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Absence of vertical transmission of *Helicobacter pylori* in an experimental murine model

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***Helicobacter pylori* (*H. pylori*) infection is acquired mainly in early childhood but the precise transmission routes are unclear. This study examined the maternal *H. pylori* infection status in order to determine the potential of perinatal transmission. These issues were investigated using an experimental murine model, the Mongolian gerbil, which has been reported to be the most suitable laboratory animal model for studying *H. pylori*. Pregnant Mongolian gerbils, infected experimentally with *H. pylori*, were divided into two groups. The stomachs of the mother and litters were isolated and assessed for the transmission of *H. pylori* at the prenatal period (2 weeks after pregnancy) and at the parturition day. The bacterial culture, polymerase chain reaction (PCR) and rapid urease test were used to examine the presence of the transmitted *H. pylori*. There was no *H. pylori* observed in any of the fetuses during pregnancy and in the litters at parturition. This suggests that vertical infection during the prenatal period or delivery procedure is unlikely to be route of mother-to-child transmission of a *H. pylori* infection.**

Key words: *Helicobacter pylori*, murine model, transmission, vertical infection

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium that infects the human gastric mucosa [8]. A chronic infection is believed to be associated with chronic active gastritis, peptic ulcers and gastric malignancies, such as mucosa-associated B cell lymphoma and adenocarcinoma [11]. In particular, this organism has been classified by the World Health Organization as a class I carcinogen [5] and previous studies have confirmed that a long-term infection with *H. pylori* induces adenocarcinoma in Mongolian gerbils [4,16]. An in-depth knowledge of the transmission routes will provide important information for future intervention strategies. In the absence of consistent and verified environmental reservoirs, predominantly person-to-person transmission has been suggested. *H. Pylori* infection is associated with poor living conditions, and possible transmission routes are fecal-oral, oral-oral, or gastro-oral. However, there is no firm evidence for any of these routes [15]. Young children are particularly vulnerable the transmission of *H. pylori* from their infected parents, particularly infected mothers [12], and it is generally believed that such transmission is influenced by the socio-economic status of the family. However, little is known regarding how and when maternal transmission occurs during the perinatal period, especially whether this occurs before or after parturition. This study examined these issues in an experimental murine model, the Mongolian gerbil, which has been reported to be the most suitable laboratory animal model for examining *H. pylori in vivo* [3].

This study was designed to determine the incidence of vertical transmission of *H. pylori* from their infected mother during the prenatal period or the delivery procedure in an experimental murine model.

Materials and Methods

Experimental design

Pregnant Mongolian gerbils were infected experimentally with *H. pylori*. The stomachs of the litters were isolated and assessed for *H. pylori* transmission during pregnancy and at parturition day. Their mother was also evaluated for the *H. pylori* infectious status. The vertical transmission of *H. pylori* was assessed using a bacterial culture assay and polymerase chain reaction (PCR).

Experimental animals

Specific pathogen-free (SPF) 3-month-old male and female Mongolian gerbils (*Meriones unguiculatus*) were obtained from the SPF Animal Facilities of College of

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Medicine, Seoul National University, Korea. All the animals were allowed one week to acclimatize in the inspection facility. Thereafter, the gerbils were kept in an isolated SPF barrier room with a regulated temperature (23 ± 1°C), humidity (50 ± 5%) and light/dark cycle (12/12 h). The animals were fed a pellet diet sterilized with 2 k Gy radiation (Purina, Korea) and sterilized water *ad libitum*. This study was performed in accordance with the Guide for Animal Experimentation by Seoul National University and approved by the Institutional Animal Care and Use Committee of Seoul National University (Seoul, Korea). All efforts were made to minimize pain or discomfort to the animals.

Preparation of *H. pylori* and inoculation

H. pylori ATCC 43504 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. The animals were inoculated twice at 3-day intervals by the oral administration of 1.0×10^9 CFU of *H. pylori* suspended in 0.5 ml of the brain-heart infusion broth. The challenged animals were confirmed to be *H. pylori*-positive by PCR of their fecal samples, as described previously [7]. The *H. pylori*-negative animals were excluded from the following study.

Vertical transmission of *H. pylori*

One week after *H. pylori* challenge, the infected females and males were transferred to each separate cage for mating. As soon as the female was confirmed to be pregnant, it was separated from the group and cared for until delivery. Six infected pregnant females were used to determine the vertical transmission. Three mothers were sacrificed 2 weeks after pregnancy. The gastric samples of the mother and fetuses were isolated and analyzed for *H. pylori* infection. In addition, another three pregnant gerbils were cared for until delivery. The stomachs of the mother and litters were isolated and assessed for the presence of *H. pylori* at the parturition day. For the negative control, an uninfected female and her litter was sacrificed at 2 weeks after pregnancy and at the parturition day, respectively. Thereafter, their gastric samples were submitted in order to determine the presence of *H. pylori* infection.

Isolation of *H. pylori*

Aliquots of the homogenate were cultured on M-BHM pylori agar medium plates and incubated under the previous described conditions [7]. In order to confirm *H. pylori* infection, the remaining homogenate was used for the following PCR procedure.

Polymerase chain reaction

The bacterial DNA was extracted from the above homogenate using the bead beater-phenol extraction method [7]. Each sample homogenate was suspended in 200 µl of Tris-EDTA-NaCl buffer (10 mM Tris-HCl, 1 mM EDTA, and 100 mM NaCl, pH 8.0). A bacterial suspension was placed in a 2.0-ml screw-cap microcentrifuge tube filled with 100 µl (packed volume) of glass beads (diameter, 0.1 mm; Biospec Products, USA) and 100 µl of phenol-chloroform-isoamyl alcohol (25 : 24 : 1) (Sigma, USA). The tube was oscillated on a Mini-Bead Beater (Biospec Products, USA) for 1 min and centrifuged (12,000 × *g*, 5 min) to separate the phases. The aqueous phase was transferred into another clean tube, into which 10 µl of 3 M sodium acetate and 250 µl of ice-cold absolute ethanol were added. The DNA was precipitated by storing the mixture at 20°C for 10 min. The harvested DNA pellets were dissolved in 60 µl of a Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA , pH 8.0) and used as the template DNA for PCR. A set of primers (HF, 5'-ACTTTAAACGCATGAAGATAT-3'; and HR, 5'-ATATTTTGACCTTCTGGGGT-3') was used to detect the specific nucleic acid of *H. pylori* [7]. The template DNA (50 ng) and 20 pmol of each primer were added to the PCR mixture tube (Bioneer, Korea) containing 1 U of Taq DNA polymerase, 250 µM each deoxynucleoside triphosphate, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $MgCl_2$, and the gel loading dye. The volume was adjusted to 20 µl with distilled water. The 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 (Thermocycler; Perkin-Elmer, USA). The PCR products were electrophoresed on 1.2% (wt/vol) agarose gel.

Rapid urease test

The gastric mucosal specimens of animals were collected aseptically and placed in a rapid urease test (Asan Pharma, Korea) to detect the presence of *H. pylori* urease. The test was performed according to the manufacturer's instructions and the results were interpreted after 24 h. A positive result was reported if a color change from yellow to pink was observed within 24 h of incubation in a 37°C incubator.

Results

While a culture of the bacterium is one of the gold standards for diagnosing *H. pylori* infection, PCR with the culture method was also used in this study to detect *H. pylori*. The culture method was not considered to be ideal for determining *H. pylori* transmission because only a small amount of bacteria were suspected to colonize the stomach, and the detection limit of the quantitative culture assay was 1×10^2 CFU/g gastric tissue. Vertical transmission was examined 2 weeks after pregnancy and at parturition day (corresponding to the transplacental or intrauterine transmission during prenatal period and the delivery transmission during

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

Infection status	Evaluated time	Subject	Detection rate of *H. pylori*		
			Culture	PCR	Rapid urease
Infected female	Pregnancy	Mothers	3/3	3/3	3/3
		Fetuses	0/23	0/23	0/23
	Delivery	Mothers	3/3	3/3	3/3
		Litters	0/21	0/21	0/21
Uninfected female	Pregnancy	Mothers	0/1	0/1	0/1
		Fetuses	0/7	0/7	0/7
	Delivery	Mothers	0/1	0/1	0/1
		Litters	0/8	0/8	0/8

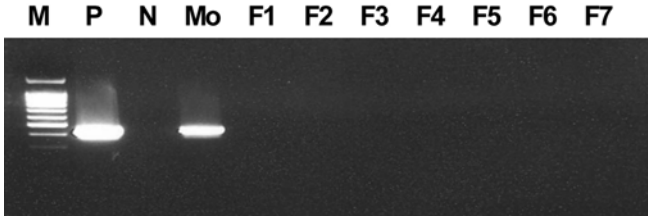

Fig. 1. Amplification of *Helicobacter* DNAs. None of the fetuses showed any evidence of *H. pylori* transmission at 2weeks after pregnancy. M, 100 bp DNA ladder; P, positive control; N, negative control; Mo: mother, F: fetuses.

birth canal passage, respectively). Each group contained three pregnant females and their litters.

Two weeks after pregnancy, the mothers were shown to have *H. pylori* infected status by the culture assay, PCR and rapid urease test (Table 1). However, their fetuses were not infected with *H. pylori* (Fig. 1 and Table 1) even though the mothers were infected with *H. pylori* by the culture assay, PCR and rapid urease test (Table 1). *H. pylori* was not detected in any of the litters or their mothers in the negative control group (Table 1).

Discussion

It is estimated that 50% of the world's population is infected with *H. pylori,* and the infection is mainly acquired in early childhood. However, the precise transmission route remains elusive. Infected mothers are generally believed to be the main source of the pathogen [14]. The epidemiology of *H. pylori* infection varies, with the prevalence being significantly higher and an incident infection occurring earlier in developing countries than in developed countries [2]. *H. pylori* infection has an obvious public health impact. Therefore, knowledge of the mode of transmission is essential for designing targeted and cost-effective prevention strategies. *H. pylori* infection is typically acquired in early childhood and usually persists throughout life unless a specific treatment is applied [6]. The definitive transmission routes have not been characterized and the principal reservoir appears to be humans. Person-to-person transmission via fecal-to-oral, oral-to-oral and gastro-to-oral routes has been suggested. In addition, many studies have also indicated that a low socioeconomic status, including domestic overcrowding in childhood is major risk factor for the higher prevalence of infections [1,9]. Little is known about how and when the maternal transmission of *H. pylori* occurs during the perinatal stage. This study examined these issues in an experimental murine model.

The results of this study indicate that the vertical transmission of *H. pylori* does not occur during pregnancy or delivery. Recent epidemiological studies in humans suggest that *H. pylori* is acquired during childhood. For example, Rothenbacher *et al*. [13] reported that the acquisition of *H. pylori* appears to occur mainly between the first and second year of life i.e. after the age of weaning. The results in this study agree with this report. Rothenbacher *et al*. [14] reported that infected parents, particularly infected mothers, play a key role in the transmission of *H. pylori* within families. The maternal contact behavior during breastfeeding might be responsible for the high frequency of maternal transmission. These results did not show that the maternal-transmission of *H. pylori* occurs during pregnancy and delivery. Hence, *H. pylori* infection is not transmitted through the transplacental route during pregnancy or from discharges from the uterus or the vagina, obstetric delivery tract during parturition. Further studies will be needed to examine other possible routes including a horizontal infection through breast-feeding, contaminating saliva and fecal-oral transmission during co-habitation.

In conclusion, this study provides new and important information on maternal transmission of *H. pylori*. The results suggest that the maternal transmission of *H. pylori* might be developed during latency or at the later postpartum stage. The acquisition of *H. pylori* infection during childhood appears to be a risk factor for the later development of gastric cancer. The prevention of *H. pylori* transmission during childhood might be an effective strategy for decreasing the incidence of *H. pylori* infections and gastric cancer.

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