

A population based study of *Helicobacter pylori* infection in a European country: the San Marino Study. Relations with gastrointestinal diseases

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Abstract

Helicobacter pylori is present worldwide but few large population studies exist on the epidemiology of the infection. A random cross sectional study was performed of *H pylori* infection in the adult population of San Marino, a European country with high gastric cancer rate, to assess its prevalence and to evaluate its relations with gastrointestinal disease. In 2237 subjects (77% of the initial sample) *H pylori* IgG antibodies were detected with enzyme linked immunosorbent assay (ELISA) and immunoblotting. A questionnaire including questions about occupation, place of birth, and smoking was given to all subjects. Dyspepsia, peptic ulcer, and gastric cancer in the subjects, relatives, and partners as well as use of drug, dental treatment/prostheses, and gastrointestinal endoscopies, were evaluated by multivariate analysis. *H pylori* prevalence was of 51%, increased with age from 23% (20-29 years) to 68% (≥ 70 years), and was higher among manual workers. *H pylori* was independently associated with ulcer (OR=1.63, 95% confidence intervals (CI)=1.16 to 2.27), H₂ antagonists (OR=1.94, 95% CI=1.21 to 3.10), and benzodiazepines (OR=1.57, 95% CI=1.02 to 2.42), dental

prostheses (OR=1.25, 95% CI =1.05 to 1.49), gastroscopy in the past five years (OR=1.50, 95% CI=1.05 to 2.14), peptic ulcer in siblings (OR=1.52, 95% CI=1.09 to 2.12), gastric cancer in father (OR=1.61, 95% CI=1.02 to 2.52). The association of seropositivity with history of ulcer, gastric cancer in family, gastroscopy, and H₂ antagonists suggests that *H pylori* is an epidemiological key factor in the pathogenesis of gastro-duodenal diseases in this area.

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Helicobacter pylori infection is now recognised as one of the most common bacterial infections in humans.¹ It is considered the main aetiological factor for chronic gastritis² and also an important determinant for ulcerogenesis,³ especially in the longterm recurrence of duodenal and gastric ulcer diseases.⁴⁻⁶ *H pylori* infection can persist for years and probably decades in most untreated patients² and seroconversion or spontaneous eradication rates are low (1% or less) in the general population.⁷ Beside the risk of peptic ulcer, *H pylori* chronic infection can lead to gastric atrophy, a precursor of gastric cancer.⁸ This sequence of events has been shown in the early studies performed in Finland and Estonia,⁹ and chronic gastritis is an important independent risk factor for development of gastric cancer.¹⁰ Case control serological studies have clearly shown that the risk for developing gastric adenocarcinoma either in Eastern or Western countries was strongly associated with an *H pylori* infection already present 10 to 20 years before.¹¹⁻¹³ Gastric cancer rates, however, differ significantly from country to country, from one region to another, and from one generation to the next, suggesting the effect of environmental rather genetic only factors. *H pylori* prevalence is higher in areas at high risk for gastric cancer¹⁴ and *H pylori* serum IgG is significantly associated with gastric cancer incidence and mortality in different countries.^{15 16} To investigate the epidemiology of *H pylori* in an entire country of the European Mediterranean area, we performed a population based study of this infection, the San Marino *H pylori* study. The republic of San Marino is located in the

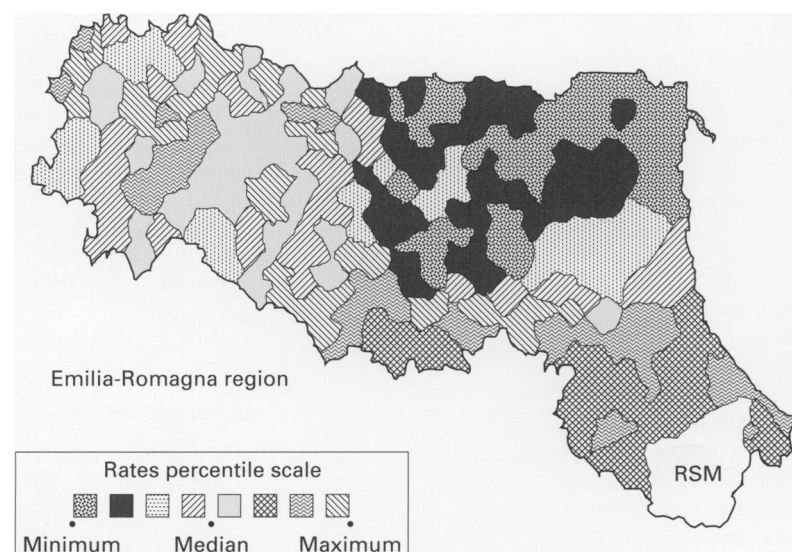


Figure 1: Gastric cancer death rates (men, 1980-3) in the Italian region Emilia-Romagna. The Republic of San Marino (RSM) is situated in the south eastern area of this region, where death rates reach the highest numbers (modified from Regione Emilia Romagna Mortality Atlas 1980-83, 1991 edition).

northern central part of the Italian peninsula and is considered the oldest republic of the world.¹⁷ San Marino is known to be a country with a high gastric cancer rate,^{18 19} as is the adjacent part of Emilia-Romagna in comparison with other areas of this region²⁰ (Fig 1). The San Marino *H pylori* study was done: (a) to determine the prevalence of *H pylori* infection and its relation with age, sex, and environmental factors such as place of birth, occupation, and smoking, (b) to identify any relation of *H pylori* with use of drugs or with previous gastrointestinal endoscopies, history of dyspepsia, peptic ulcer, and gastric cancer in the subjects of the study as well in their close relatives and partners.

Methods

Study population

Between June 1990 and July 1991 a cross sectional study was carried out on the adult population (≥ 18 years) living in the nine districts of the Republic of San Marino (17 000 inhabitants). The Republic of San Marino was chosen for this study for several specific reasons: (a) the population sampled comprised all the adult inhabitants of a country with different social classes, professions, dietary habits, life styles, and where socio-demographic characteristics are very similar to those of Western European countries; (b) the population is very stable because of strict immigration laws as well as economic incentives for the residents; (c) in San Marino there is a higher incidence of clinically relevant gastroduodenal diseases, such as peptic ulcer and gastric cancer (25 of 10 000 and 8 of 10 000 per year, respectively, in 1990)²¹ with respect to Italy and other European countries. Subjects for the study were selected from the National Register of residents in San Marino at January 1990, after a random stratified sampling with proportional allocation by age, sex, and district. Estimated prevalence of antibodies by age reported in a previous study²² were used to determine sample size.²³ Data on gastric cancer incidence during the period 1988 to 93 were obtained from the files of the endoscopy unit of the department of internal medicine of the San Marino Hospital.

Six months before starting the study, mass media were used to inform the San Marino population about the object of the study. A letter explaining the purpose of the study was sent to all the enrollees. A telephone call was made later to each subject to organise the medical interview at the division of internal medicine of the Istituto di Sicurezza Sociale of San Marino. All of the subjects who participated in the study were interviewed by the same physician using a specific standardised questionnaire. The questionnaire contained items concerning demographic, socio-economic, and life style characteristics of the subject including age, sex, occupation, place of birth, smoking, use of drugs (such as non-steroidal anti-inflammatory drugs (NSAIDs),

antacids, antibiotics, benzodiazepines, and H₂ receptor antagonists). Previous diagnosis of dyspepsia (defined according to Colin-Jones *et al*²⁴), peptic ulcer or gastric cancer, as well as gastroscopic examinations and present gastrointestinal symptoms in the subject were recorded. The subjects were also questioned about the presence of gastrointestinal diseases in their family (mother, father, brother or sister, husband or wife). The study was approved by the ethical board of the Istituto di Sicurezza Sociale of San Marino and all subjects gave informed consent to interviews and blood collection.

Serological examination

Serum samples obtained from each subject at the time of interviewing were stored at -70°C . All samples were tested by enzyme linked immunosorbent assay (ELISA) for evaluation of IgG antibodies to *H pylori* proteins. The antigen used was a centrifuged sonicate of two strains, one from serogroup 1 and the other from serogroup 3 by the Lior schema.²⁵ A dilution of 1:100 of each serum was tested in duplicate, as previously described.²⁶ The cut off value used to define a positive serum by ELISA was an optical density of 0.350, which gives a sensitivity of 95%. All positive samples were subsequently tested by immunoblotting. The tests were performed by a BioRad apparatus using whole cells antigens. An anti-IgG conjugate labelled with peroxidase was used at a dilution of 1/500 and 4-chloro-1-naphthol was used as a substrate. A sample was considered positive for *H pylori* IgG antibodies if a 120 000 to 130 000 molecular weight protein band was detected or if five bands among those usually present were visualised. Subjects with a positive immunoblot test were considered to have *H pylori* infection.

Statistical analysis

The data were analysed by a χ^2 test (Mantel-Haenszel) and logistic regression using the SPSS-X statistical package.²⁷ The χ^2 test was used to examine the association between *H pylori* infection and the presence of several variables in a monivariate analysis and to choose the relevant variables to be used in multivariate analysis. Logistic regression was used to evaluate the relation between each independent variable of the study and the frequency of *H pylori* infection with adjustment for other variables.

TABLE I Study population by age and sex (San Marino Study, 1990-1, n=2237)

Age group	Men (n)	Women (n)	Total
20-29	214	247	461
30-39	201	261	462
40-49	213	229	442
50-59	168	191	359
60-69	152	156	308
>70	100	105	205
Total	1048	1189	2237

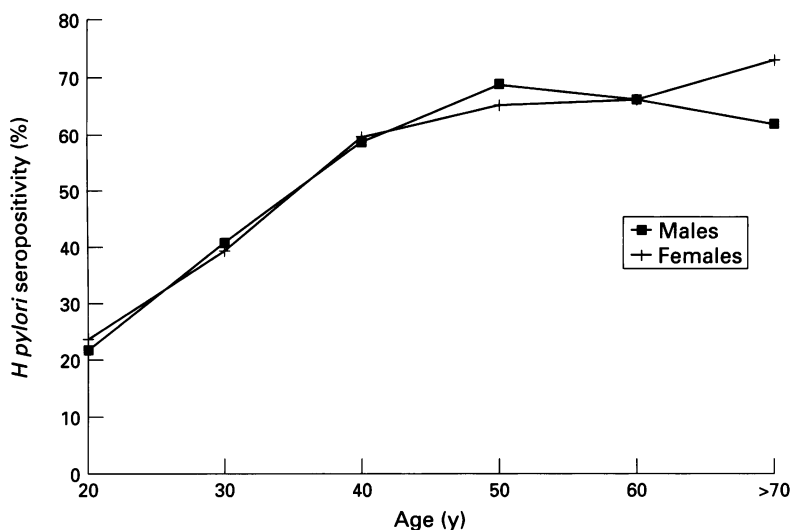


Figure 2: *H pylori* seropositivity by age and sex in the Republic of San Marino (San Marino *H pylori* Study, 1990-1, $n=2237$). The prevalence of the infection increases significantly with age, in a roughly linear fashion, with no sex differences.

Analysis of non-responders

Non-responders were contacted again while the survey team was in the area. In each district a sample of 10% of non-responders was chosen and interviewed briefly by telephone. A proportional allocation was made by sex, age, and reason for no response (the latter classified as not willing to participate or uncertain to participate in the study).

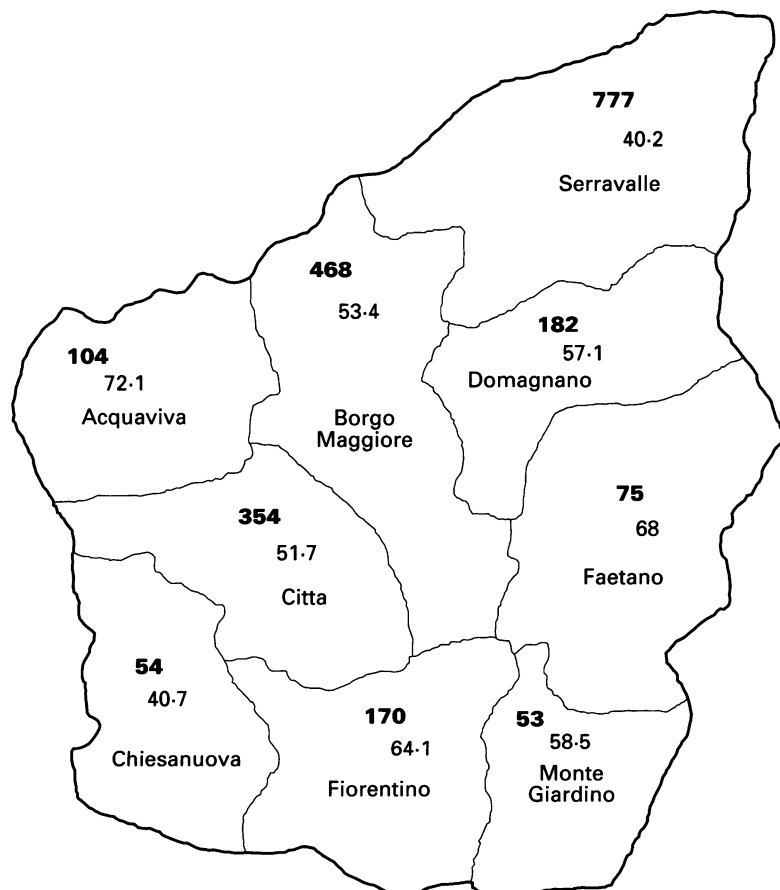


Figure 3: *H pylori* seropositivity by district in the Republic of San Marino (nine districts). Bold type: sampled subjects. Normal type: per cent of seropositive subjects (San Marino *H pylori* Study, 1990-1, $n=2237$). There are large differences in seropositivity between districts, and higher prevalence is found in districts with highest gastric cancer incidence (see text).

TABLE II *H pylori* seropositivity and gastric cancer incidence (1988 to 93) (San Marino Study, 1990-1, $n=2237$)

Districts	Gastric cancer cases	<i>H pylori</i> positive (%)	Gastric cancer rate/10 000 residents
Acquaviva	8	72.1	70.42
Faetano	5	68	70.12
Florentino	1	64.1	6.68
Montegiardino	4	58.5	67.79
Domagnano	13	57.1	67.6
Borgomaggiore	15	53.4	32.88
Citta'	18	51.7	44.73
Chiesanuova	3	40.7	42.67
Serravalle	41	40.2	58.93

Results

The sample population consisted of 2904 subjects, the response rate was of 77%, and the final sample studied was of 2237 subjects. Table I shows the age and sex distribution of the sample. Non-responders represented 23% of 2904 subjects sampled in this study and they were most often in the youngest and oldest class of age (33% and 36% respectively). The non-respondent sample consisted of 164 subjects. Non-responders showed a lower prevalence of positive history for gastritis and peptic ulcer than responders. No significant differences were seen between the two groups for other important surrogate variables.

H pylori infection was detected in 1137 or 51% of the sample studied. Figure 2 shows the distribution of seropositivity by age. The prevalence of *H pylori* infection was similar in both sexes (50.9% in males *v* 50.8% in females, $p>0.6$), thus the data are shown with sex merged. Seropositivity increases significantly with age ($p=0.001$), from 23% in the youngest group (20-29 years) to 68% in the oldest (≥ 70 years), in a roughly linear fashion from 20 years to 50 years. Figure 3 shows the prevalence of seropositivity in the nine districts of San Marino. It differed considerably from 40% in the Serravalle and Chiesanuova districts to 72% in the Acquaviva district. Table II shows the distribution of seropositivity in the different districts together with the corresponding gastric cancer incidence during the period 1988 to 93. With regard to place of birth, seropositivity was associated with southern regions for those subjects born outside San Marino ($p<0.01$), with a trend to increase from north to south (Table III). Data on socioeconomic characteristics showed that *H pylori* infection tended to be more frequent among blue collar workers ($p>0.001$), especially those doing manual work (miners 78% of nine, road sweepers 65% of 37, plumbers/painters 61% of 83, house keepers 60% of 288, cooks 58% of 26) with

TABLE III *H pylori* seropositivity by birth place in the San Marino Study

Place of birth	Seropositive (n)	%
South Italy	16/21	76.2
Central Italy	197/310	63.5
North Italy	213/359	59.3
San Marino	676/1396	48.4
Foreign	35/151	23.1
Total	1137/2237	50.8

$p<0.01$.

TABLE IV Results of logistic regression analysis applied to anamnestic factors and drug use (San Marino Study, 1990-1, n=2237)

Factor	Regression coefficient β (SEM)	Odds ratio	95% Confidence intervals	p Value
Dyspepsia*	0.002 (0.12)	1.00	0.79 to 1.27	0.986
Peptic ulcer*	0.486 (0.17)	1.63	1.16 to 2.27	0.005
H ₂ antagonists*	0.661 (0.24)	1.94	1.21 to 3.10	0.006
Antacids*	-0.219 (0.24)	0.80	0.50 to 1.28	0.363
Benzodiazepines*	0.454 (0.22)	1.57	1.02 to 2.42	0.037
Dental treatment*	-0.254 (0.10)	0.78	0.64 to 0.94	0.012
Dental prosthesis*	0.225 (0.09)	1.25	1.05 to 1.49	0.015
Gastrointestinal surgery	0.490 (0.10)	1.63	1.34 to 1.99	0.001
Endoscopy:				
Last month	-0.344 (0.49)	0.71	0.27 to 1.85	0.482
Last 3 months	-0.042 (0.45)	0.96	0.40 to 2.32	0.926
Last 6 months	0.689 (0.38)	1.99	0.94 to 4.19	0.069
Last 12 months	0.387 (0.24)	1.47	0.92 to 2.36	0.111
Last 5 years	0.406 (0.18)	1.50	1.05 to 2.14	0.023

*1=yes; 2=no.

respect to white collar workers (physicians 15% of 13, clerks 21% of 81, secretaries 37% of 179, nurses 38% of 34, general managers and lawyers 38% of 50, teachers 39% of 67, shop workers 50% of 276). High prevalence of infection was also noted among social workers (74% of 15). With regard to lifestyle habits, seropositivity was associated with use of H₂ receptor antagonists (9.9% of 1137 *v* 3.5% of 1098, $p < 0.001$) and of benzodiazepines (7.1% of 1132 *v* 3.8% of 1096, $p < 0.001$) while for antacids the difference did not reach the significance value (5.6% of 1136 *v* 3.9% of 1097, $p < 0.058$). No significant differences were found for smoking and use of NSAIDs or antibiotics.

Table IV shows the results of logistic regression analysis with *H pylori* seropositivity as the dependent variable and some anamnestic factors not highly intercorrelated (contingency coefficient under 0.5) as independent variables. A significant direct association was seen between a history of peptic ulcer and *H pylori* infection (OR=1.63). Subjects who had gastrointestinal endoscopy in the past five years showed a higher risk for *H pylori* infection (OR=1.50) with respect to those who never had endoscopy. Use of H₂ antagonists was associated with a higher frequency of *H pylori* infection (OR=1.94). No significant association was seen with other drugs (NSAIDs, antacids, antibiotics) except benzodiazepines (OR=1.57). There was a negative correlation between dental treatment and *H pylori* infection; those who received dental treatments showed the lowest rate of seropositivity (OR=0.78), while those who had permanent dental prostheses presented a significant higher rate (OR=1.25). No significant associations were seen with presence of caries. Table V summarises the frequency of seropositivity among subjects with anamnestic significant

TABLE V *H pylori* seropositivity and gastric diseases/surgery, dental prostheses/therapy, drugs use (San Marino Study, 1990-1, n=2237)

Factors	No	Positive (%)
Peptic ulcer	295	70
Gastrointestinal surgery	851	61
H ₂ antagonists	151	75
Endoscopy (last 5 years)	203	63
Benzodiazepines	122	66
Dental prostheses	1176	56
Dental treatment	598	45

risk factors. Table VI shows the results of logistic regression analysis applied to the history of gastrointestinal diseases in close relatives of the population under study. Presence of gastric cancer in the father was directly correlated with a higher *H pylori* seropositivity rate (60 of 1137 *H pylori* positive subjects had a father with gastric cancer *v* 33 of 1100 *H pylori* negative subjects, OR=1.61, 95% CI=1.02 to 2.52). An inverse correlation was found between history of dyspepsia in the father and *H pylori* infection in the subject (OR=0.50). There was a positive correlation between *H pylori* seropositivity and some gastrointestinal diseases in siblings, significant for peptic ulcer (124 *H pylori* positive subjects had a sibling with peptic ulcer *v* 76 *H pylori* negative subjects, OR=1.52, 95% CI=1.09 to 2.12), but not for dyspepsia (OR=1.36, $p = 0.058$). No significant relation was seen between seropositivity and the presence of gastrointestinal diseases in husband or wife.

Discussion

H pylori infection is present throughout the world. The available epidemiological data are based on studies performed on blood donors, on subjects presenting themselves to health centres or on volunteers.²⁸ This kind of recruitment may introduce bias because the selected people may not be representative of the general population. There is only one published study that is based on a random sample of a large population²⁹ but this study was retrospectively conducted and the demographic information had been obtained during two previous studies carried out in a single Welsh town. The Eurogast Study Group has recently published data on the variability of prevalence of *H pylori* infection and associated risk factors among asymptomatic subjects in 17 different populations.³⁰ There are also the cross sectional studies done in southern China³¹ and Saudi Arabia,³² but these studies have not been designed to represent the entire population of the country. The study performed by Al-Moagel *et al* in Riyadh City is interesting, however, as it shows many similarities with our study: it was conducted recruiting subjects randomly from the records of general practitioners of Primary Health Care Centres (even if there were only 557 subjects, recruited from a single town of a tropical region), it was undertaken to evaluate the prevalence of *H pylori* in different age groups, and it looked for any relation of seropositivity with socioeconomic status, use of drugs, smoking, and gastrointestinal symptoms in the subject. The distinguishing features of the San Marino *H pylori* study consist of: (a) a very large sample size with the highest percentage of responders (77%) reported to date, which can be considered truly representative of all the adult residents in this country; (b) a standard protocol for recruiting and interviewing with a specific questionnaire (focused on gastrointestinal diseases in the subject and their close relatives) for all the subjects in a single

TABLE VI Results of logistic regression analysis applied to family history of gastric diseases (San Marino Study, 1990-1, n=2237)

Factor	Regression coefficient β (SEM)	Odds ratio	95% Confidence intervals	<i>p</i> Value
Dyspepsia father*	-0.682 (0.18)	0.50	0.35 to 0.72	0.001
Dyspepsia mother*	-0.279 (0.18)	0.76	0.53 to 1.08	0.121
Dyspepsia brother/sister*	0.311 (0.16)	1.36	1.00 to 1.87	0.058
Ulcer father*	0.161 (0.13)	1.17	0.91 to 1.52	0.230
Ulcer mother*	-0.343 (0.21)	0.71	0.47 to 1.07	0.107
Ulcer brother/sister*	0.421 (0.17)	1.52	1.09 to 2.12	0.011
Cancer father*	0.475 (0.23)	1.61	1.02 to 2.52	0.037
Cancer mother*	0.320 (0.28)	1.38	0.79 to 2.38	0.260
Cancer brother/sister*	0.236 (0.30)	1.27	0.70 to 2.28	0.427

*:1=yes; 2=no.

centre of a country where high frequency of gastroduodenal diseases has been reported.

The clinical interest for this kind of population based study is justified by the finding that *H pylori* infection is associated with gastric cancer³³ and it is an independent risk indicator of gastric adenocarcinoma.³⁴ Therefore, the eventual confirmation of the provocative hypothesis for the role of *H pylori* as relevant cofactor in the pathogenesis of gastric malignancies³⁵ could modify the clinical approach to the patient with *H pylori* infection.³⁶

In our study the analysis of non-responders showed that subjects who did not participate presented a lower prevalence of previous diagnosis of dyspepsia and peptic ulcer. Thus, even if the inferential validity has to be restricted to the types of people who were willing to participate fully in the study, it is worthwhile to note that three quarters of the active population fall in this group. Moreover when we analysed questionnaires for gastrointestinal symptoms we only found epigastric pain in 173 (7.8%) of 2237 responders (data not shown). This result balances the possibility of having introduced a bias for a higher frequency of gastroduodenal diseases in the subjects who participated in the study with respect to non-responders. Therefore peptic ulcer patients are not over-represented in our study population. For diagnosis an ELISA test with a first generation antigen was used as a screen test. The low cut off value used gave a high sensitivity but low specificity. All the positive samples were subsequently tested by immunoblot and 9.8% of the samples were eliminated. This approach, while time consuming, gave us a high degree of sensitivity and specificity.

The San Marino *H pylori* Study shows that over half of the adult population of this country is infected by *H pylori*. This prevalence is higher than in Italy or in other Western European countries. We found that *H pylori* seropositivity increases significantly with age, confirming previous studies.^{22 28-32 34} This pattern has been explained by a cohort effect.^{7 37} The hypothesis for this cohort effect is also supported in our study by the finding that the curve of *H pylori* seropositivity by age tended to parallel that seen for the increase of economic and sanitation conditions in San Marino during the past 50 years.²¹

With regard to demographic and socio-economic factors, we found that subjects born in

southern regions and those who were manual workers (blue collars) tended to have a higher rate of infection. The finding that social workers with a high educational standard have a higher rate of seropositivity (74%) than subjects with a similar socioeconomic status but a different type of job, emphasises the relevance of direct person to person spread of the infection as recently shown in nurses³⁸ and in cohabiting children.³⁹ These findings suggest that poor hygiene standards and low socioeconomic status (which frequently reflects the first) are important factors for the acquisition of *H pylori* in the first years of life, confirming previous findings on differences between developed and developing countries⁴⁰ and on the importance of overcrowding and close person to person contacts in childhood.⁴¹

Concerning the use of drugs, in the multivariate analysis the prevalence of infection remained associated significantly with previous treatment with H₂ antagonists (excess of risk: 94%) and benzodiazepines (excess of risk: 56%). The association with H₂ antagonists is in agreement with the results reported in a recent case control study on the risk for gastric adenocarcinoma.³⁴ These data could be interpreted either as indirect evidence for an underlying asymptomatic peptic ulcer disease in the *H pylori* positive subject or as a result of symptomatic treatment with H₂ antagonists of the ulcer like symptoms induced by the *H pylori* associated gastritis.⁴² In fact patients presenting with peptic ulcer and non-ulcer dyspepsia have high prevalence rates of *H pylori* infection, and often show anxious personality patterns^{43 44}; therefore they are also frequently treated with sedatives for their dyspeptic symptoms.

H pylori infection was significantly higher in subjects who reported gastrointestinal endoscopy in the past five years. This finding can be explained either with a possible *H pylori* transmission by endoscopic instruments as previously shown⁴⁵ or with an indirect effect of longstanding dyspeptic symptoms in *H pylori* positive subjects, which induced them to have endoscopy. Earlier studies reported the presence of *H pylori* in dental plaque⁴⁶; this finding induced us to look for an association of the infection in subjects who had had dental treatment or permanent dental prostheses. Multivariate analysis showed that *H pylori* seropositivity was directly associated with the presence of dental prostheses, while those who had regular dental treatment (and therefore have probably eliminated or reduced their dental plaque) showed the lower rates of *H pylori* infection (Tables IV and V). Therefore, having permanent dental prostheses could infer a higher risk of infection for the subject, by possible continuous ingestion of *H pylori*.⁴⁷ The logistic regression analysis also confirmed the relevance of *H pylori* infection in the natural history of gastroduodenal diseases. In fact significant associations with seropositivity were found not only for peptic ulcer in the subjects of the study (excess of risk: 63%), but also in their close relatives as regards peptic ulcer in siblings (excess of risk: 52%) and

gastric cancer in the father (excess of risk: 50%). In contrast, *H. pylori* seropositivity was not significantly associated with gastro-duodenal diseases in partners.

In the San Marino *H. pylori* study the lack of association of seropositivity with history of dyspepsia in the subject and the inverse association seen in subjects whose fathers had dyspepsia confirm the data of previous serological studies^{32, 48} but are apparently in contrast with histological studies that show *H. pylori* infection in over half of the patients with non-ulcer dyspepsia. It is necessary to emphasise, however, that in our study the diagnosis of dyspepsia in the subjects, as well in their close relatives, was not based on endoscopic or histological findings as were those of peptic ulcer or gastric cancer. Given that the concordance of clinical diagnosis of dyspepsia with histological gastritis can be as low as 30%, it is conceivable that in the San Marino *H. pylori* Study we did not find an association of dyspepsia with *H. pylori* infection. This could be caused by an over-estimation of the presence of *H. pylori* gastritis in seronegative subjects.

No conclusions can be made on the direct relations between gastric carcinoma or colon cancer and *H. pylori* seropositivity because of the small number of subjects recruited who had these diseases (respectively five and four subjects of 2237 cases in the study). Italy is one of the European countries with the highest death rates for gastric cancer⁴⁹ (males: 27×100 000 inhabitants in 1980–3), which accounts for over 14 000 deaths per year in 1987 (10% of all the cancer deaths).⁵⁰ Furthermore in Italy there is a wide geographical variability for gastric cancer mortality⁵¹ and high risk areas are located in central northern regions. One of these regions, Emilia-Romagna, showed a crude death rate for men of 48×100 000 inhabitants in 1980–3, with noticeable prevalence for men in its south eastern areas²⁰ (Fig 1). The same trend was confirmed in the following years with a death rate of 43×100 000/year in 1987–9.⁵² The Republic of San Marino is located in the highest risk area of this region, and in this small independent country a gastric cancer death rate of 68×100 000 inhabitants in 1987–90 has been reported.²¹ Moreover, previous studies in San Marinense families with gastric carcinoma have shown a higher frequency of this disease in first degree relatives of the affected families.¹⁹ The results of our San Marino *H. pylori* Study shows that during 1988–93 the highest gastric cancer incidence were generally seen in the districts with higher seropositivity rates, even if a significant linear correlation could be shown only in seven of nine districts (Table II). This trend is in agreement with the results obtained in a recent multicentre international study,¹⁶ however, which showed a significant relation between *H. pylori* seropositivity and cumulative rates for both gastric cancer incidence and mortality.

In conclusion we performed for the first time a cross sectional serological population based study of *H. pylori* infection in a complete

European Mediterranean country, the Republic of San Marino. *H. pylori* infection increases significantly with age, is associated with a Southern place of birth, and manual work and it is more widespread in those districts of San Marino with higher gastric cancer incidence. *H. pylori* seropositivity was associated with anamnestic factors such as peptic ulcer, use of H₂ antagonists and benzodiazepines, presence of permanent dental prostheses, gastrointestinal endoscopy in the past five years, and a positive history of peptic ulcer in siblings. We also found that subjects with a *H. pylori* seropositivity rate reported a high frequency of gastric cancer in their father. Because the prevalence rates of peptic ulcer, atrophic gastritis, and gastric cancer in San Marino are higher in comparison with those seen in the Italian population,⁵³ prospective endoscopic and histopathological investigations in these San Marinense subjects could confirm a possible role of *H. pylori* infection, together with genetic,¹⁸ environmental,¹⁹ and dietary factors⁵⁴ in the pathogenesis of these diseases.

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