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Helicobacter pylori Seropositivity among 963 Japanese Brazilians According to Sex, Age, Generation, and Lifestyle Factors

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Seropositivity of anti-*Helicobacter pylori* antibody (*HP*+) was examined among Japanese Brazilians. The study was announced through 18 Japanese community culture associations in São Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis in 2001. Among 969 participants, 963 individuals aged 33–69 years were analyzed. The overall *HP*+% was 48.1% (95% confidence interval, 44.9–51.3%). There was no difference in *HP*+% between 399 males and 564 females (49.6% and 47.0%, respectively). The *HP*+% increased with age; 35.3% for those aged 33–39 years, 46.2% for those aged 40–49 years, 46.5% for those aged 50–59 years, and 56.9% for those aged 60–69 years, but no differences were observed among the generations (Issei, Nisei, and Sansei) for each 10-year age group. Mogi das Cruzes, a rural area, showed a higher *HP*+%. Length of education was inversely associated with the positivity; the odds ratio (OR) relative to those with eight years or less of schooling was 0.61 (0.42–0.89) for those with 12 years or more. The associations with smoking and alcohol drinking were not significant. Fruit intake was associated with the *HP*+%; the OR relative to everyday intake was 1.38 (1.05–1.83) for less frequent intake, while intake frequencies of green tea, miso soup, and pickled vegetables (tsukemono) were not. Multivariate analysis including sex, 10-year age group, residence, education, and fruit intake showed that all factors except sex were significant. This is the largest study of *HP* infection among Japanese Brazilians, and the results indicated a similar pattern of age-specific infection rate to that for Japanese in Japan.

Key words: *Helicobacter pylori* — Seropositivity — Japanese Brazilians — Lifestyle factors

Since *Helicobacter pylori* (*HP*) was identified in 1982, the bacterium has been widely accepted as a pathogen related to digestive ulcer, atrophic gastritis, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[1–4] *HP* infection occurs worldwide, and the prevalence is higher in developing countries than developed countries.[5,6] It is thought that improved social and sanitary conditions prevent person-to-person transmission, though no clear evidence exists as to which route of infection or source of *HP* predominates.[6,7] Several studies indicated that crowded living conditions, family size, and sharing a bedroom predispose to *HP* infection.[8] Most investigators believe that *HP* is transmitted directly by fecal-oral and/or oral-oral route.[5–7] An increase in the seropositivity with age was observed in developed countries, and this is considered due to the birth cohort effect, in that the older generations were under poorer sanitary conditions during their childhood.[9]

Brazil has the world's largest population of Japanese outside Japan. The first group of 781 Japanese immigrants arrived in Brazil in 1908. According to a report by the Japanese Ministry of Foreign Affairs, the number of permanent residents of Japanese nationality in Brazil was 83 803 in 1998 and the population of Japanese descendants is estimated to be 1.3 million. Most Japanese Brazilians belong to Japanese communities, which preserve Japanese language and culture through many activities such as Japanese language school for children, Japanese folk dance parties (bon-odori), culinary and music festivals, and sport competitions (undo-kai). The communities also play an important role in health promotion through health checkup and education.

A previous study on Japanese Brazilians reported that the *HP* infection rate evaluated by means of a serological test among those aged 40–59 years in São Paulo city was 76.8% (129/168), 71.6% for 81 males and 81.7% for 87 females.[10] The comparison between men aged 40–49 years from São Paulo and from five areas (Ninohe, Yokote, Saku, Katsushika, and Ishikawa) in Japan revealed

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that the persistent *HP* infection rates were similar.[10] The present study aimed to determine the *HP* infection rate more precisely according to sex, age, and generation, for Brazilians of full Japanese ancestry, and to examine the associations of the infection with lifestyle factors such as smoking, alcohol drinking, and dietary habits. A serological test was used in this study, as adopted in other studies.

SUBJECTS AND METHODS

Study subjects Subjects were apparently healthy adult Japanese Brazilians, who voluntarily participated in this study, from four different cities, São Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis. Those with a history of disease such as ulcer and stomach cancer were not excluded. In São Paulo, about 350 000 Japanese descendants reside, and 47 associations named for each prefecture of Japan (Kenjin-kai), Japanese co-operative societies, country clubs of Japanese Brazilians, and many other non-profit Japanese associations have been founded. The city of Mogi das Cruzes is 70 km northeast from São Paulo city, and contains a population of Japanese Brazilians estimated at 6000 families, who live by growing vegetables. The total population of Mirandopolis is 25 000 and Japanese Brazilians account for about 2000. Cattle farming is common in this region. Curitiba is the capital city of Parana state, an urban area, where approximately 12 000 Japanese Brazilian families reside.

With the approval of association directors, Japanese Brazilians aged 35 to 69 years were identified from the lists of 18 Japanese Brazilian associations (12 in São Paulo, 4 in Curitiba, 1 in Mogi das Cruzes, and 1 in Mirandopolis). In São Paulo, where more than 100 associations exist, 12 associations that responded in the predetermined period were selected. The members of these associations were invited to participate in February to March 2001 through a standardized letter informing them of the study aims, procedure, and confidentiality. In Mogi das Cruzes, the study was also announced by a local newspaper. After written informed consent had been obtained, lifestyle data and blood sample were taken in rooms of each association on the occasions of festivals and sports competitions in March to May 2001.

The applicants numbered 662 in São Paulo city, 90 in Curitiba city, 110 in Mogi das Cruzes city, and 107 in Mirandopolis city. Six applicants aged less than 30 years or more than 69 years were excluded from the analysis, but 9 applicants aged 33–34 years were included. The remaining 963 subjects (399 males and 564 females) of full Japanese ancestry were selected for the analysis.

Anti-*HP* antibody test A 10 ml sample of venous blood was obtained from each participant. The blood samples were centrifuged and frozen at −20°C according to an identical protocol. An anti-*HP* IgG antibody test, High-Molecular-weight Campylobacter-Associated-Protein (HM-CAP) ELISA ("Detaminor *H. pylori* antibody," Enteric Products, Inc., Westbury, NY) was used for the identification of *HP*-infected participants.[11, 12] The test was conducted at SRL Co., Ltd. in Japan, where routine measurement of the IgG antibody has been established. A value of 2.3 EV (ELISA Value) or over was regarded as *HP* infection-positive.

Lifestyle data Information on lifestyle factors was obtained using a self-administered questionnaire checked by interviewers. The questionnaire included items on demographic characteristics, familial origin by prefecture, educational background, personal and family history, habitual smoking and alcohol drinking, reproductive history, physical exercise and dietary habits. Food intake frequency was questioned using five categories; never, 1–3 times per month, 1–2 times per week, 3–4 times per week, and every day. The food items were rice, beans, bread/spagetti, potatoes, beef, chicken, fish, pork, bacon/sausage, salty meat/fish, egg, cheese/yogurt, raw vegetables, yellow vegetables, tomato, fruit, fruit juice, sweet, frozen food, spicy food, miso soup, pickled vegetables (tsukemono), and soybean food. Intake frequencies of milk, coffee and tea were questioned using four categories; never, occasionally, 3–4 times a week, and every day. Information on tea consumption was obtained in terms of the kind of tea (green, black, mate, and others).

Statistical analysis *HP* seropositivity was analyzed according to sex, age, residence and generation. The 95% confidence intervals (95%CI) of the percentage were calculated by assuming a binomial distribution. The antibody-positive rate was tested by a χ^2 test. Age-sex-adjusted odds ratio (OR) and 95%CI were calculated according to an unconditional logistic model. In the model, the association was examined for education coded as primary (≤7 years), secondary (8–11 years), and university (≥12 years); habitual smoking as never smokers, former smokers, and current smokers including those who quit in the past 1 year; alcohol drinking as non-drinkers, former drinkers, and current drinkers including those who quit within the past 1 year; and food frequencies as every day and less frequent. These calculations were conducted by the computer program STATA Version 7 (STATA Corp., College Station, TX).

RESULTS

The seropositive rates of anti-*HP* IgG antibody according to characteristics of the participants are shown in Table I. The overall rate was 48.1% (95%CI, 44.9–51.3%); 49.6% (44.6–54.6%) for men and 47.0% (42.8–51.2%) for women. The difference between the sexes was not significant (χ^2=0.65, *df*=1, *P*=0.420). Age was a strong predictor for *HP* infection (χ^2=13.98, *df*=3, *P*=0.003). A dif-

ference in seropositivity rate was observed among the four cities (χ^2=7.67, *df*=3, *P*=0.053). Mogi das Cruzes, a rural area, had the highest rate, 60.0%, while the rate was 45.9% for São Paulo, 50.0% for Curitiba, and 47.7% for Mirandopolis. There was no significant difference among the generations; 52.6% (42.2–62.8%) for the first generation (Issei), 48.2% (44.6–51.8%) for the second generation (Nisei), and 42.7% (33.0–52.8%) for the third generation (Sansei) (χ^2=1.98, *df*=2, *P*=0.372). Table II shows the comparisons among the generations according to 10-year age group. For the age group 40–49 years, the rate was the highest for Issei, and the lowest for Sansei, but the difference between any two of the three generations was not statistically significant. Though the rate for age group 60–69 years of Sansei was 75.0%, this included only 4 subjects.

Table III shows sex-age-adjusted OR of antibody positivity for lifestyle factors. The subjects with the highest education level (≥12 years) were found to have an OR of 0.61 (95%CI, 0.42–0.89). The OR was 1.26 (0.85–1.85) for former smokers and 1.14 (0.78–1.68) for current smokers. The analysis for males showed similar OR of smoking habits; 1.22 (0.84–1.77) and 1.14 (0.65–1.86), respectively. Alcohol consumption was not statistically significant for either sex or both combined. The OR of fruit intake relative to every day was 1.38 (1.05–1.81) for less frequent intake. Green tea, miso soup, pickled vegetables, as well as the food items not listed in Table III, were

Table I. Seropositive Rate of Anti-*Helicobacter pylori* IgG Antibody (*HP*+%) According to Characteristics of Study Subjects

	Subjects	% for the total	*HP*+% (95%CI)	*P* value
Sex				
Males (aged 33–69 years)	399	41.4	49.6 (44.6–54.6)	0.420
Females (aged 34–69 years)	564	58.1	47.0 (42.8–51.2)	(χ^2=0.65, *df*=1)
Age in years				
33–39	85	8.8	35.3 (25.2–46.4)	
40–49	275	28.5	46.2 (40.2–52.2)	0.003
50–59	355	36.9	46.5 (41.2–51.8)	(χ^2=13.98, *df*=3)
60–69	248	25.7	56.9 (50.4–63.1)	
Residence (city, state)				
São Paulo, São Paulo	656	68.1	45.9 (42.0–49.8)	
Curitiba, Parana	90	9.3	50.0 (39.2–60.7)	0.053
Mogi das Cruzes, São Paulo	110	11.4	60.0 (50.2–69.2)	(χ^2=7.67, *df*=3)
Mirandopolis, São Paulo	107	11.1	47.7 (37.9–57.5)	
Generation[a)]				
Issei	97	10.7	52.6 (42.2–62.8)	0.372
Nisei	763	79.2	48.2 (44.6–51.8)	(χ^2=1.98, *df*=2)
Sansei	103	10.7	42.7 (33.0–52.8)	
Total	963	100	48.1 (44.9–51.3)	

a) Issei, immigrant; Nisei, second generation; Sansei, third generation.

Table II. Seropositive Rate of Anti-*Helicobacter pylori* IgG Antibody (*HP*+%) According to Age and Generation

Age	Issei[a)]		Nisei[a)]		Sansei[a)]	
	n	*HP*+% (95%CI)	*n*	*HP*+% (95%CI)	*n*	*HP*+% (95%CI)
33–39	0	—	56	32.1 (20.3–45.9)	29	41.4 (23.5–61.0)
40–49	24	54.2 (32.8–74.4)	208	46.1 (39.2–53.1)	43	41.8 (27.0–57.8)
50–59	30	43.3 (25.4–65.5)	298	47.3 (41.5–53.1)	27	40.7 (22.4–61.2)
60–69	43	58.1 (42.1–72.9)	201	56.2 (49.0–63.1)	4	75.0 (19.4–99.3)

a) Issei, immigrant; Nisei, second generation; Sansei, third generation.

Table III. Age-sex-adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CIs) of Lifestyle Factors for *Helicobacter pylori* Seropositivity (*HP*+)

	n	*HP*+%	OR	95%CI
Education				
Primary (≤ 8 years)	237	56.1	1	Ref.
Secondary (9–11 years)	223	52.9	0.94	0.63–1.38
University (12≤ years)	503	42.1	0.61	0.42–0.89
Smoking				
Never	704	47.0	1	Ref.
Former	132	53.8	1.26	0.85–1.85
Current	127	48.8	1.14	0.78–1.68
Alcohol drinking				
Never	676	48.9	1	Ref.
Former	52	46.3	0.84	0.47–1.49
Current	232	46.1	0.89	0.64–1.23
Rice				
Every day	832	48.9	1	Ref.
Less frequent	131	42.7	0.78	0.54–1.14
Beef				
Every day	132	42.1	1	Ref.
Less frequent	831	48.9	1.26	0.86–1.83
Chicken				
Every day	56	48.2	1	Ref.
Less frequent	907	48.0	1.00	0.58–1.73
Fruit				
Every day	634	45.9	1	Ref.
Less frequent	329	52.2	1.38	1.05–1.81
Raw vegetables				
Every day	752	48.1	1	Ref.
Less frequent	211	47.9	0.99	0.73–1.36
Yellow vegetables				
Every day	450	49.3	1	Ref.
Less frequent	513	46.9	0.92	0.71–1.20
Fruit juice				
Every day	215	46.9	1	Ref.
Less frequent	748	48.4	1.06	0.78–1.45
Milk				
Every day	575	48.7	1	Ref.
Less frequent	388	47.1	0.98	0.75–1.28
Coffee				
Every day	667	49.0	1	Ref.
Less frequent	296	45.9	0.88	0.66–1.16
Green tea				
Every day	749	48.6	1	Ref.
Less frequent	214	46.2	0.85	0.63–1.17
Miso soup				
Every day	131	49.0	1	Ref.
Less frequent	832	48.0	1.09	0.75–1.60
Pickled vegetables				
Every day	74	47.3	1	Ref.
Less frequent	889	48.1	0.97	0.88–1.07

Table IV. Multivariate Analysis for Anti-*Helicobacter pylori* Positivity

	OR[a)]	95%CI[b)]
Sex		
Males	1	Ref.
Females	0.85	0.64–1.11
Age in years		
33–39	1	Ref.
40–49	1.64	0.98–2.73
50–59	1.47	0.88–2.44
60–69	2.02	1.16–3.54
Residence		
São Paulo	1	Ref.
Curitiba	1.20	0.76–1.88
Mogi das Cruzes	1.58	1.03–2.41
Mirandopolis	0.81	0.52–1.27
Education		
Primary	1	Ref.
Secondary	0.90	0.60–1.35
University	0.59	0.39–0.88
Fruit		
Every day	1	Ref.
Less frequent	1.39	1.05–1.83

a) Odds ratio adjusted for the listed five factors.
b) 95% confidence interval.

not associated with the *HP* antibody status. Only 3 participants ate salty meat/fish every day. The categorization of salty meat/fish intake frequency yielded no difference in seropositivity between the two groups.

The significant factors identified by univariate analysis, as well as sex and residence, were analyzed by using a multivariate logistic regression model (Table IV). Consistent with the univariate analysis, schooling longer than 12 years (OR=0.59, 0.39–0.88) and less frequent intake of fruit (OR=1.39, 1.05–1.83) were significantly associated with persistent *HP* infection. The age group of 60–69 years was found to have a two times higher risk than the age group of 33–39 years. The multivariate analysis showed an increase risk for Japanese Brazilians living in Mogi das Cruzes (OR=1.58, 1.03–2.41) relative to São Paulo, but not for the other rural area, Mirandopolis.

DISCUSSION

Japanese Brazilians reportedly had a similar mortality/incidence rate of stomach cancer to that for Japanese, which was about 50% higher than Brazilians as a whole. The standardized mortality rate of Japan-born Brazilians in

the city of São Paulo 1979–81 was 86 in males and 79 in females compared with Japanese, but 166 in males and 150 in females compared with all the São Paulo residents.[13] The incidence rate of stomach cancer for Japan-born Brazilians in São Paulo also showed a similar tendency.[14]

As described, the anti-*HP* IgG antibody-positive rate reported by Tsugane *et al.* was 76.8% (95%CI, 69.7–82.9) for 168 Japanese Brazilians in São Paulo aged 40–59 years.[10] Since the study was conducted in 1989, their birth year ranged between 1930 and 1949. No other studies have been reported for Japanese Brazilians to date. This study was a middle-sized study, which allowed us to estimate age-specific positive rates, revealing an increase in the rate with age. The rate for those born before 1950 in the present study was lower than that of the previous study.[10] The difference was probably due to the different population sampling. Since the participation rate could not be defined in this study and was 57% in the previous study,[10] precise comparison between the two studies is difficult. However, both indicated that the rate for Nisei was similar to that for Issei.

It is important to discuss whether or not the present study subjects reflected all Japanese Brazilians in the year 2001. Almost all Brazilians of full Japanese ancestry belong to at least one Japanese association. Although precise comparisons were not possible, the education level of the participants indicated that the sampled Japanese Brazilians were not far from the whole Japanese Brazilian population. Even if potential study subjects were sampled randomly, the participation rate might be far from 100%, as shown in the previous study.[10] This sampling framework seems to be a practical method for a large study with limited funding.

A study of *HP* seropositive rate in 418 asymptomatic Japanese in Sapporo in 1990 showed that those born before 1950 had a 70 to 80% infection rate, while the rate for those born after 1950 decreased with their year of birth.[15] A study of 4361 workers residing on the coast of Honshu island in 1996 showed that the seropositive individuals amounted to 21.6% for those aged 19–29 years, 29.5% for those aged 30–39 years, 46.0% for those aged 40–49 years, and 53.0% for those aged 50–59 years.[16] Another study of 1815 blood donors in Akita, Miyagi, Iwate, and Okinawa showed a slightly higher positive rate, but a similar tendency in term of age-specific positive rate.[17] A difference in the positive rate was reported among different areas in Japan,[18] but our study found Japanese Brazilians had a similar rate to those for Japanese in Japan reported in the largest study.[16] Their prefecture of origin was included in the questionnaire, but marked differences were not observed among the prefectures. This may be because the difference in the rate decreases from generation to generation.

The age-specific rate is important not only for *HP* infection control, but also for the prediction of stomach cancer incidence. A decreasing trend in the positive rate with birth year among Japanese Brazilians was analogous to that for Japanese in Japan[16, 17] and in Northern Ireland.[19] The pattern was different from that for Caucasians in the United States, showing a lower rate for the elderly, and that in developing countries, showing a higher rate from childhood. This is the first report that Japanese Brazilians have a similar decreasing tendency to Japanese in Japan. In Brazil, the children of a low socioeconomic level were reported to have a higher seropositive rate.[20] The prevalence of *HP* antibody was 61.8% among blood donors,[21] which was slightly higher than that observed for the present participants.

It was clearly demonstrated that age is a factor that determines the seropositive rate, but not the generation, as shown in Table II. There was no significant difference between any two of the positive rates according to age group among Issei, Nisei, and Sansei, and the trend among the three generations was not significant (e.g., $P=0.351$ for age group 40–49 years by a logistic model with a variable, 1 for Issei, 2 for Nissei, and 3 for Sansei). The results suggest that birth place did not affect the risk of infection. Japanese Brazilians have maintained Japanese culture, and their unchanged lifestyle may be one of the reasons why there is little difference in the infection rate among the generations.

This study indicated that education level is associated with the positive rate. Since education level is a surrogate index for income and social class, it has no etiological meaning. If the level were closely related to determinants such as sanitary conditions, it would show a strong association. Otherwise, weak or no association would be observed. For Japanese, an insignificant weak association was reported.[22]

Among the lifestyle factors, those who ate fruit every day had a significantly reduced risk of seropositivity. A significant association was reported by some authors,[23, 24] though studies on Japanese found no association.[22, 25] A study of *interleukin 1B C-31T* polymorphism for outpatients showed that the association between *HP* infection and the polymorphism was modified by fruit intake frequency, though the effect was not significant.[26] Further studies with biological markers should reveal the role of fruit intake in the biological mechanism of *HP* infection.

In this study, smoking was not significantly associated with the seropositive rate, though the estimated OR was more than unity. Past studies on the association with smoking are inconsistent.[5] However, a logical explanation for the inconsistency may be possible. The eradication rate of *HP* was worse in smokers than in non-smokers.[27, 28] A positive association was observed among patients.[23, 25] Studies of inhabitants showed no association,[22, 29] except

for the studies in North Ireland,[19] and Scotland,[30] where the overall infection rate was high (50.5% and 67.8% from the figures in the paper,[30] respectively). An association with smoking was reported for Japanese Americans in Seattle, though their infection rate was not high (27.5% in males and 29.1% in females).[31] Since in most cases the infection occurs in childhood, smoking is not a risk factor for the infection, but it could affect the spontaneous elimination or coincidental eradication by antibiotic medication. Accordingly, the association is hardly detected in a population with a low prevalence rate, because the association is diluted by the uninfected individuals. The inconsistency among the studies on inhabitants might be partly explained by this logic, though it is not applicable to the Japanese[22] and Japanese Brazilians in this study. Recently, we found that the association between smoking and *HP* seropositivity was marked for those with certain genotypes of *interleukin 1B C-31T* and *myeloperoxidase* (*MPO*) polymorphisms.[26, 32] These gene-environment interactions may provide a clue to resolve the inconsistency.

In summary, this is the first study to demonstrate the sex-age-specific *HP* seroprevalence rate in a Japanese Brazilian population. Those aged 33–39 years had a lower positive rate than older groups, and there was no difference among the generations (Issei, Nisei, and Sansei) for the same age groups. Every-day fruit intake was a low risk factor for persistent infection. Education level, possibly reflecting socioeconomic status, was associated with seropositivity. We are now examining the association between persistent *HP* infection and genetic polymorphisms in the present subjects.

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REFERENCES

1) Labenz, J. and Borsch, G. Evidence for the essential role of *Helicobacter pylori* in gastric ulcer disease. *Gut*, **35**, 19–22 (1994).
2) Kuippers. E. J., Uyterlinde, A. M., Pena, A. S., Roosendaal, R., Pals, G., Nelis, G. F., Festen, H. P. M. and Meuwissen, S. G. M. Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet*, **345**, 1525–1528 (1995).
3) Munoz, N. Is *Helicobacter pylori* a cause of gastric cancer? An appraisal of the seroepidemiological evidence. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 445–451 (1994).
4) Dunn, B. E., Cohen, H. and Blaser, M. J. *Helicobacter pylori*. *Clin. Microbiol. Rev.*, **10**, 720–741 (1997).
5) Brown, L. M. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol. Rev.*, **22**, 283–297 (2000).
6) Goodman, K. and Correa, P. The transmission of *Helicobacter pylori*. A critical review of the evidence. *Int. J. Epidemiol.*, **24**, 875–887 (1995).
7) Cave, D. R. How is *Helicobacter pylori* transmitted? *Gastroenterology*, **113**, S9–S14 (1997).
8) Webb, P. M., Knight, T., Greaves, S., Wilson, A., Newell, D. G., Elder, J. and Forman, D. Relation between infection with *Helicobacter pylori* and living conditions in childhood: evidence for person to person transmission in early life. *Br. Med. J.*, **308**, 750–753 (1994).
9) Banatvala, N., Mayo, K., Megraud, F., Jennings, R., Deeks, J. J. and Feldman, R. A. The cohort effect and *Helicobacter pylori*. *J. Infect. Dis.*, **168**, 219–221 (1993).
10) Tsugane, S., Fahey, M. T., Hamada, G. S., Kabuto, M. and Miyakawa, V. Y. *Helicobacter pylori* infection and atrophic gastritis in middle-aged Japanese residents of São Paulo and Lima. *Int. J. Epidemiol.*, **28**, 577–582 (1999).
11) Evans, D. J., Evans, D. G., Graham, D. Y. and Klein, P. D. A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology*, **96**, 1004–1008 (1989).
12) Matsuo, K., Hamajima, N., Tominaga, S., Suzuki, T., Nakamura, T., Matsuura, A. and Kitayama, K. *Helicobacter pylori* IgG antibody test established in the United States showed a substantial lower sensitivity for Japanese population. *Am. J. Gastroenterol.*, **95**, 1597–1598 (2000).
13) Tsugane, T., Gotlieb, S. L. D., Laurenti, R., De Souza, J. M. P. and Watanabe, S. Cancer mortality among Japanese residents of the city of Sao Paulo, Brazil. *Int. J. Cancer*, **45**, 436–439 (1990).
14) Tsugane, T., De Souza, J. M. P., Costa, M. L., Jr., Mirra, A. P., Gotlieb, S. L. D., Laurenti, R. and Watanabe, S. Cancer incidence rates among Japanese immigrants in the city of Sao Paulo, Brazil, 1969–78. *Cancer Causes Control*, **1**, 189–193 (1990).
15) Asaka, M., Kimura, T., Kudo, M., Takeda, H., Mitani, S., Miyazaki, T., Miki, K. and Graham, D. Y. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. *Gastroenterology*, **102**, 760–766 (1992).
16) Kikuchi, S., Kurosawa, M. and Sakiyama, T. *Helicobacter pylori* risk associated with sibship size and family history of gastric diseases in Japanese adults. *Jpn. J. Cancer Res.*, **89**, 1109–1112 (1998).

17) Fukao, A., Komatsu, S., Tsubono, Y., Hisamichi, S., Ohori, H., Kizawa, T., Ohsato, N., Fujino, N., Endo, N. and Iha, M. *Helicobacter pylori* infection and chronic atrophic gastritis among Japanese blood donors: a cross-sectional study. *Cancer Causes Control*, **4**, 307–312 (1993).
18) Tsugane, S., Kabuto, M., Imai, H., Gey, F., Tei, Y., Hanaoka, T., Sugano, K. and Watanabe, S. *Helicobacter pylori*, dietary factors, and atrophic gastritis in five Japanese populations with different gastric cancer mortality. *Cancer Causes Control*, **4**, 297–305 (1993).
19) Murray, L., McCrum, E. E., Evans, A. E. and Bamford, K. B. Epidemiology of *Helicobacter pylori* infection among 4742 randomly selected subjects from Northern Ireland. *Int. J. Epidemiol.*, **26**, 880–887 (1997).
20) Oliveira, A. M. R., Queiroz, D. M. M., Rocha, G. A. and Mendes, E. N. Seroprevalence of *Helicobacter pylori* infection in children of low socioeconomic level in Belo Horizonte, Brazil. *Am. J. Gastroenterol.*, **89**, 2201–2204 (1994).
21) Rocha, G. A., Queiroz, D. M. M., Mendes, E. N., Oliveira, A. M. R., Moura, S. B., Barbosa, M. T., Mendes, C. C., Lima, G. P., Jr. and Oliveira, C. A. Indirect immunofluorescence determination of the frequency of anti-*H. pylori* antibodies in Brazilian blood donors. *Braz. J. Med. Biol. Res.*, **25**, 683–689 (1992).
22) Tsugane, S., Tei, Y., Takahashi, T., Watanabe, S. and Sugano, K. Salty food intake and risk of *Helicobacter pylori* infection. *Jpn. J. Cancer Res.*, **85**, 474–478 (1994).
23) Fontham, E. T. H., Ruiz, B., Perez, A., Hunter, F. and Correa, P. Determinations of *Helicobacter pylori* infection and chronic gastritis. *Am. J. Gastroenterol.*, **90**, 1094–1101 (1995).
24) Goodman, K. J., Correa, P., Aux, H. J. T., Ramirez, H., DeLany, J. P., Pepinosa, O. G., Quinones, M. L. and Parra, T. C. *Helicobacter pylori* infection in the Colombian Andes: a population-based study of transmission pathways. *Am. J. Epidemiol.*, **144**, 290–299 (1996).
25) Hamajima, N., Inoue, M., Tajima, K., Tominaga, S., Matsuura, A., Kobayashi, S. and Ariyoshi, Y. Lifestyle and anti-*Helicobacter pylori* immunoglobulin G antibody among outpatients. *Jpn. J. Cancer Res.*, **88**, 1038–1043 (1997).
26) Hamajima, N., Matsuo, K., Saito, T., Tajima, K., Okuma, K., Yamao, K. and Tominaga, S. Interleukin 1 polymorphisms, lifestyle factors, and *Helicobacter pylori* infection. *Jpn. J. Cancer Res.*, **92**, 383–389 (2001).
27) Bertoni, G., Sassatelli, R., Nigrisoli, E., Tansini, P., Bianchi, G., Casa, G. D., Gagni, A. and Bedogni, G. Triple therapy with azithromycin, omeprazole, and amoxicillin is highly effective in the eradication of *Helicobacter pylori*: a controlled trial versus omeprazole plus amoxicillin. *Am. J. Gastroenterol.*, **91**, 258–263 (1996).
28) Kamada, T., Haruma, K., Komoto, K., Mihara, M., Chen, X., Yoshihara, M., Sumii, K., Kajiyama, G., Tahara, K. and Kawamura, Y. Effect of smoking and histological gastritis severity on the rate of *H. pylori* eradication with omeprazole, amoxicillin, and clarithromycin. *Helicobacter*, **4**, 204–210 (1999).
29) The EUROGAST Study Group. Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations. *Gut*, **34**, 1672–1676 (1993).
30) Woodward, M., Morrison, C. and McColl, K. An investigation into factors associated with *Helicobacter pylori* infection. *J. Clin. Epidemiol.*, **53**, 175–182 (2000).
31) Namekata, T., Miki, K., Kimmey, M., Fritsche, T., Hughes, D., Moore, D. and Suzuki, K. Chronic atrophic gastritis and *Helicobacter pylori* infection among Japanese Americans in Seattle. *Am. J. Epidemiol.*, **151**, 820–830 (2000).
32) Hamajima, N., Matsuo, K., Suzuki, T., Nakamura, T., Matsuura, A., Tajima, K. and Tominaga, S. Low expression myeloperoxidase genotype negatively associated with *Helicobacter pylori* infection. *Jpn. J. Cancer Res.*, **92**, 488–493 (2001).