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行政院衛生署九十一年度委託研究計畫

以碳-13 尿素呼氣試驗建立正確的兒童及青少年幽門螺旋桿菌感染流行病學資料

委 託 研 究 成 果 報 告

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目　　錄

中文摘要：

幽門螺旋桿菌(Hp)，已被認為和上消化道多項病理變化有關。由於研究顯示，大多數地區的 Hp 感染大多發生在孩童及青少年時期，且年輕時候得到 Hp 感染會顯著增加成人時得到胃癌的機率，因此，孩童時期 Hp 感染準確的流行病學資料包括盛行率、感染途徑，受感染年齡及新感染獲得率等，就成為世界各國衛生保健及疾病預防非常重要的項目之一。雖然現今以孩童為對象之流行病學研究，仍大多以血清學檢查為主，但是血清學檢查用於偵測孩童及青少年 Hp 感染的準確度則尚未經過確認。我們發現對年齡較小的國小兒童，血清學檢查的誤差很大，會引起對以往相關流行病學資料準確性的懷疑。

本研究目的在於：1) 同時以碳 13 呼氣試驗及血清學來檢測與比較孩童及青少年 Hp 感染。前瞻性逐年調查及確認國內國小及國中 (7 至 15 歲) 之孩童及青少年 Hp 感染"真正的"盛行率；2) 探討 Hp 感染在台灣地區傳播(transmission)之相關因子，做為國內制訂公共衛生政策及傳染病防治工作方針的參考。

我們選定羅東地區之一國小及國中為研究範圍。對每一例學生均以班級為單位，排定時間接受抽血及碳 13 尿素呼氣試驗。全部對象在四週內完成血液收集與編號及呼氣試驗。每一對象均被要求留下姓名、性別、學號、家庭住址、及電話，以便後續追蹤、訪談之用。每位參與研究學生之家長，均被要求填寫一份針對 Hp 感染流行病學相關之問卷，之後加以回收做統計分析。對收集之血液做血清學檢測，而收集之碳 13 尿素呼氣樣品則以質譜儀做 $^{13}C/^{12}C$ 之比值測定。將所得有關血清學檢驗及碳 13 呼氣試驗之結果，與問卷調查所得之流行病學相關資料，輸入統計研究所之電腦中，以 SAS system 分析之。針對有消化不適等症狀，且 Hp 陽性的學童及家屬，鼓勵其接受胃鏡檢查，在獲得同意的情況下，收集學童及家屬的組織檢體做 Hp 菌株的培養，將培養出來的 Hp 菌株做分子生物學比對 (RFLP 及 RAPD geno-typing)，以探討 Hp 傳播的來源。

總共收錄國小（一至六年級）共 780 人，國中（一至三年級）共 629 人及老師 150 人完成本研究。血清學檢查在各年齡學童的靈敏度由 7 歲至 15 歲分別為 33, 41, 50, 59, 68, 63, 65, 66, 及 70%，而老師則可高至 90%。若以血清學來篩檢，則各年齡學童感染 Hp 的盛行率為 5.5, 8.6, 6.8, 11.8, 12.3, 15.3, 11.9, 14.5 及 15.2%，而老師為 58.7%。若以碳-13 呼氣檢查為金標準來校正血清學的誤差，則真正感染 Hp 的盛行率便提高為 13.6, 14.5, 13.6, 16.7, 17.9, 18.8, 16.4, 20.4 及 20.7%，而老師組則維持

在 57.3%。此結果顯示血清學檢查在小孩群體的靈敏度是不夠的，會導致一些流行病學資料的誤差。若要得到較正確的資料，應該使用碳 13 呼氣試驗做進一步的確認。此外，呼氣檢查時 T0 基礎值一定要收集和分析，若只用 T30 的呼氣值來做判斷，會造成 5%的誤差。再者，和 Hp 感染傳播的正相關因子有年齡大小及家中共同生活的小孩數，然而有較多比例的傳染可能來自社區或學校。

中文關鍵詞(至少三個)：幽門螺旋桿菌、碳-13尿素呼氣試驗、血清學、流行病學

Abstract

Because *H. pylori* infection is contracted primarily in childhood, epidemiological studies among pediatric populations are imperative. Serologic immunoassays based on *H. pylori* antigens require validation in the pediatric population under evaluation. The aims of this prospective study are: (1) to compare the suitability of serological test with ^{13}C-urea breath test as a epidemiological screening tool in children and adolescents; (2) to investigate the "true" prevalence rate of *H. pylori* infection in the population whose ages between six and fifteen; (3) to explore the risk factor of transmission of *H. pylori* infection in Taiwan. The study population included 780 students of one primary school, 629 students of one junior high school and 150 teachers. Blood samples were collected from each student and teacher for the serological test. ^{13}C-urea breath test was adopted as gold standard. Result: The sensitivity of serology in the students with age of 7, 8, 9, 10, 11, 12, 13, 14 and 15, were 33, 41, 50, 59, 68, 63, 65, 66, and 70%, respectively, while this value in the teachers were 90%. The "crude" prevalence was 5.5, 8.6, 6.8, 11.8, 12.3, 15.3, 11.9, 14.5, and 15.2% in each age group of students and 58.7% in the teachers. However, after corrected by the data of ^{13}C-urea breath test, the "true" prevalence raised to 13.6, 14.5, 13.6, 16.7, 17.9, 18.8, 16.4, 20.4, and 20.7% in each age group of students. The reference value in the teachers was 57.3%. The mean δ^{13}C value of baseline measurement of adults was significant lower than that of children (-24.6±1.3 vs. –20.9±1.2; $p<0.01$). This is probably because the Taiwanese children tend to consume more meats, egg, cane sugar, and corn products. If only a single 30-min sample was adopted to determine the *H. pylori* status, a further 5% false-positive for children and 1% false-negative for adults results would occur. When logistic regression analysis was applied on some variables for the serology-based prevalence of *H.*

*pylor*i infection in children, age and number of children living together were two significant positive coefficients at 5% level. In siblings group, 1 of 3 (33.3%) had identical strains; 2 of 3 (66.6%) had non-identical strain within family. In parent-offspring group, 2 of 6 (33.3%) had identical strains; 4 of 6 (66.7%) had non-identical strain. Conclusion: It is concluded that the serological test is not sensitive enough as an epidemiological screening tool for *H. pylori* infection in children. The baseline measurement in ^{13}C-UBT for detection of *H. pylori* infection should not be omitted. Age and number of children living together may be two significant positive coefficients. The major transmission route within family might be community-acquired, but intrafamiliar spreading of *H. pylori* infection would also play a role.

Keyword: *Helicobacter pylori*, ^{13}C-urea breath test, serology, epidemiology

Introduction

Helicobacter pylori (*H. pylori*), a spiral microaerophilic Gram-negative bacterium isolated in 1983 [1], is now known as the most common gastrointestinal bacterial infection worldwide. It is the principal cause of chronic gastritis [2] and is strongly associated with peptic ulcer disease [3] as well as gastric lymphoma (MALT type) [4], and gastric cancer [5-6]. In developed countries, infection occurs in more than 50% of adults, whereas developing countries have infection rates reaching 90% [7-8]. Among those with *H. pylori* infection, eradication therapy alters the natural history of recurrences with attendant morbidity and death, which previously required lifelong maintenance therapy. Perhaps the greatest concern with regard to infection with *H. pylori* is the increased risk for the development of gastric cancers in adulthood. This is particularly relevant because infection dating from childhood appears to enhance the risk of carcinogenesis [5, 9-10]. Because *H. pylori* infection is contracted primarily during the childhood years, additional epidemiological studies among pediatric populations are imperative [11].

Two categories of diagnostic methods for *H. pylori* infection are distinguished: invasive tests to detect the microorganisms in a biopsied samples of the gastric mucosa obtained at endoscopy [12-15], and noninvasive tests to obviate the need for endoscopy[16-21]. These diagnostic tests have been applied to diagnose *H. pylori* infection in adults. Deep sedation or even general anesthesia is sometimes required for endoscopy in children, while this procedure remains valuable in pediatric patients with symptoms suggesting peptic ulcer. Noninvasive tests, such as urea breath test (UBT), have been proved to be equally accurate in diagnosing *H. pylori* infection in children. The validation of an inexpensive,

easy-to perform, sensitive, specific, and noninvasive diagnostic test for *H. pylori* infection in children and adolescents is of paramount importance to enhance our presently limited understanding of *H. pylori*-related diseases. Currently available tests for *H. pylori* infection in children may be not optimal tools for use in large-scale epidemiological research. Serologic immunoassays based on *H. pylori* antigens require validation in the pediatric population under evaluation [23-27].

The aims of this prospective study were: (1) to compare the suitability of serological test with ^{13}C-urea breath test as a epidemiological screening tool in children and adolescents; (2) to investigate the "true" prevalence rate of *H. pylori* infection in the population whose ages between six and fifteen; (3) to explore the risk factor of transmission of *H. pylori* infection in Taiwan.

Materials and Methods

Study population and study design

The study population included students of one primary school and one junior high school in Lo-Tong area. The teachers of these two schools were recruited as adult control. Participation in this study was voluntary. Informed consent of parents was obtained in each case. Blood sample was collected from each student for the serological test. ^{13}C-urea breath test was performed in each student, too. The parents of students were asked to answer a questionnaire concerning the basic epidemiological data and possible factors related to the transmission of *H. pylori*. All of these results were analyzed by a SAS system.

Data collection

Self-administered questionnaire

The parents of the children were asked to fill out a standardized questionnaire, which contained questions about sociodemographic factors, housing and living conditions, and other factors that was suspected to be potentially related to *H. pylori* infection.

Serology test

HEL-p II test kit (Amrad, Boronia, Victoria, Australia) for determination of *H. pylori* IgG antibody was used in this study. The HEL-p II test is an indirect ELISA immuno-assay involving four separate steps. Initially, test serum or plasma is diluted in specimen diluent buffer and allowed to react with *Helicobacter pylori* antigen bound to the microtitre well. Removal of the unreacted antibodies by washing allows the specifically bound antibody to be detected by an enzymatic method. Conjugated sheep anti-human IgG-HRPO reacts with this bound patient IgG. Unreacted conjugate is removed with a

subsequent washing step. Tetramethylbenzidine (TMB) substrate is converted enzymatically to a blue color with the rate of conversion of this substrate from colorless to a blue color being proportional to the amount of specific antibody bound. H_2SO_4 is used to terminate the enzymatic reaction converting the blue to a yellow color which is measured spectrophotometrically. According to the manufactory's statement, the sensitivity of the HEL-p II test is 96% and the specificity is 93%.

^{13}C-urea breath test

^{13}C-urea breath test modified from European standard protocol was performed in each case. Briefly, a baseline sample of expired breath in a 20 ml vacutainer was obtained by using a disposable plastic straw. Patients then drank 100 ml milk intended to delay gastric emptying. After 10 minutes, 100 mg of ^{13}C-urea (99% pure, Isotech, USA) in 50 ml of tap water was swallowed and distributed within the stomach by turning the patient to the left then, the right decubitus position. One point breath sample was collected, 30 min post ingesting the ^{13}C-urea, in an identical manner to the baseline sample. All samples were taken in duplicate and sent to INER where an isotope ratio mass spectrometer (Bureau of Stable Isotope Analysis Ltd. England) was used for analysis. The technician performing the analysis was unaware of the results or the status of *H. pylori* in the patients. The results were expressed as excess δ $^{13}CO_2$ excretion per mil by subtracting the baseline pre-^{13}C-urea breath sample result. The positive breath test was defined as excess δ $^{13}CO_2 >$ 5 per mil. The ^{13}C-UBT was adopted as gold standard in this study for the evaluation of the "true" prevalence.

Totally, 663 adults (age 20~80) with dyspepsia in other studies and 1082 children (age 7~15) in this epidemiological study were enrolled for the evaluation of the clinical importance of the baseline measurement of ^{13}C-UBT.

The $\delta^{13}C$ values of common Taiwanese foodstuffs were determined by continuous-flow isotope ratio mass spectrometry. In brief, the tin capsules of food samples were sealed into sphere shape and loaded into ANCA-NT system (Europa Scientific, UK). $\delta^{13}C_{PDB}$ values were measured against a laboratory's own reference material (wheat flour, $\delta^{13}C_{PDB}$ = -22.5 per mil), which was calibrated against an international Standard (IAEA stable Isotope Reference Material Code IAEA-CH-6, sucrose, $\delta^{13}C_{PDB}$ = -10.4 per mil).

Endoscopy

The members from the families of these index patients were screened by UBT first. The positive subjects, reflecting active *H. pylori* infection, were advised to receive further panendoscopic examination. Among them, forty-two members with positive UBT agreed to receive endoscopic biopsy. Members of these families, including siblings, parents, offspring and spouses received UBT. All of these them had positive *H. pylori* infection confirmed by culture and histology for samples from endoscopic biopsy of stomach. Each member of the same family was performed with different endoscope and biopsy forcep to avoid possible cross contamination. The endoscopes were disinfected with an automatic washing machine (Endo Thermo Disinfector, Olympus Co., Tokyo, Japan). The biopsy forceps were sterilized with ethylene oxide and disinfected as described above.

PCR-RFLP fingerprinting of chromosomal DNA

The oligonucleotides used for PCR amplification of specific fragments were 5'-AGGAGAATGAGATGA (forward) and 5'-ACTTTATTGGCTGGT (reverse) for a 2.4-kb *ureA-ureB* fragment. PCR reaction mixture contained approximately 0.1ug of gnomic DNA, 2units of Taq polymerase(Qiagen), 20 pmole of a pair of primers and 10mM of dNTPs(Pharmasia) in 50ul. PCR

was perfomed by 94°C for 4 min to initiate denaturation and followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C, 1 min. Following PCR, 10ul aliquots were removed and subjected to restriction digest with Mspl (for Msp1 polymorphism), HaeIII (for HaeIII polymorphism) (restriction enzyme from BM). After amplification the DNA products were ethanol precipitated and resuspended in 25 μl of sterile distilled water, and 3 μl of product was electrophoresed on a 1% agarose gel to ensure homogeneity and to assess the yield. The gel was stained with ethidium bromide then examined by transillumination and photographed.

RAPD

The PCR reaction mixture contained approximately 10ng of genomic DNA, 3mM $MgCl_2$, 1 unit of Taq polymerase(QIAGEN), 20pmole of primer and 200uM of dNTPs(Pharmasia) in 25ul. PCR was performed by 94°C for 4 min to initiate denaturation and follored by 45 cycles of denaturation at 94C ^{o} for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. Following PCR, 10ul aliquotes were removed and electro[horesed on 2.5% agarose (FMC, Rockland, LE) gels for 1 hour and following ethidium bromide staining. The gels were photographied by using ultraviolet light transilluminator.

Statistical analyses

In this study, statistical analyses were performed using on SAS system. The stepwise logistic regression analysis was performed with various items affecting the *H. pylori* infection.

Results

Totally, 780 students of the primary school (LT), 629 students of one junior high school (TK) and 150 teachers were enrolled into this study. The variation of sensitivity of serology and the prevalence of *H. pylori* in the different age of students and teachers are summarized in the table 1. The sensitivity of serology in the students with age of 7, 8, 9, 10, 11, 12, 13, 14 and 15, were 33, 41, 50, 59, 68, 63, 65, 66, and 70%, respectively, while this value in the teachers were 90%.

The "crude" prevalence was 5.5, 8.6, 6.8, 11.8, 12.3, 15.3, 11.9, 14.5, and 15.2% in each age group of students and 58.7% in the teachers. However, after corrected by the data of ^{13}C-urea breath test, the "true" prevalence raised to 13.6, 14.5, 13.6, 16.7, 17.9, 18.8, 16.4, 20.4, and 20.7% in each age group of students. The reference value in the teachers was 57.3%.（Table 2）

The Taiwanese food could be divided into two groups: the higher δ^{13}C value group (range from –10.4 to –20.6 per mil), and the lower δ^{13}C value group (range from –22.3 to –31.5 per mil). The mean δ^{13}C value of baseline measurement of adults was significant lower than that of children (-24.6±1.3 vs. –20.9±1.2; $p<0.01$). This is probably because the Taiwanese children tend to consume more meats, egg, cane sugar, and corn products. If only a single 30-min sample was adopted to determine the *H. pylori* status, a further 5% false-positive for children and 1% false-negative for adults results would occur.

When logistic regression analysis was applied on some variables for the serology-based prevalence of *H. pylor*i infection in children, age and number of children living together were two significant positive coefficients at 5% level. However, age was not a significant positive coefficient for the

UBT-based prevalence rates in this study, because the UBT-based prevalence rates reached a much higher level than the serology-based prevalence in the age groups of 7~9.

Five students with *H. pylori* infection were enrolled as index patients. Nine family members in these 5 families with positive UBT and culture of *H. pylori* were enrolled. Totally, the number of relationships of index patient with each family were 3 pairs of siblings, and 6 pairs of parent-offspring.

The results of *H. pylori* strain typing are shown on the Table 5. In siblings group, 1 of 3 (33.3%) had identical strains; 2 of 3 (66.6%) had non-identical strain within family. In parent-offspring group, 2 of 6 (33.3%) had identical strains; 4 of 6 (66.7%) had non-identical strain. In overall, 3 of 9 (33.3%) were identical; 6 of 9 (66.7%) were non-identical strains.

Discussion

At present, precise details concerning the bacterial, host, and environmental factors that lead to the development of disease complications are lacking. Because *H. pylori* infection is contracted primarily during the childhood years, additional epidemiologic studies among pediatric populations are imperative. To achieve this, accurate diagnosis of *H. pylori* infection in children is essential. At present, diagnosis of *H. pylori* infection in children still largely depends on the endoscopic biopsy of the gastric tissues for culture and urease test. These methods are previously regarded as the gold standard. However, the invasive nature limits its wide use in children, especially for the young children and infants. This invasive method has made it impossible to perform proper epidemiologic studies in this important population. The availability of a safe, valid, noninvasive test in children is essential if the epidemiology of *H. pylori* is to be properly evaluated. Noninvasive diagnostic tests, including ^{13}C-UBT and serology, were recently developed and shown to be promising in establishing the diagnosis of *H. pylori* infection in children.

Serologic immunoassays based on *H. pylori* antigens require validation in the pediatric population under evaluation because cutoff values established in adult subjects are often higher than antibody levels present in infected children [31]. In addition, commercially available serologic tests demonstrate lower accuracy compared with testing in the research setting [32-33]. Most commercially available IgG antibody to *H. pylori* kits performed equally. It must be used carefully as a test for diagnosis and therapeutic monitoring in children. Probably due to the duration of infection and the difference in immunity and bacterial load, the antibody levels in children are different from the adults. Moreover, spontaneous clearance of *H. pylori* may occur in some children with persistent antibody, thus results in false positive serological

tests.

In adults the carbon 13-labeled urea breath test has been shown, initially by Graham et al. [18] and subsequently by other groups, to be a safe and reliable method for the diagnosis of *H. pylori* infection [19]. Rowland et al. reported the sensitivity and specificity of ^{13}C-UBT may be as high as 100% and 97.6% if the subjects were fasting [30]. ^{13}C-UBT is of good diagnostic accuracy in children. It offers a feasible way for diagnosing *H. pylori* infection, screening of asymptomatic population, and monitoring the therapeutic effects in children who can successfully follow the procedure of ^{13}C-UBT.

Currently, serological test is the most popular epidemiological screening tool in adult. However, it must be used with caution in the childhood years. In this study, we found the sensitivity of the serological test became decreasing to only about 30 ~ 50% in the young children which age between 7 ~ 10.

The natural ^{13}C abundance of food is not constant, this difference in different food is determined by the isotopic fractioning of one of the two major biochemical pathway that can be used by plants for photosynthesis. Studies in animals and humans have shown that protracted use of ^{13}C-enriched food increases ^{13}C abundances in the body. Because there is a natural background of $^{13}CO_2$, the labeled CO_2 is actually the amount of $^{13}CO_2$ in excess of the $^{13}CO_2$ abundance before the labeled substrate was administered. The test signal is therefore the difference between the initial abundance of ^{13}C and that after ingestion of labeled substrate.

Some investigators have suggested that the *H. pylori* infective status can be determined from a single 30-min post-dose breath sample of ^{13}C-UBT. However, our study showed the baseline measurement in ^{13}C-UBT for detection of *H. pylori* infection should not be omitted. There is variation in

mean $\delta^{13}C$ value of baseline measurement in different age group and geographic area. These values may also be used as a dietary guideline for patients in the prevention of various diseases.

In our study, high strain diversity of *H. pylori* infection was noted in our society. The higher diversity of *H. pylori* strains in the siblings might imply that the major transmission route within family might be community-acquired. While, two identical strains in the siblings of one family might mean that intrafamiliar spreading is also possible besides the major mode of community-acquisition. This possibility will need to be investigated with more cases under well-designed study. But, each sibling might also get infected from one of his (or her) parents, respectively, which caused diversity between them. In order to solve this question, the results of parent-offspring group with two parents and two children may provide more valuable clues.

Two of 6 parent-offspring group had identical strain. This was also another indirect evidence to speculate that intrafamiliar spreading of *H. pylori* infection would also play a role. However, to investigate the *H. pylori* strains of both of their mother and father simultaneously will be important to answer more exactly. For example, a daughter having identical *H. pylori* strain as father but different to her mother in one family was noted in this study.

It is concluded that the serological test is not sensitive enough as an epidemiological screening tool for *H. pylori* infection in children. The baseline measurement in ^{13}C-UBT for detection of *H. pylori* infection should not be omitted. Age and number of children living together may be two significant positive coefficients. The major transmission route within family might be community-acquired, but intrafamiliar spreading of *H. pylori* infection would also play a role.

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Table 1. Variation of sensitivity of serology and the prevalence of *H. pylori* in the different age of students and teachers:

Student age	7	8	9	10	11	12	13	14	15	Teacher
Total number (T)	110	186	132	102	106	144	312	172	145	150
Positive (P)	5	11	8	10	13	17	33	23	21	77
Negative (N)	94	154	114	82	87	112	257	135	114	53
False positive (FP)	1	5	1	2	0	5	4	2	1	11
False negative (FN)	10	16	9	7	6	10	18	12	9	9
Sensitivity (%)	33.3	40.7	50.0	58.5	68.4	63.0	64.7	65.7	70.0	89.5

Table 2. Prevalence rates of *H. pylori* infection in the different age groups of students and teachers

Student age	7	8	9	10	11	12	13	14	15	Teacher
Prevalence 1 (%)*	5.5	8.6	6.8	11.8	12.3	15.3	11.9	14.5	15.2	58.7
Prevalence 2 (%)†	13.6	14.5	13.6	16.7	17.9	18.8	16.4	20.4	20.7	57.3

* prevalence 1 (P+FP/T) means the result is base on serological data only
† prevalence 2 (P+FN/T) means the result is corrected by the data of ^{13}C-urea breath test.

Table 3. $\delta^{13}C_{PDB}$ Values of Common Taiwanese Food

High ^{13}C abundance food		Low ^{13}C abundance food	
	$\delta^{13}C_{PDB}$(mean±SD(n))		$\delta^{13}C_{PDB}$(mean±SD(n))
Meats		Vegetables	
Pork	-16.2±0.2(3)	Cabbage	-25.0±0.3(3)
Chicken	-18.7±0.4(3)	Sweet potato	-25.2±0.1(3)
Beef	-12.2±0.2(3)	Carrot	-23.9±0.4(3)
Seafood		Soy bean	-25.5±0.1(3)
Shrimp	-16.4±0.1(3)	Cauliflower	-23.7±0.5(3)
Sardine	-17.0±0.1(3)	Grains and cereals	
Salmon	-16.8±0.1(3)	Rice	-26.8±0.4(3)
Mackerel	-17.6±0.1(4)	Wheat flour	-24.1±0.2(3)
Goldline fish	-20.6±0.5(3)	Black sesame	-27.3±0.5(3)
Egg		Oat flour	-22.5±0.2(3)
Yolk powder	-17.1±0.2(3)	Fruits	
Whole egg powder	-17.3±0.1(3)	Papaya	-24.7±0.2(3)
Cane sugar	-10.5±0.1(3)	Orange	-25.0±0.2(3)
Pine apple	-12.4±0.1(5)	Grape	-22.3±0.2(3)
Non fat dry milk	-20.3±0.1(3)	Guava	-27.0±0.6(3)
Whole cow milk	-17.7±0.3(3)	Olive oil	-29.3±0.1(4)
Corn flour	-10.4±0.0(4)	Soy oil	-31.5±0.3(3)
Corn oil	-18.9±0.1(4)		

Table 4. Distribution of $^{13}CO_2/^{12}CO_2$ isotope ratios in baseline samples of breath for the children and adults

Baseline ($\delta^{13}CO_2$)	Children	Adults
-29	0	0
-28	0	7
-27	0	19
-26	0	53
-25	1	151
-24	3	230
-23	22	135
-22	158	51
-21	301	12
-20	334	4
-19	192	0
-18	55	0
-17	10	0
-16	5	1
-15	1	0
-14	0	0
N:	1082	663
Average:	-20.9	-24.6
S.D.	1.2	1.3

Table 5 The results of DNA typing of *Helicobacter pylori* in 9 pairs of 5 families

	Identical pattern		Non-identical pattern	
Siblings	1/3	(33.3%)	2/3	(66.7%)
Parent-offspring	2/6	(33.3%)	4/6	(66.7%)
Total	3/9	(33.3%)	6/9	(66.7%)