Seroepidemiology of *Campylobacter pylori* Infection in Various Populations

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Campylobacter pylori **infection has been recognized as being strongly associated with chronic gastritis and
duodenal ulceration, but the prevalence of** *C. pylori* **infection in a normal population is not known. A serological
survey was conducted in four countries with different geographical and socioeconomic status, in a randomly
chosen population as representative as possible, by using an enzyme-linked immunosorbent assay (ELISA) with
a sonic extract of two strains as the antigen. The test had a specificity of 94% when 600 ELISA units was used
as the threshold. In France, few children were infected before the age of 10 years. The prevalence then
increased gradually to 36.7% in the sixth decade of life. This increasing prevalence of infection with age was
also observed in Algeria, Vietnam, and the Ivory Coast but at a higher rate (80 to 90%). In Vietnam, as in
France, few children were infected, whereas in Africa,** *C. pylori* **infection occurred earlier. The prevalence of
infection did not differ with sex for a particular age group; it also did not differ with respect to gastric
symptoms, smoking and drinking habits, or urban or rural residence when these potential risk factors were
studied. The epidemiological data available on peptic ulcer disease in developing countries compared with
developed countries led to the speculation that infection with** *C. pylori* **is not a sufficient condition to develop
this disease.**

Campylobacter pylori is now known to be an etiologic
agent of type B gastritis as defined by histologic criteria (2).
However, this condition can be asymptomatic. In Finland
and Estonia, population surveys found that antral gastritis
increased with age from less than 20% at 20 years of age to
approximately 50% at 50 years of age (22). Diagnosis of *C.
pylori* and of gastritis are based on examination of biopsy
specimens obtained by fiber-optic endoscopy (13). Recently,
a serological approach has been developed which allows
epidemiological studies to be performed on a large scale.
Serology has been made possible by using a bacterial sonic
extract, since *C. pylori* is an organism distinct from other
bacteria, as shown by DNA-RNA hybridization and protein
profile studies.

Therefore, we have used serology to study the epidemiol-
ogy of *C. pylori* in various populations to determine the
extent of *C. pylori* infection.

MATERIALS AND METHODS

Subjects tested. The sera were collected in October 1987
from healthy individuals chosen so as to be representative of
the population at large.

In France, the sera were collected from 1,000 consecutive
adults presenting themselves to a health checkup center in
the city of Bordeaux. All French workers benefiting from the
Social Security system (health insurance service) are invited
every 5 years to have an extensive health checkup. The
population was from the Gironde region (75% urban), and
which people are invited each month for a checkup is
decided on the basis of the month of birth, followed by the
year of birth. The cycle is repeated every 5 years. For
children, the sera were collected from patients with surgical

problems other than gastrointestinal ones who were referred
to the emergency room of the Children's Hospital as well as
to a private clinic in Bordeaux.

In Algeria, the sera were collected from blood donors at
the blood bank of the Oran Hospital Center. Sera from
children were collected in an outpatient clinic.

In Vietnam, the sera were collected at the Ho Chi Minh
City blood bank. For children, the sera were collected in an
outpatient clinic of the Children's Hospital No. 2.

In the Ivory Coast, the sera were collected from various
groups during a study designed to determine the prevalence
of B hepatitis in the Ivory Coast by cluster sampling.

All sera were maintained frozen at −20°C before being
tested. Information about the age and sex of a patient was
noted in all the countries. In France, the questionnaire also
included questions about smoking and drinking habits, past
and present histories of gastric disease, and place of resi-
dence.

Method. All sera were tested by using an enzyme-linked
immunosorbent assay (ELISA) technique. The antigen was a
centrifuged sonic extract of two strains of serogroups 1 and
3 by the Lior schema (H. Lior, A. D. Pearson, D. L.
Woodward, and P. Hawtin, Abstr. 3rd Int. Workshop Cam-
pylobacter Infect., abstr. no. 120, 1985). This serogrouping
schema is based on heat-labile antigens detected by slide
agglutination with absorbed sera. A dilution of 1:100 of each
serum was tested in duplicate. The anti-immunoglobulin G
conjugate (Nordic, Tilburg, The Netherlands) labeled with
peroxidase was used at a dilution of 1:10,000 in phosphate-
buffered saline plus bovine serum albumin. 2,2'-Azinoben-
zothiazoline sulfonate and H_2O_2 were used as the substrate.
The optical density obtained was transformed into ELISA
units by using a regression curve made by progressively
diluting a strongly positive serum as described by Goodwin

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No. of sera tested:
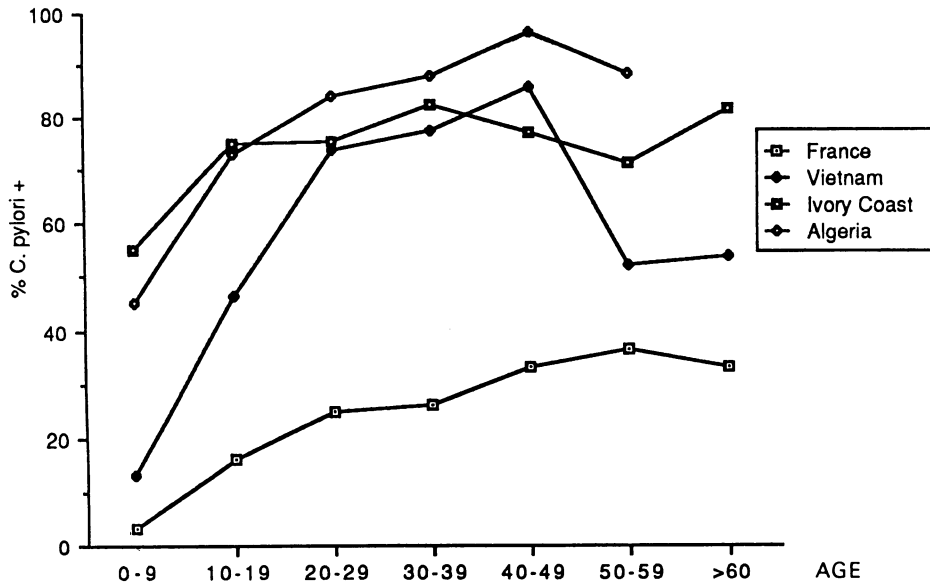
	0-9	10-19	20-29	30-39	40-49	50-59	>60
France	113	98	105	229	285	324	45
Viet Nam	61	43	92	72	50	21	26
Ivory Coast	116	100	78	40	22	7	11
Algeria	42	59	83	49	27	17	0

FIG. 1. Distribution of positive sera for *C. pylori* immunoglobulin G antibodies by age and country of origin.

et al. (7). A negative control and a weakly positive control were also included. A plate was rejected if the value of the weakly positive control was above 1 standard deviation of the mean (optical density, 0.9) measured on 40 different occasions.

According to previous results obtained with patients' sera known to be *C. pylori* positive or negative (18), the threshold of 400 ELISA units gives a sensitivity of 89% and a specificity of 90%. For this epidemiological study, the threshold was increased to 600 ELISA units (sensitivity, 84%; specificity, 96%).

For statistical purposes, the chi-square test was used.

RESULTS

Among the 2,215 serum specimens tested, 1,035 (46.7%) were positive (>600 ELISA units). The repartition of the positive sera by the patient's age is presented in Fig. 1, both in reference to the country and globally. A common feature in all countries was that prevalence increased with age. In France, very few children were infected (3.5%) in the first decade. The prevalence increased markedly to 16.3% in the second decade and to 24.8% in the third decade. After 40 years of age, the number of infected people stabilized around 35%. In Vietnam, Algeria, and the Ivory Coast, prevalence also increased with age but the main difference observed was a higher prevalence of the infection. In Vietnam, relatively few children were infected (13.1%). As in France, most people became infected between 10 and 30 years of age but more than 75% of the population had antibodies after this age.

In Algeria and in the Ivory Coast, 80 to 90% of the subjects were infected in adulthood and the first contact with *C. pylori* occurred earlier in life than in France and Vietnam. In the first decade, 45.2% had antibodies in Algeria and 55.2% had antibodies in the Ivory Coast.

Another common finding in all these countries was a slight decrease in the numbers of subjects with high antibody levels in the population over 60 years of age in France and over 50 years in the other countries; however, this difference was not statistically significant.

The repartition of the positive cases by sex and age stratification for the four populations studied as a whole is presented in Fig. 2. There was no statistically significant difference observed in any age group.

In France, where the association of a high antibody level with previous or actual gastric symptoms, smoking and drinking habits, and place of residence (urban or rural) was studied, no statistically significant association with a positive serological result was noted.
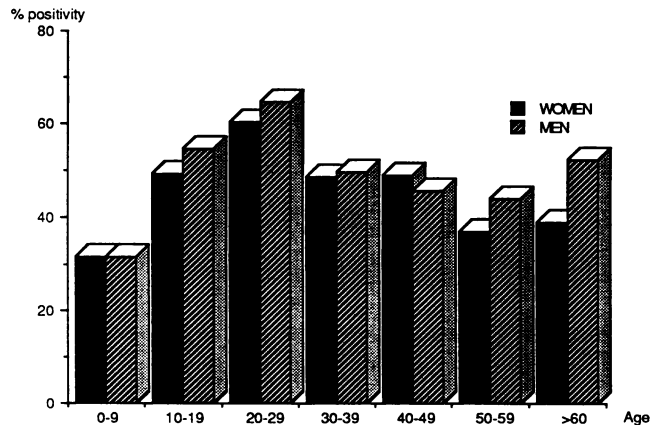

FIG. 2. Distribution of positive sera for *C. pylori* immunoglobulin G antibodies by age and sex for the four populations studied as a whole.

Quantitative data concerning the titers obtained showed that the titers were distributed in two main groups: the negative (<600 ELISA units) (53.2%) and the strongly positive (>1,000 ELISA units) (37.9%). Only 193 serum samples (8.7%) were in an intermediary position.

DISCUSSION

In an epidemiological study, the first question which arises is the representativeness of the population. In all three countries except the Ivory Coast, the subjects were not randomly selected. The French adult population seems to be reasonably representative but, in fact, excludes people who do not work and several categories of workers, such as the farmers, who do not belong to the same health-care system. In addition, even if a random selection of the population were invited for a checkup, part of the population would not come. Nevertheless, it is admitted that this population is better than that of the blood bank with respect to representativeness. The Algerian adult population studied consisted of donors of blood to a blood bank; it must be noted, however, that in this country members of the family of a patient needing a transfusion are asked to donate their blood. In Vietnam, the blood bank operates by paying adult volunteers, and therefore, a bias exists. The sera from the Ivory Coast, including those from children, were collected randomly by cluster sampling. For children from all four countries, patients referred to the hospital or outpatient clinic were tested.

The value of the technique used can also be questioned. Different antigens have been used for *C. pylori* serology: whole bacteria treated by formaldehyde (19) or heated (14); sonic extract (3, 17; J. Kaldor, W. Tee, P. McCarthy, J. Watson, and B. Dwyer, Letter, Lancet i:921, 1985); acid glycine extract (7, 15, 23); and high-molecular-weight antigen (9; J. Dent, C. A. M. McNulty, J. S. Uff, M. W. L. Gear, and S. P. Wilkinson, Letter, Lancet i:1002, 1988). We used a sonic extract in this study. By immunoblotting, it has been found that some proteins are shared with *C. jejuni*, and therefore, cross-reactions of moderate intensity can theoretically be observed (15, 16). We tested sera which are strongly positive for *C. jejuni*, and we did not observe such cross-reactions. However, it is not known if repeated infection with *C. jejuni* (as is true for populations from developing countries) would increase the antibody response to the few antigens shared with *C. pylori* to such a magnitude that the test would give a false-positive result. A way to verify this hypothesis would be to standardize the test in the population to be studied. In practice, such a verification is difficult because the facilities for gastroduodenoscopy and culture are lacking in the majority of cases. Furthermore, we predict that it would be difficult to find enough *C. pylori* negative controls to determine cross-reactivity. We have increased the threshold for positivity in this study to avoid false-positives. The distribution of the titers observed does not favor the occurrence of many false-positives.

A slight decrease in prevalence has been noted in the oldest age groups. It was more important in the Vietnamese population; this fact has not been explained. The same findings were noted in a Thai population which was 75% positive between 30 and 49 years of age and 60% positive between 50 and 64 years of age (G. I. Perez-Perez, D. N. Taylor, P. Echeverria, and M. J. Blaser, Abstr. 5th Int. Workshop Campylobacter Infect., abstr. no. 43, 1989).

Four populations were studied. One population is from a developed country (France) but is not homogeneous, since immigrants are working in the country and it was not possible to screen the population by ethnic status. The other populations are from developing countries which, on the contrary, are very homogeneous with respect to race and sociocultural conditions: Arab-Berbers in Algeria, Mongoloids in Vietnam, and Sudanese in the Ivory Coast. There is a marked difference between the results in developed and developing countries, with the prevalence in the latter group being at least twice that in France at its peak. We can even postulate that virtually every individual in Algeria will be infected sometime in his lifetime. This high prevalence in a strictly Muslim country does not favor the role of pork as a contaminant. A few studies on the prevalence of *C. pylori* infection have already been performed but they always used a limited number of subjects and most of them used a unique population. Jones et al. (11) were the first to present serological data on sera obtained from 607 control individuals and to note an increasing prevalence with age. Confirmation was made by endoscopy of healthy volunteers (C. P. Dooley, P. Fitzgibbons, H. Cohen, M. D. Appleman, G. I. Perez-Perez, and M. J. Blaser, Gastroenterology 92:A102, 1988). Morris et al. (14), using serology, found 40 to 70% of Melanesians infected with *C. pylori* versus 15% of blood donors of unknown race from New Zealand. Graham et al. (8), with a [^{13}C]urea breath best, showed that Chinese (60%) and Indians (46%) were more likely to be infected than individuals from the United States. In eastern Africa, *C. pylori* was frequently found in a group of patients referred for endoscopy (75% of patients) (20). Dwyer et al. studied a population of aborigines from Australia who are known to lack duodenal ulcers (1). Their results showed that only 0.5% were *C. pylori* positive (5) in contrast to 15% for white Australians (4). These data led to the conclusion that *C. pylori* may be necessary for the development of a duodenal ulcer. The data that we obtained in developing countries show a very high rate of *C. pylori* infection, and it is inconceivable that all these people had or will develop a duodenal ulcer. *C. pylori* infection is, therefore, not a sufficient condition for developing a duodenal ulcer.

The epidemiology of ulcer disease in these particular developing countries is not known. Few studies have addressed this question in other places. A high frequency of severe peptic ulcers was found in rural Haitian men (6). However, the population studied corresponded to patients with epigastric pain, and therefore, the frequency in the general population is not known. In southern India, data concerning a population-based study of 11,540 urban adults are available. The prevalence rate of peptic ulcer was 7.5/1,000, with a male-to-female ratio of 2.4/1. However, the diagnostic method was not endoscopy but X-ray examination after a barium meal, and only the individuals with symptoms of peptic ulcer were examined (12).

The epidemiology of duodenal ulcer among black Africans was studied by Tovey and Tunstall (21). Two areas have a high prevalence rate, the western coast and the eastern region, whereas the frequency is low in the savannah areas. In Australia, the annual incidence rate was calculated for the entire country by looking at cimetidine prescriptions. In 1981, the annual incidence rate of duodenal ulcers was 3.8/1,000 (10). This rate varies from 1.4 to 3.3/1,000 in European countries.

This lack of related information concerning peptic ulcer disease in the population tested does not allow us to draw definitive conclusions about the role of *C. pylori* in this disease. We can only speculate that *C. pylori* is not a sufficient condition for development of the disease.

Epidemiology of *C. pylori* infection is a key point in developing a more comprehensive approach to the natural history of duodenal ulcer disease. We found a serological method using ELISA to be a good tool for this purpose.

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