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Observational Study

Family-based *Helicobacter pylori* infection status and transmission pattern in central China, and its clinical implications for disease prevention

Family-based *H. pylori* infection and transmission

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Abstract

BACKGROUND

Helicobacter pylori has characteristics of family cluster infection; however, its family-based infection status, related factors, and transmission pattern in central China, a high-risk area for *H. pylori* infection and gastric cancer (GC), have not been evaluated. We investigated family-based *H. pylori* infection in healthy households to understand its infection status, related factors, and patterns of transmission for related disease prevention.

AIM

To investigate family-based *H. pylori* infection status, related factors, and patterns of transmission in healthy households for related disease prevention.

METHODS

Blood samples and survey questionnaires were collected from 282 families including 772 individuals. The recruited families were from 10 selected communities in the greater Zhengzhou area with different living standards, and the family members' general data, *H. pylori* infection status, related factors, and transmission pattern were analyzed. *H. pylori* infection was confirmed primarily by serum *H. pylori* antibody arrays; if patients previously underwent *H. pylori* eradication therapy, an additional ¹³C-urea breath test was performed to obtain their current infection status. Serum gastrin and pepsinogens (PGs) were also analyzed.

RESULTS

Blood samples and survey questionnaires were collected from 282 families including 772 individuals. The recruited families were from 10 selected communities in the greater Zhengzhou area with different living standards, and the family members' general data, *H. pylori* infection status, related factors, and transmission pattern were analyzed. *H. pylori* infection was confirmed primarily by serum *H. pylori* antibody

arrays; if patients previously underwent *H. pylori* eradication therapy, an additional ¹³C-urea breath test was performed to obtain their current infection status. Serum gastrin and pepsinogens (PGs) were also analyzed.

CONCLUSION

In our study sample from the general public of central China, the *H. pylori* infection rate was 54.27%, but in 87.23% of healthy households, there was at least 1 *H. pylori*-infected person; in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this area. Individuals with a higher education level showed significantly lower infection rates; no other variables affected infection rates.

Key Words: *Helicobacter pylori*; Atrophic gastritis; Family clustering infection; Gastric cancer; Gastrin; Pepsinogen

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Core Tip: *Helicobacter pylori* has characteristics of family cluster infection. However, few studies have investigated family-based infection status and pattern of intrafamilial transmission in the general public of central China. In our study, the *H. pylori* infection rate was 54.27%, but in 87.23% of healthy households, there was at least 1 *H. pylori*-infected person; in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this area. Intrafamilial infection status and patterns of transmission are important causes of *H. pylori* spread, indicating the urgent need for family-based infection control and related disease prevention.

INTRODUCTION

Chronic *Helicobacter pylori* infection is the major cause of chronic gastritis, peptic ulcers, and gastric cancer (GC), and is also closely associated with a number of extra-gastrointestinal (GI) diseases^[1-3]. The *H. pylori* infection rate in China is about 50%, but the infection rate varies widely in different regions due to economic development, age, lifestyle habit, and sanitary conditions^[4-6]. *H. pylori* has characteristics of family cluster infection^[7]. Most *H. pylori* infections are acquired during childhood and adolescence, and infection will persist for decades unless proper treatment is received.

Mounting evidence has demonstrated that transmission of *H. pylori* is mainly by oral-oral and fecal-oral routes, and water sources^[8,9], and intra-familial spread is the major source of *H. pylori* transmission^[10-12]. The infected parent, especially the mother, is thought to play an important role in its transmission^[9,13]. When parents are infected with *H. pylori*, the infection rates of their children markedly increase; spread has also been demonstrated between spouses and among siblings^[14-18]. Therefore, diagnosis and treatment of the whole family have important clinical implications for preventing related diseases^[19]. Recently, the notion of “family-based *H. pylori* infection control and management” has been introduced to China as a practical strategy to curb *H. pylori* intra-familial transmission and the development of related diseases^[3]. However, relatively few studies have been performed to investigate the family-based infection status, related factors, and pattern of intrafamilial transmission in the general population.

Type I (cytotoxin-associated protein-negative [CagA-], vacuolating cytotoxin-positive [VacA+]) *H. pylori* infection causes severe gastric inflammation and can induce carcinogenesis^[20,21]. Our previous study of 3572 patients admitted to the hospital showed that the *H. pylori* infection rate was 75.9% in this area of central China. The infection rate was further confirmed by investigation of 523 endoscopy-confirmed patients (76.9%), of whom 72.4% (291/402) had type I *H. pylori* infection and 27.5% had type II *H. pylori* infection. Importantly, 88.4% of GC patients were *H. pylori*-positive, of whom 84.2% had type I infection; only 11.6% of GC patients were *H. pylori*-negative^[22]. At present, the genotype of family based-*H. pylori* infection in the healthy household is

unclear, as well as its relationship with GC epidemiological markers such as gastrin-17 (G-17), pepsinogen (PG) level, and PG I/II ratio (PGR).

Henan province in central China is one of the high-risk areas for *H. pylori* and GC, with an *H. pylori* infection rate of 49.6%^[23] and GC incidence of 42.52/100000^[24]. The capital city, Zhengzhou, has a population of 12 million, but there has been no large-scale family-based *H. pylori* intrafamilial transmission survey, and the factors that affect *H. pylori* spread and cause disease are unclear.

Therefore, we investigated family-based *H. pylori* infection status, factors related to bacteria spread, bacteria genotype, and patterns of transmission for the residents in this area, and analyzed their impact on GC epidemiological markers including G-17, PGI, PGII, and PGR. The results of this study will provide an understanding of *H. pylori* infection status in the household and refine eradication strategies for the prevention of related diseases.

MATERIALS AND METHODS

Study population and data collection

From September 2020 to April 2021, blood samples and questionnaires were collected from family members of 10 selected communities in the greater Zhengzhou area; each community enrolled 20-30 families, with all members participating. The 10 communities were selected based on high, middle, and low living standards to prevent biased selection of the population; these included two high-income communities, six middle-class communities, and two communities originating from rural areas. Specifically, the study included two communities located in the Guancheng District, namely Lufu Pavilion and Houjiadong Street communities; three communities located in Jinshui District, namely Chengbei Road, Jiagang, and Huilong Digital City communities; four communities in the New East Zhengzhou District, namely Hanhai Qingyu, Zhengzhou Academy of Aviation Administration, Henan University of Finance, Economics and Law, and Nanxi Fudi communities; and one community in the Central Zhengzhou District, namely Kowloon City community.

A total of 282 families (family size ≥ 2 persons) including 772 individuals participated in the survey. The inclusion criteria were: family members being long-term residents living in the Zhengzhou area, with no age limit; all family members being willing to participate by providing blood samples and filling out the questionnaire; at least 2 people composing the family unit, but with no limitation on how many people are living in the same household; and all family members being willing to provide written informed consent. An infected family was defined as a household with various family members infected with *H. pylori*, ranging from only 1 person to all family members being infected, and a family could be composed of only a couple, with or without children. Exclusion criteria were: pregnant and breastfeeding females; people with mental illness; or people who refused to fill out the questionnaire or sign the consent form.

This study was approved by the Ethics Committee of People's Hospital of Zhengzhou University (No. 53, 2021). All subjects provided written informed consent; for minor subjects, written informed consent was given by their legal guardian. This study was registered in the China Clinical Trial Registry (www.chictr.org.cn; No. ChiCTR2100052950), and the protocol is freely available from the website after registration.

Subject enrollment and questionnaire

Before and during enrollment, an introduction brochure or information booklet for the study was distributed to the community center staff, who were responsible for distributing and helping recruit community family members for onsite registration. A registration website was also open to community members for whole family-based registration; registration could not be carried out for only a single individual. A questionnaire was filled out either online or onsite by each of the participating family members. Blood samples were collected from each participating member for *H. pylori*, gastrin, and PG analyses; if necessary, the ^{13}C -urea breath test (UBT) was subsequently performed by appointment.

Based on the purpose of this study, the questionnaire included the following 17 items: age; sex; family ethic; number of family members; professions; marriage status; socioeconomic data; dining history; living habits; lifestyle; disease history; medication history; presence of GI symptoms; *H. pylori* eradication history; history of gastroscopy; infection history of other family members; and treatment history (Table 1).

***H. pylori* infection status, gastrin, and PG analyses**

Three milliliters of fasting venous blood were collected from all subjects in the morning. Blood samples were centrifuged at $1000 \times g$ for 10 min, (80-2 centrifuge; Jiangsu Zhongda Instrument Technology Co., Ltd., Jiangsu, China), and samples were either analyzed on ice on the same day or stored at -80°C for subsequent analyses. Serum anti-*H. pylori* antibodies (detecting CagA, VacA, UreA, UreB via *H. pylori* enzyme-linked immunosorbent assay (ELISA) kit [Blot Biotech Co., Ltd., Shenzhen, Guangdong, China]) and G-17, PGI, PGII levels, as well as PGR (via PGI, PGII, G-17 ELISA kits; Biohit Biotechnology, Helsinki, Finland) were measured by an ELISA kit following the manufacturer's instructions as previously reported^[22]. If patients had previously undergone *H. pylori* eradication therapy, an additional ^{13}C -UBT was performed to obtain their current infection status (^{13}C -UBT Diagnostic Kit; Beijing Boran Pharmaceutical Co., Ltd., Beijing, China).

Statistical analyses

Data were analyzed using SPSS for Windows version 25 (IBM Corp, Armonk, NY, United States). Continuous variables are expressed as the mean \pm standard deviation, whereas categorical variables are described as percentages or frequencies. The measurement data were compared by the *t*-test, and the enumeration data were compared by the χ^2 test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic information of the enrolled families

As shown in Table 1, a total of 282 families including 772 members participated in this study. Among them, 419 had *H. pylori* infection, giving an overall infection rate of 54.27% (419/772); among the infected individuals, 328 (42.49%, 328/772) were infected with type I strains and 91 (11.79%, 91/772) were infected with type II strains (Figure 1C). Type I strains accounted for 78.28% (328/419) of cases, and type II strains accounted for 21.7% (91/419) of infected individuals.

In total, 330 (42.75%) of the study participants were male, with an average age of 44.56 ± 20.19 years, and 442 (57.25%) were female, with an average age of 45.95 ± 18.74 years ($P > 0.05$). The age range of the enrolled individuals was 3 years to 90 years, with the youngest and oldest infected individuals aged 5 years and 87 years, respectively. As shown in Figure 2, the infection rates of individuals under the age of 18 were 23.26% (20/86), and the age groups of 51-60 and 61-70 years had the highest infection rates of 63.01% (92/146) and 65.95% (93/141), respectively. Compared with age groups under 18-years-old, the infection rate was significantly higher in groups above 18-years-old ($P < 0.05$), but there was no difference in infection rates among groups above 18-years-old ($P > 0.05$).

Among the questionnaire variables (Table 1), annual household income, history of smoking, history of alcohol consumption, dining location, presence of GI symptoms, and family history of gastric disease and GC did not affect infection rates ($P > 0.05$), but individuals with a higher education level showed significantly lower infection rates ($P < 0.05$).

H. pylori infection status of the enrolled families

The average family size of the study cohort was 2.74 persons per households, and the family size ranged from as few as 2 persons per family to as many as 6 per family (Figure 1A). In this survey, 2- and 3-person households accounted for 80.85% (228/282) of the families enrolled.

As shown in Figure 1B-F, *H. pylori*-infected individuals were distributed in 246 of the 282 families with varying numbers of members infected, ranging from only 1 person to all family members infected. The family infection rate was 87.23% with at least 1 person infected in a family unit (246/282). In 12.77% of the 282 households, no family members were infected (36/282) (Figure 1B), but in 67 of the 246 infected families, all members were infected (27.24%, 67/246). Among these 67 all member-infected households (Figure 1F), 46 households were infected with the same type I strains (68.66%, 46/67), 1 household was infected with type II strains (1.49%, 1/67), and 20 households had mixed type I and II strain infection (29.85%, 20/67). In 53.66% (132/246) of families with at least 2 members infected, 59.09% (78/132) of these families were infected with type I strains, 5.30% (7/132) were infected with type II strains, and 35.61% (47/132) were infected with mixed type I and II strains (Figure 1D).

***H. pylori* infection status between couples**

H. pylori infection status between couples is shown in Figure 3A-E. In all, 244 of the 282 families had both spouses, and the infection rate of both spouses was 34.84% (85/244); further, 17.21% (42/244) couples were not infected, and 47.95% (117/244) had only a single spouse infection (Figure 3A). Among the 117 families with infection of only 1 spouse, 75.21% (88/117) were infected with type I strains and 24.79% (29/117) were infected with type II strains (Figure 3B). Of these spouses, the husband was infected in 49.57% (58/117) of cases and the wife was infected in 50.53% (59/117) of cases ($P > 0.05$; Figure 3C). Furthermore, among the 85 families with both husband and wife co-infected with *H. pylori*, 68.24% (58/85) were infected with the same type of strain (Figure 3D), of whom 63.53% (54/85) were infected with type I strains, 4.71% (4/85) were infected with type II strains, and 31.76% (27/85) had mixed type I and type II infection. Significantly more couples were infected with the same type of strain than with mixed strains ($P < 0.05$). In addition, with the increase in marriage duration, the infection rate of both husband and wife was significantly increased ($r = 0.98$, $P < 0.05$; Figure 3E).

Parental infection and infection status of children and adolescents

In the 51 families with both parents and children younger than 18 years of age, as shown in Table 2, the infection rate of children was 23.08% (6/26) when both parents were *H. pylori*-infected; however, when both parents were not infected, the infection rate of children was 18.18% (2/11) ($P > 0.05$). When only the mother was infected, the infection rate of children was 45.45% (5/11); no child was infected (0/9) when only the father was infected ($P > 0.05$).

Table 3 shows the infection status of the 51 families comprising both parents and children, for a total of 190 individuals. Among these family members, the infection rates were as follows: father, 62.75% (32/51); mother, 62.75% (32/51); grandfather, 50.00% (2/4); grandmother, 66.67% (8/12); maternal grandfather, 25.00% (1/4); maternal grandmother, 37.50% (3/8); and other relatives, 66.67% (2/3).

Comparison of G-17, PGI, and PGII levels, and PGR with different types of *H. pylori* infection

To determine the impact of *H. pylori* infection on common GC epidemiological markers (e.g., G-17, PGI, PGII, and PGR) in healthy households, we assayed their levels during *H. pylori* infection. As shown in Table 4, compared to *H. pylori*-negative groups, PGI levels were significantly higher and PGR was significantly lower in *H. pylori* type I infections compared with *H. pylori*-negative groups ($P < 0.05$). However, there were no differences in G-17, PGII level, and PGR between type II *H. pylori* infection and *H. pylori*-negative groups ($P > 0.05$). The levels of G-17, PGI, and PGII were significantly higher, and PGR was significantly lower in *H. pylori*-infected groups than in *H. pylori*-negative groups ($P < 0.05$).

DISCUSSION

H. pylori infection rates vary greatly among different countries and regions^[25]. Although numerous studies have demonstrated that intrafamilial transmission is one of the most important sources of *H. pylori* spread^[2,19,26], there are few studies on the characteristics

and pattern of family-based *H. pylori* infection for disease prevention and control^[9,27,28]. Therefore, focusing on family-based *H. pylori* infection control and management would be a novel approach to reduce the related diseases and GC burden in a society.

In this work, we analyzed *H. pylori* infection status in a total of 772 individuals from 282 families in the Zhengzhou area. The results showed that despite an overall infection rate of only 54.27%, in as high as 87.23% of the surveyed families (246/282), there was at least 1 person infected, and in 27.07% (67/246) of these infected families, all family members were infected; further, 34.55% (85/246) of these families were infected with the same type of strain. Therefore, this study provides new evidence showing the importance of *H. pylori* infection control, which has substantive public health implications, and suggests that intrafamilial infection is a major source of *H. pylori* transmission. Thus, preventing intrafamilial spread is critical to eliminate the source of infection in order to reduce the development of related diseases.

Over the past several decades, the social and family structure in China has changed dramatically. The latest national statistics^[29] (2020) revealed that the nation has a population of 1.41 billion and 492 million families with an average family size of 2.62 persons/family, which is much smaller than it was in 1990, when China had 1.13 billion citizens and 278.6 million families, with an average family size of 4.05 persons/family^[29]. Due to the previous nationwide “one-children-per-family policy” (between 1982 and 2016), most families in China only have 1 child and two generations. As these children do not have siblings, transmission among siblings within a family unit did not appear to be the major route of transmission in the current analysis. *H. pylori* spread from parent or grandparent to children is probably more important for bacteria transmission. Although the current results provide a snapshot of *H. pylori* infection status in the general public, a nationwide large-scale investigation is needed to explore the nationwide infection status and develop policies for related disease prevention.

In single factor analysis, we noted that the highly infected age groups were between 31 years and 70 years, and infection rates increased along with age and duration of

marriage. Annual household income, history of smoking, history of alcohol consumption, dining location, and family history of gastric disease or GC were not different between the infected and non-infected groups, but individuals with a higher education level showed a lower infection rate. A 2020 all-ages population-based cross-sectional study^[30] in Wuwei county in northwestern China showed that the prevalence of *H. pylori* infection was closely associated with socioeconomic conditions, sanitary situations, dietary habits of the participants in the city, eating at school, and drinking untreated water; these were the main factors explaining the rising infection rate in junior-senior high school students. The results indicated that close contact is associated with increased infection risk. In addition, differences in geographic location, study population, lifestyle habit, and sanitary conditions are important factors that greatly contribute to *H. pylori* infection^[23,31,32].

Similar results were obtained from other regions, such as one community-based study^[33] in Vietnam in 2017 on familial clustering in a multiple-generation population. The study showed that high monthly income, not regularly being fed chewed food, and being breastfed were protective factors against *H. pylori* infection. Risk factors for *H. pylori* infection in children were not regularly handwashing after defecation, *H. pylori*-infected mother and grandfather, father's occupation, mother's education, and household size. Other factors such as number of siblings, infected fathers, regularly sharing a bed, group living, and antibiotic use were not found to be significant risk factors for infection.

H. pylori-infected family members are a possible source of continued transmission, which is an important health threat for uninfected family members^[7,34,35]. A 2003 study^[9] in the Stockholm area of Sweden using bacterial isolates for family-based DNA fingerprinting technique demonstrated a high proportion of shared strains among siblings, and between spouses, but also showed different strains in a portion of subjects (8%). Similar results were also reported by a 2009 study in Bangladesh^[27]. In the current analysis, we found that among the 67 all member-infected households (Figure 1F), 68.66% (46/67) were infected with *H. pylori* type I strains, 1.49% (1/67) were infected

with type II strains, and 29.85% (20/67) had mixed type I and II strain infection. These data support the notion that intrafamilial transmission is the primary transmission route, but exogenous infection outside the family can occur, indicating that there may be multiple sources of transmission. In 244 couples comprising both husband and wife, 34.84% (85/244) of them were co-infected, and 68.24% (58/85) of households were infected with the same strain. With an increase of marriage duration, the infection rate of both husband and wife was significantly increased, suggesting that there was also cross-infection between husband and wife.

One unexpected result was that when both parents were infected, the infection rate of children was 23.08% (6/21), whereas when both parents were not infected, the infection rate of children was 18.18% (2/11), although the difference did not reach statistical significance. This result was slightly different from our previous concept that parental *H. pylori* infection is an independent factor for infection in young children, and that mothers play an important role in *H. pylori* transmission to their descendent^[14-16,28]. However, the current results likely reflect the current infection status in this region, as the gradually improved living standard, sanitary condition, use of tap water, and avoiding chewing food to feed children over the past several decades in China's urban family have resulted in a reduced infection rate. This is in line with the fact that the overall *H. pylori* infection rate is declining in most of China's urban areas^[3,23]. Another possibility is that the current cohort had relatively small numbers of children and adolescents, which may not have generated enough power for statistical significance. In addition, we were unable to perform bacterial DNA fingerprinting to confirm if the strains were identical, so the genotype that precise bacterial strains transmit within a family unit has yet to be determined. Future large-scale investigations are needed to confirm the current conclusion.

Type I *H. pylori* strain accounted for 78.28% of the infected population in this survey, similar to the results of our previous study in patients admitted to the hospital, which showed that type I *H. pylori* infection accounted for 72.4% (291/402) of the infected patients, and type II was 27.6%^[22]. When compared to *H. pylori*-negative groups, G-17,

PGI, and PGII levels were higher in *H. pylori*-infected groups. G-17, PGI, and PGII levels were significantly higher and PGR was significantly lower in the type I *H. pylori*-infected groups than in *H. pylori*-negative groups. The levels of G-17 and PG II were significantly higher, and PGR was significantly lower in the type I *H. pylori*-infected groups than in the type II-infected groups. These results are in line with our previous endoscopy results from inpatients, and indicate that both type I and type II *H. pylori* strains increase G-17 Level, whereas only type I *H. pylori* infection affects PGI and PGII levels and the PGR in this geographic area^[22].

Type I infection and reduced PGR are risk factors for gastric mucosal precancerous lesions and GC^[20,22]. Therefore, these results have important clinical implications, as the abnormal expression of gastric markers was noted in a portion of individuals in healthy households infected with *H. pylori* before they sought medical examinations. It was unclear if this group of individuals had gastric mucosal precancerous lesions; thus, further examinations by endoscopy may be required for confirmation. The results of this study also provide another line of support showing that family-based *H. pylori* infection control and management may be an important strategy for infection control and related disease prevention^[3,19,36,37].

Although this pilot work provides novel information regarding family-based *H. pylori* infection status, it had some limitations. First, the investigation was performed with a relatively small number of families, and in some groups, especially children and adolescents, the number of samples was not large enough to reach statistical significance; thus, future large-scale, multiple region sampling would provide more convincing data. Second, this was a cross-sectional study without data from endoscopy to confirm *H. pylori* infection-related disease status and pathological changes in gastric mucosa; therefore, some in-depth information was missing and the work was performed in a Chinese setting, which may produce findings not applicable to other areas. Third, type I and II *H. pylori* genotype concordance through antibody array analysis only provided a very general evaluation. As we did not obtain bacteria strain culture and DNA fingerprinting data, *H. pylori* intrafamilial transmission was unable to

be evaluated precisely to assess the heterogeneity of *H. pylori* strains within families. Thus, future studies are needed to evaluate the *H. pylori* DNA fingerprinting pattern for more precise evaluation. Even with these limitations, this study provides novel points and information on family-based *H. pylori* infection characteristics, which merit further large-scale exploration.

CONCLUSION

The current results provide snapshots of family-based *H. pylori* infection status in central China. The high infection rate and coincidence of people infected with *H. pylori* within a family unit indicate the status and pattern of intrafamilial transmission, which provide a novel option for *H. pylori*-related disease prevention that requires further investigation and intervention. The concept is applicable not only to Chinese residents but also to other communities with high infection rates.

ARTICLE HIGHLIGHTS

Research background

Helicobacter pylori has characteristics of family cluster infection; however, its family-based infection status, related factors, and transmission pattern in central China have not been evaluated.

Research motivation

We evaluated family-based *H. pylori* infection status, related factors, and interfamilial transmission pattern in healthy households in central China, a high-risk area for *H. pylori* and gastric cancer (GC).

Research objectives

To investigate family-based *H. pylori* infection and identify a better approach for *H. pylori* infection control and related disease prevention.

Research methods

H. pylori infection was confirmed primarily by serum antibody arrays in 282 enrolled families, including a total of 772 family members. If patients previously underwent *H. pylori* eradication therapy, an additional ^{13}C -urea breath test was performed to obtain their current infection status. Serum levels of gastrin and pepsinogens (PGs) were also analyzed.

Research results

In our study sample from the general public of central China, the *H. pylori* infection rate was 54.27%. In 87.23% of healthy households, there was at least 1 *H. pylori*-infected person, and in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this geographic area. Among many variables, only individuals with a higher education level showed lower infection rates. *H. pylori* infection was also correlated with abnormal gastrin-17, PGI, and PGII levels and PGI/PGII ratio.

Research conclusions

H. pylori infection in healthy households is very common in central China, and poses an important health threat to uninfected family members. The intrafamilial infection status and patterns of transmission represent one important source of *H. pylori* spread, and indicate the urgent need for family-based infection control and related disease prevention.

Research perspectives

The results of this study provide new information on family-based *H. pylori* infection status in central China, and support the novel concept of family-based *H. pylori* infection control and management. This concept is also likely to benefit other *H. pylori* and GC prevalent areas.

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