylori* was not detected in any litters of the negative control group.

**DISCUSSION**

Half of the world's population is estimated to be infected with *H pylori* and the infection is mainly acquired in early childhood but the exact routes of transmission remain elusive. Infected mothers are generally considered to be the main source of the pathogen[15,21,22]. The epidemiology of *H pylori* infection is variable, with prevalence being significantly higher and incident infection occurring earlier in developing countries compared with developed countries[17,25,24]. There is an obvious public health impact of *H pylori* infection and thus, to design targeted and cost-effective prevention strategies, elucidation of the mode of transmission for this bacterium is crucial[23].

### Table 1 Results of cultures and PCR for assessment of transmission of *H pylori* during pregnancy and at parturition day

| Infection status | Evaluated time | Subject | Detection rate of *H pylori* (No. of positive/No. of animal) | Culture | PCR |
|------------------|----------------|---------|-------------------------------------------------|---------|-----|
| Infected female  | Pregnancy      | Mothers | 3/3                                             | 5/3     |     |
|                  |                | Fetuses | 0/23                                            | 0/23    |     |
|                  | Delivery       | Mothers | 3/3                                             | 3/3     |     |
|                  |                | Fetuses | 0/21                                            | 0/21    |     |
|                  | 1-wk old       | Mothers | 3/3                                             | 3/3     |     |
|                  |                | Fetuses | 5/21                                            | 7/21    |     |
|                  | 3-wk old       | Mothers | 3/3                                             | 3/3     |     |
|                  |                | Litters | 11/23                                           | 16/23   |     |
| Uninfected female| Pregnancy      | Mothers | 0/1                                             | 0/1     |     |
|                  |                | Fetuses | 0/7                                             | 0/7     |     |
|                  | Delivery       | Mothers | 0/1                                             | 0/1     |     |
|                  |                | Litters | 0/9                                             | 0/9     |     |
|                  | 1-wk old       | Mothers | 0/1                                             | 0/1     |     |
|                  |                | Litters | 0/7                                             | 0/7     |     |
|                  | 3-wk old       | Mothers | 0/1                                             | 0/1     |     |
|                  |                | Litters | 0/7                                             | 0/7     |     |

Figure 3 Amplification of Helicobacter DNAs. All fetuses showed no transmission of *H pylori* at 2 wk after pregnancy. P: Positive control; N: Negative control; Mo: Mother; F: Fetuses.

Figure 4 Amplification of specific nucleic acids for *H pylori*. All litters showed no transmission of *H pylori* at parturition day. P: Positive control; N: Negative control; Mo: Mother; L: Litters.

Figure 5 At 3 wk postpartum, some of litters delivered from infected mothers revealed positive reaction. P: Positive control; N: Negative control; Mo: Mother; L: Litters.
is known that Helicobacter pylori infection is typically acquired in early childhood and usually persists throughout life unless specific treatment is applied[12-14,26]. Definitive modes of transmission have not yet been characterized and the principal reservoir appears to be humans. Person-to-person transmission via fecal-oral, oral-oral and gastro-oral routes have been proposed[6-11]. Numerous studies also indicate low socioeconomic status, including domestic overcrowding in childhood, as major risk factors for higher infection prevalence rates[16,17,27,28]. Little is known about when and how often maternal transmission of H. pylori occurs during the perinatal stage. In the present study, we examined these issues in an experimental murine model.

The results of the vertical-transmission experiment indicated that vertical transmission of H. pylori did not occur at the pregnant and delivery stage. However, they revealed 33.3% and 69.6% at the lactating and weaning stage respectively. Recent epidemiological studies in humans suggest that acquisition of H. pylori occurs during childhood. For example, Rothenbacher et al[29] reported that H. pylori acquisition seems to occur mainly between the first and second year of life: that is, after the age of weaning. Our results are in agreement with this report. Also, Rothenbacher et al[22] reported that infected parents, especially infected mothers, play a key role in the transmission of H. pylori within families. Maternal contact behaviour during the breastfeeding period may be responsible for the high frequency of maternal transmission[30]. Our results also showed that the maternal-transmission of H. pylori was not observed during pregnancy and delivery stage, but detected at lactating and weaning stage. On the basis of these findings, vertical infection during pregnancy or at delivery is unlikely as a route of mother-to-child H. pylori infection. We suggested that H. pylori infection by the transplacental route during pregnancy does not occur and that H. pylori transmission by discharges of the uterus or vagina, obstetric delivery tract, during parturition does not occur. H. pylori might be acquired through breast-feeding, contaminated saliva and fecal-oral transmission during co-habitation.

In conclusion, the present study provides new and important information on maternal transmission of H. pylori. This study implied that maternal transmission of H. pylori might be developed during latency or a later postpartum stage. Data from human children are limited, because most H. pylori-infected children have no symptoms and it is difficult for a paediatrician to examine such asymptomatic children invasively. In the present study, we examined these issues in an experimental murine model, the Mongolian gerbil model that has been reported as an optimal laboratory animal model to study H. pylori in vivo[18]. We analysed the stomachs of many infant Mongolian gerbils directly and we believe that it is meaningful to use our results to speculate when H. pylori infection occurs in human children. The acquisition of H. pylori infection during childhood seems to be a critical risk factor for the later development of gastric cancer. The prevention of transmission of H. pylori during childhood could provide an effective strategy to decrease H. pylori infection and gastric cancer.
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