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ORIGINAL PAPER

Association Between *Helicobacter pylori* Infection in Mothers and Birth Weight

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Abstract *Helicobacter pylori* infection may cause intrauterine growth restriction (IUGR). However, it is unknown whether the growth of children from *H. pylori*-infected mothers is also affected or whether transmission of infection from mother to child occurs. This study aimed to determine if maternal *H. pylori* infection was associated with IUGR and low birth weight in a mouse model, and whether transmission of infection from mother to infant occurs. Female C57BL/6 mice were inoculated with *H. pylori* ($n = 18$) or water (control; $n = 18$) via gavage. Mice were mated at 6 weeks postinfection, with half of the mice sacrificed after 2 weeks of gestation. The remaining mice gave birth and a third of the litter was weighed and sacrificed at birth, during milk feeding (1.5 weeks), and during solid feeding (4 weeks). Stomachs of all mice and whole foetuses were cultured for the presence of *H. pylori*. There were no differences in litter size or foetus weight between control and *H. pylori*-infected mice. Pups from infected mothers had a lower weight during milk feeding (control, 5.91 ± 0.23 g; *H. pylori*, 4.59 ± 0.16 g; $p < 0.05$) and solid feeding (control, 12.73 ± 0.58 g; *H. pylori*, 10.01 ± 1.02 g; $p < 0.05$). *H. pylori* was not detected by culture in the pups at any age. *H. pylori* infection in mothers was associated with a decrease in infant weight during milk feeding and after weaning. Transmission of infection from mother to infant was not detected by culture, suggesting that decreased baby weight may be due to decreased milk supply or altered nutrition from the mother.

Keywords *Helicobacter pylori* · Birth weight · Transmission · Mouse model

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Introduction

It is thought that approximately half of the world's population is infected with *Helicobacter pylori* (*H. pylori*) [1], making this one of the most common infections in humans. *H. pylori* infection has been recognized as a major cause of various gastroduodenal diseases [2], such as chronic gastritis, peptic ulcer disease, and gastric cancer [1]. In children, *H. pylori* infection has been associated with recurrent abdominal pain (in cases of erosive gastritis or ulcer disease), iron deficiency, and subnormal growth at puberty and smaller stature compared to noninfected children [3–5]. At present, association between *H. pylori* and small stature remains to be confirmed. Recently, an association between *H. pylori* infection and morning sickness [6, 7], as well as intrauterine growth restriction, has also been suggested [8]. To prevent these health problems it is important to prevent infection or eradicate it at an early age. Therefore an understanding of whether *H. pylori* infection is transmitted from mother to child and the health impact this has on the child is required.

The route of transmission of *H. pylori* is unknown, but it is thought to be person-to-person [9]. Possible routes of person-to-person transmission are gastric-to-oral and fecal-to-oral [10]. The gastric-to-oral route of transmission has been postulated as a result of vomiting and gastroesophageal reflux, whereas fecal-to-oral transmission has been associated with contamination of water supplies or poor hygiene [10]. Sibling-to-sibling infection, spouse-to-spouse infection, and father-to-child infection have been assessed but seem to be less important compared to mother-to-child infection [11–13]. Recent studies have suggested that the inadvertent ingestion of foods contaminated with cockroach excreta and the presence of houseflies are potential routes of *H. pylori* transmission [14, 15]. However, no environmental reservoir has consistently been identified [9]. The main risk factors for *H. pylori* infection have recently been determined as household crowding and poor socioeconomic conditions in childhood [16].

H. pylori infection is predominantly acquired during early childhood [10, 17], and a recent study suggested that *H. pylori* acquisition mainly occurs before the second year of life [17]. Most evidence suggests that mothers play a key role in transmission of infection to their children [18]. Previous research is conflicting as to whether breastfeeding protects against *H. pylori* infection. One study suggested that breastfeeding did not protect the child from transmission [19], while another study suggested that specific human IgA antibodies in breast milk may play a crucial role in delaying the onset of *H. pylori* infection by maintaining the integrity of the gastric acid barrier, throughout the weaning period [20]. A recent editorial on the epidemiology of *H. pylori* proposed that more prospective studies are needed to determine age-specific rates of infection, the relevant antecedent risk factors, and the route by which *H. pylori* is transmitted [9]. Studies of the route of *H. pylori* transmission in humans are difficult due to a number of reasons. The main difficulties include nonexisting clinical symptoms to diagnose acute infection, lack of highly selective culture media for use in epidemiological field conditions, and the absence of a reproducible serotyping system. Furthermore, studies of transmission in humans are time-consuming and complicated, as there is no sensitive diagnostic test for use in children younger than 2 years of age [9]. Therefore the aim of the current study was to use a mouse model to investigate whether transmission of *Helicobacter* occurs in utero, at birth, during milk feeding, or after weaning. In addition, this study aimed to determine how *H. pylori* infection of the mother during pregnancy influences the birth size of the infant.

Methods

Experimental design

Female C57Bl/6 mice (aged 8 weeks) were inoculated via orogastric gavage. Mice were either inoculated with 0.1 ml water (control mice; $n = 18$) or 0.1 ml of 10^9 *H. pylori*/ml (Sydney strain 1, infected mice; $n = 18$). At 6 weeks postinfection mice were mated, and once impregnation was evident mice were placed in separate cages. Mice were housed in a controlled-temperature laboratory at 24°C with 14 hrs light per day and were allowed free access to water and a standard chow diet (Ridley AgriProducts, Murray Bridge, South Australia). The study was approved by the Children, Youth and Women's Health Service Animal Ethics Committee and the study complied with the Australian Code of Practice for the Care and Use of Animals.

Approximately 2 weeks after mating and during the third trimester of pregnancy, half of the pregnant noninfected control mice ($n = 9$) and half of the *H. pylori*-infected mice ($n = 9$) were sacrificed. Foetuses were removed and litter size and weights were recorded. The remaining mice gave birth 19–23 days after mating and litter sizes were recorded. One-third of the pups from each litter were sacrificed immediately after birth. The second third of the pups from each litter were sacrificed halfway through the milk-feeding period (9–11 days after birth), while the remaining pups were sacrificed approximately 4 weeks after birth (1 week after weaning) together with the adult female mice. Pup weights were assessed at each time point.

Tissue collection and preparation

Adult mice and pups older than 12 days were asphyxiated with carbon dioxide followed by cervical dislocation. Foetuses and neonatal animals (<12 days) were sacrificed by decapitation, due to resistance to the effect of carbon dioxide [21]. Stomachs from adult mice and pups were excised. The stomach was cut open, and the contents were removed by washing in saline and then homogenized using an Ultra Turrax homogenizer (Janke & Kunkel, Staufen, Germany) for culture of *H. pylori*. When pregnant mice were sacrificed the foetuses were removed, homogenized in saline, and cultured. The stomach and foetus homogenates from all mice were serially cultured and plated out in duplicate on *Helicobacter*-selective agar. All plates were incubated in an atmosphere controlled at 5% CO_2 and 10% O_2, 95% humidity, and a temperature of 37°C for 7 days. Results are expressed as colony forming units (CFU) per gram of tissue for *H. pylori*.

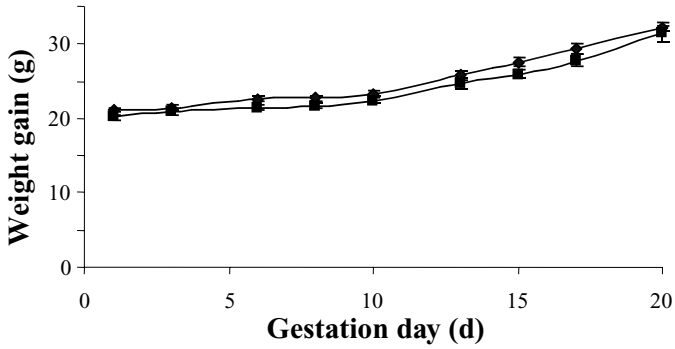

Fig. 1 Weight gain of control mice (diamonds; $n = 9$) and *H. pylori*-infected mice (squares; $n = 9$). Data are mean ± standard error

Statistical analysis

Litter size and body weight were compared between control and *H. pylori* mice using paired *t*-tests. All analysis was performed using the SigmaStat Statistical software version 3.01 (SPSS Inc., Chicago, IL, USA), and differences were considered to be statistically significant at $p < 0.05$.

Results

Gastric homogenates of adult mice infected with *H. pylori* were positive for infection after sacrifice, with an average bacterial load of 2.52×10^6 CFU/g of gastric tissue. Gastric tissue from all noninfected control adult mice was *Helicobacter* negative. Gastric tissues from all foetuses, newborns, milk-feeding pups, and weaned pups were *Helicobacter* negative with culture. Therefore there was no evidence of transmission from infected mothers to their pups at any age.

There were no significant weight differences between the groups of mice during pregnancy ($p > 0.05$; Fig. 1). In the groups of mice that were sacrificed during pregnancy, there were no significant differences between foetus weights (control, 0.23 ± 0.09 g; *H. pylori*, 0.18 ± 0.05 g; $p > 0.05$) and litter size (control, 5.9 ± 0.6; *H. pylori*, 6.3 ± 0.9; $p > 0.05$). All mice sacrificed during pregnancy showed a 100% success rate of pregnancy and uterine implantation. Remaining noninfected and *H. pylori*-infected mice gave birth 19–23 days after mating. There were no significant differences in litter size (control, 5.71 ± 0.42; *H. pylori*, 6.11 ± 0.47; $p > 0.05$). There was also no difference between newborn weights, however, during milk feeding and 4 weeks after birth (weaned) pups from *H. pylori*-infected mothers were significantly smaller than pups from noninfected mothers ($p < 0.001$ and $p < 0.05$, respectively; Table 1).

Four of nine (44%) *H. pylori*-infected mothers killed all their babies after birth and before the 11-day follow-up time point, whereas only one (11%) noninfected control mouse killed her pups. This, however, was not statistically different (*z*-test, $p > 0.05$). The high rate of infanticide in the infected group did not affect the statistical calculation during the weaning period, as it is expected that the pups will have an increased weight gain since there are fewer infants competing for the mother's milk; despite this, we found that there was a significant decrease in infant weight.

Discussion

In this study a mouse model was used to study if *H. pylori* infection was transmitted from mother to infant and whether infection in mothers affected pregnancy and postnatal outcomes. The results showed that *H. pylori* infection is not transmitted to infants as determined by culture, but it did restrict growth of mice pups, as milk feeding and weaned pups from infected mothers were significantly smaller than pups from noninfected mice. Interestingly, growth was not delayed in foetuses and newborn pups of infected and noninfected mothers. This may be explained by a similar study conducted by our group [22], where we characterized the changes in metallothionien (MT) of foetuses from mothers infected with *H. pylori*. We demonstrated that foetal MT levels were increased twofold compared to control foetuses whose mothers were not infected. Since MT is a zinc binding protein with antioxidant activities, these findings suggest that the induction of MT facilitates the movement of zinc into the foetus, where it is required for the processes underlying

Table 1 Weight of newborn, milk feeding, and weaned pups from noninfected control and *H. pylori*-infected mothers

Mother	Newborn (g)	Milk feeding (g)	Weaned (g)
Control ($n = 9$)	1.21 ± 0.02 ($n = 13$)	5.9 ± 0.23 ($n = 15$)	12.73 ± 0.58 ($n = 15$)
H. pylori ($n = 9$)	1.28 ± 0.03 ($n = 16$)	4.5 ± 0.16*($n = 12$)	10.01 ± 1.02** ($n = 10$)

Note. Data are mean ± SE. Significant difference (*$p < 0.05$, **$p < 0.001$) compared to age-matched, noninfected mice.

growth and development [23], which may compensate for any size differences that *H. pylori* infection may cause to the foetuses and/or newborns. However, the results from the present study suggest that maternal *H. pylori* infection caused a growth delay in older pups. It may be speculated that maternal *H. pylori* infection negatively influenced the quality or quantity of the breast milk, resulting in poorer nutrition to the pups. Another explanation for why pups of infected mothers were smaller than pups of noninfected mothers may be that pups were infected, however with a low nondetectable number of bacteria, compromising the growth. It is speculated that gastritis induced by *H. pylori* infection in the present study may have led to reduced maternal food intake and therefore resulted in decreased pup size. However, as there were no significant differences in the weights of the pregnant mice, the association is unlikely.

The present study did not find any evidence of transmission from *H. pylori*-infected mothers to their foetuses or pups, suggesting that the transmission rate in C57BL/6 mice is low, at least before 4 weeks after birth. When comparing the mouse model to humans it should be remembered that mice are more likely to transmit infection via the oral-fecal route, as it has been proven that adult mice transmit *H. pylori* via feces but not by shared water and food [24]. The human transmission route is not yet established. As weaned pups from *H. pylori*-infected mothers were smaller than pups from noninfected mothers, it may be speculated that pups are not only compromised during the period of milk feeding, but also restricted longer term. It is not known from the present results if the pups catch up on growth after 4 weeks of age.

It has been suggested previously that natural immunosuppressive mechanisms that allow normal pregnancy appear to be altered in concomitance with *H. pylori* infection, resulting in a reduction in embryo implantation and foetal development [25]. However, the present study failed to find significant differences regarding litter size between the groups. An interesting observation of the current study, however, was that 44% of *H. pylori*-infected mothers killed all their babies during the first week and a half of their lives, whereas only 11% of noninfected control mothers killed their pups. As cannibalism of young mice is considered common under stressed or unsafe conditions [26], the results of this study suggest higher levels of stress in *H. pylori*-infected mice, as all other conditions were kept constant between the groups. The higher stress level of *H. pylori*-infected mice may also reduce the quality and/or quantity of their breast milk and, thereby, negatively affect growth of the pups.

There are limitations of the present study, where only one method (culture) for detection of *H. pylori* was applied. Thus, the present data are too preliminary to draw general conclusions about the mode of *H. pylori* transmission. Further studies are warranted to address this question more thoroughly using larger numbers of mice and different techniques for detection of the bacteria as well as assessing vertical transmission of newborn mice in adulthood to confirm the presence or absence of *H. pylori*.

The results of the current study did not support the recent findings of significantly lower foetal weights in *H. pylori*-infected mice compared to noninfected controls [25]. This may be explained by the different mice strains used: one is germ free, the duration of infection prior to conception is only 3 weeks in the Rossi et al. [25] paper so an acute *H. pylori* infection during pregnancy is more likely, and a different strain of bacteria was used. Furthermore, the results of the current study cannot confirm that perinatal *H. pylori* infection increases the risk of babies being born small for gestational age [8]. However, the results indicate that maternal *H. pylori* infection causes growth restriction of pups during milk feeding and after weaning. This is consistent with other studies [4, 16, 27–29] suggesting that *H. pylori* infection is associated with short stature, although the mechanism is unclear. A possible mechanism is that the accompanied iron deficiency anemia caused by *H. pylori* infection may cause a delay in pubertal growth [4]. Another explanation may be related to intrauterine growth restriction caused by *H. pylori* infection, which is linked with an increase in symptoms including dyspepsia, nausea, and vomiting because of the underlying undiagnosed peptic ulcer disease, therefore affecting maternal appetite and hence restricting the growth of the foetus [8]. Our results also showed no evidence of *H. pylori* transmission from infected mothers to pups before 4 weeks after birth, but as there was reduced growth of pups during milk feeding and 1 week after weaning, it is suggested that maternal *H. pylori* infection restricts growth of pups due to yet unknown reasons, and that growth of youth may not catch up after weaning. Further work is warranted to investigate the postparturition factors including alterations in breast milk to establish why pups from *H. pylori*-infected mothers are smaller than pups from noninfected mothers. In addition, human studies are needed to assess the relationship of maternal *H. pylori* infection and the growth of children, as this has not been investigated previously.

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