

Seroepidemiology of *Helicobacter pylori* infection in vegans and meat-eaters

M. J. WEBBERLEY¹, J. M. WEBBERLEY^{2*}, D. G. NEWELL³, P. LOWE¹
AND V. MELIKIAN¹

¹Dudley Road Hospital, Birmingham

²Sandwell District Hospital, West Bromwich

³PHLS, Centre for Applied Microbiology and Research, Porton Down, Salisbury

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SUMMARY

An enzyme-linked immunosorbent assay has been used to diagnose serologically the prevalence of *Helicobacter pylori* infection in Asian life-long vegans. There was no difference in the seropositivity between these individuals and a group of age- and sex-matched Asian meat-eaters, indicating the meat consumption is not a risk factor for *H. pylori* infection. However, both Asian groups had a higher prevalence of infection than age- and sex-matched Caucasian meat-eaters. Additionally, the Asian individuals had a wider range of specific IgG antibody concentrations than the Caucasians. This did not appear to be due to antigenic cross-reactivity between *H. pylori* and *Campylobacter jejuni*. The significance of these observations to the establishment of cut-off levels for the serodiagnosis of certain ethnic groups is discussed.

INTRODUCTION

The acquisition and transmission of *Helicobacter pylori* infection is poorly understood. Epidemiological evidence supports person-to-person spread of the organism within close communities and families [1, 2]. Volunteer studies confirm that colonization can follow ingestion [3, 4], thus implicating food and/or water as potential sources of infection. The evidence for a water source [5], suggesting a faecal-oral route, is accumulating. Nevertheless, there appears to be an association between occupational exposure to animals or meat and *H. pylori* colonization [6, 7]. To date, evidence for an animal reservoir is unconvincing with rare isolations of *H. pylori* from domestic animals, in particular the pig [8], being reported. In order to investigate the presence of meat in the diet as a risk factor, the prevalence of *H. pylori* infection in Asian life-long vegans, Asian meat-eaters and Caucasian meat-eaters has been determined. *H. pylori* infection in these populations was assessed using a non-invasive serological test for circulating anti-*H. pylori* IgG antibodies. Because of known variations in the prevalence of infection with age, comparisons were also made in individuals matched for age and sex.

* Author for correspondence and reprint requests: Dr J. M. Webberley, Department of Microbiology, Worcester Royal Infirmary, Castle St, Worcester WR1 3AS, UK.

MATERIALS AND METHODS

Patients

Asian life-long vegans ($n = 68$), Asian meat-eaters ($n = 105$) and Caucasian meat-eaters ($n = 68$) were recruited from routine medical out-patient clinics. Vegans were defined as individuals who had never eaten meat, fish or eggs but may have consumed dairy products. Meat-eaters were defined as individuals who regularly ate fish and/or meats. Childhood eating habits were included in these definitions. It was possible that vegans may have handled meat in the process of preparing foods for other family members but this information was not available.

All subjects were asymptomatic for gastrointestinal diseases and were of similar socio-economic backgrounds. Serum was obtained from 10 ml of clotted venous blood and stored at $-20\text{ }^{\circ}\text{C}$ until required.

Serological assay

An enzyme-linked immunosorbent assay (ELISA), using acid extractable surface antigens from *H. pylori* NCTC 11638 was used to measure serum anti-*H. pylori* specific IgG antibodies as previously described [9], excepting that the human serum dilution was 1 in 200. The specific antibody concentration was determined using a standard curve of antibody mass against optical density. A threshold of $10\text{ }\mu\text{g/ml}$ was used to determine seropositivity. The sensitivity and specificity of this ELISA system to detect *H. pylori* infection in dyspeptic patients is 93 and 96% respectively [10].

A similar assay was used to measure anti-*Campylobacter jejuni* IgG antibodies using acid extractable common surface antigens for *C. jejuni* 81116 [11]. No threshold for seropositivity for anti-*C. jejuni* IgG antibodies has been established.

RESULTS

Overall the Caucasian population studied had a significantly ($P < 0.01$) lower seropositivity than either of the Asian populations (Table 1). Moreover, those Caucasians who were seropositive had a higher mean specific IgG concentration, while that of the seronegative group was lower, than either of the Asian populations. There was no difference in the prevalence of seropositivity or IgG levels between the life-long vegans and the meat-eating Asians.

These trends were confirmed in the age- and sex-matched groups. Fifty-four of the Asian vegans were age- and sex-matched with Asian meat-eaters (Table 2). There was no difference in the anti-*H. pylori* IgG antibody levels, or the seropositivity, between these two groups. Sixty-five of the Asian meat-eaters were age and sex matched with Caucasian meat-eaters (Table 2). Again significantly higher antibody levels were detected in the Asian group ($P < 0.01$), and 32% of Asians were found to be seropositive compared with 17% of Caucasians.

The differences in specific IgG concentrations between the Asian and Caucasian groups were clearly evident. In the Caucasians the results generally were either strongly positive or negative using an established cut-off of $10\text{ }\mu\text{g/ml}$ (Fig. 1). However amongst both Asian vegans and meat-eaters a wide spread of antibody concentrations was observed (Fig. 2).

All sera were also investigated for anti-*C. jejuni* IgG antibodies. No threshold

Table 1. *The seropositivity and specific IgG concentrations in Asian vegans and Asian and Caucasian meat-eaters*

Group*	No.	Age range	Percent positive	Specific IgG $\mu\text{g/ml}$ mean (\pm s.d.) median (range)	
				Seropositive	Seronegative
AV	68	18-67	36	66.4 (30.5)	2.0 (2.3)
				90 (11.6-90)	1.2 (0.2-9.4)
AME	105	20-69	32.1	53.5 (33.1)	1.6 (1.9)
				55 (10.1-90)	0.7 (0.2-8.8)
CME	68	22-70	17.6	72.3 (26.5)	0.6 (0.8)
				90 (14.3-90)	0.4 (0.2-4.9)

* AV, Asian vegans; AME, Asian meat-eaters; CME, Caucasian meat-eaters.

Table 2. *The seropositivity and specific IgG concentrations in age- and sex-matched groups*

Group	No.	Percent positive	Specific IgG $\mu\text{g/ml}$ mean (\pm s.d.) median (range)	
			Seropositive	Seronegative
AV	54	35	60.8 (32.3)	2.2 (2.4)
			90 (11.6-90)	1.0 (0.2-9.4)
AME	54	33	55.3 (36.0)	1.7 (2.0)
			90 (10.1-90)	0.6 (0.2-8.8)
AME	65	32	53.9 (33.8)	1.9 (2.1)
			55 (10.1-90)	0.8 (0.3-8.8)
CME	65	16.9	75.6 (25.3)	0.5 (0.8)
			90 (14.3-90)	0.4 (0.2-4.9)

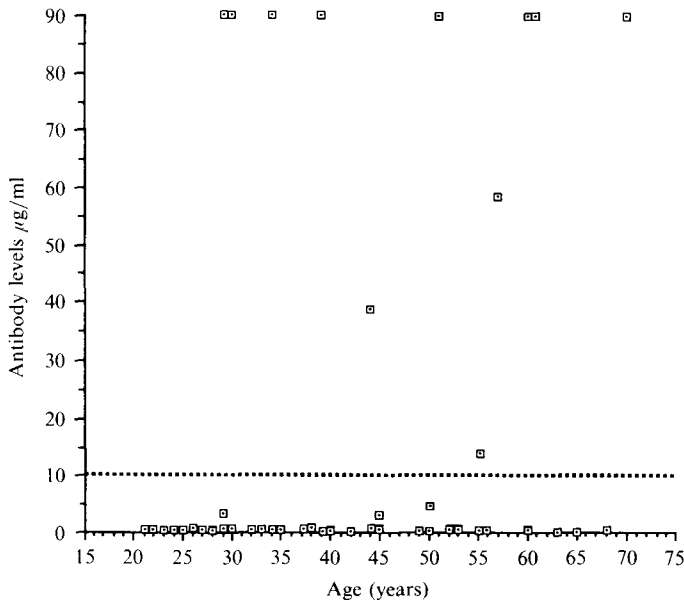


Fig. 1. The serum anti-*H. pylori* IgG antibody concentrations in Caucasian meat-eaters, age- and sex-matched to Asian meat-eaters.

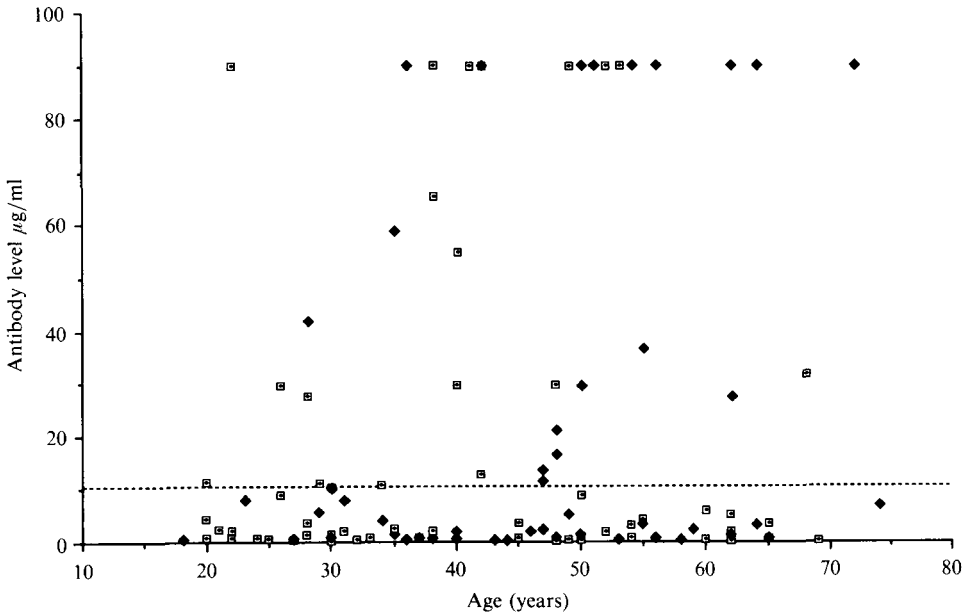


Fig. 2. The serum anti-*H. pylori* IgG antibody concentrations in age- and sex-matched Asian vegans (◆) and meat-eaters (□).

for seropositivity for this assay has been previously established. However, one individual convalescing from a confirmed *C. jejuni* infection, and who was unrelated to the current study, was used as a positive control. This patient had a pre-infection antibody level of 0.2 µg/ml and a convalescing (19 days post onset of symptoms) antibody level of 3.2 µg/ml. Only one Caucasian, but five meat-eating Asians and eight life-long vegans had anti-*C. jejuni* antibody levels of over 1 µg/ml. There was, however, no correlation between raised levels of antibodies to *C. jejuni* and *H. pylori*, in any group.

DISCUSSION

The validity of seroepidemiological methods, for determining the prevalence of gastric colonization with *H. pylori* in populations, is now widely accepted [12]. Such assays indicate a higher proportion of seropositive individuals among abattoir workers, veterinary surgeons and meat workers compared with controls [6, 7], which raises the possibility of meat being a source of *H. pylori*. However, a number of other epidemiological factors can influence prevalence of infection including social group and age so that the identification of suitable controls for such investigations becomes difficult. We, therefore, investigated the eating of meat as a risk factor because suitable population controls were readily identifiable. The similar prevalence of seropositivity in Asian vegans and Asian meat-eaters suggests that meat ingestion is not a significant risk factor for *H. pylori* infection. All dietary habits had been life-long, eliminating the possibility of childhood exposure to meat in the vegan population. In view of the species restriction of susceptibility to *H. pylori* colonization, the absence of meat ingestion as a risk factor seems reasonable. Apart from man, *H. pylori* has only been isolated from

non-human primates [13] and rarely from the pig [8]. It seems, therefore, that an animal reservoir is unlikely.

The significant difference between seropositivity in the Asian and Caucasian groups is of major importance. Previous studies also suggest that certain racial or geographically-defined populations have high prevalences of *H. pylori* infection [5]. This is assumed to be a reflection of infection acquired early in life and a consequence of differences in standards of living or sanitation [5]. In our study Asians were age- and sex-matched with Caucasians, eliminating age-related seropositivity as a variable. Moreover, there is no indication that the differences seen in our study are a reflection of socio-economic status, which can be considered similar in both racial groups attending out-patient clinics in this region of the United Kingdom. However, Asians frequently live in extended families. Previously, intrafamilial clustering of *H. pylori* infection [1] has been observed. The higher prevalence of raised antibodies against *C. jejuni* in Asians (4.6%) compared with Caucasians (1.4%) indicates recent incidences of campylobacteriosis in these populations. *C. jejuni* is largely a food-borne infection in the United Kingdom and could be considered an indicator of poor hygiene standards. So the racial differences in *H. pylori* infection could reflect different hygiene practices as well as perhaps larger family groups. The importance of such life styles in our study needs further investigation.

The use of a serological test which gives quantitative results allowed this study to highlight differences in the distribution of antibody levels, as well as seropositivity, between Asians and Caucasians. The reasons for such variations in the spread of antibody levels are unknown. Antigenic cross-reactivity between *C. jejuni* and *H. pylori* may be a complication in the interpretation of seroepidemiological data in populations where *C. jejuni* is endemic. Nevertheless, it is obvious that the generally raised antibody levels were not, in this study, a consequence of *C. jejuni* antigenic cross-reactivity. However, alternative causes of the detection of non-*H. pylori* specific antibodies cannot be eliminated and the introduction of serological assays based on defined, rather than complex, antigens may reduce this problem. It is also likely that this spread of antibody levels is not an artifact and reflects exposure to antigen without acquisition of chronic infection in these individuals. These results, therefore, have important implications for the criteria for seropositivity in various ethnic groups and suggest that the threshold for seropositivity, established for this routinely used serodiagnostic assay, should be increased for Asians in the United Kingdom.

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