HELICOBACTER PYLORI

Evidence that *cagA⁺ Helicobacter pylori* strains are disappearing more rapidly than *cagA⁻* strains

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Background and aims: The prevalence of *Helicobacter pylori* colonisation in populations in developed country has been declining, as shown by community based serological surveys of adults in Vammala, Finland in 1973 and 1994. In this study, we determined whether the proportion of subjects colonised by *cagA⁺* or *cagA⁻ H pylori* strains has changed as the overall prevalence of *H pylori⁺* has declined.
Methods: We examined 911 sera from Vammala's study for antibodies to the CagA antigen of *H pylori* using a truncated CagA protein as the antigen in an ELISA and we examined the trend in acquisition and carriage of *cagA⁺* strains.
Results: As expected, the prevalence of carriage of both *cagA⁺* and *cagA⁻* strains fell between 1973 and 1994 (p<0.001). However, the prevalence of *cagA⁺* strains among those <45 years declined (34% to 8%) significantly (p<0.001) more than for *cagA⁻* strains (12% to 6%). Of 221 subjects with paired serum samples, 12 (5.4%) changed *H pylori* status; the estimated seroconversion and reversion rates were 0.4% and 0.13% per year, respectively. Except for the few individuals who changed serostatus, absolute antibody levels to *H pylori* antigens, including CagA, changed little over the 21 year period.
Conclusions: The decline in CagA seroprevalence predominantly reflects declining acquisition of *cag⁺* strains in younger subjects. In addition, these data confirm that *H pylori* acquisition chiefly occurs during childhood but continues to occur during adulthood, albeit at low rates, in developed countries.

T
here is increasing evidence that gastric colonisation of humans with *Helicobacter pylori* has been present at least for centuries, and is probably ancient (summarised by Blaser[1]). In developing countries, nearly all adults are *H pylori*[+2 3] but in developed countries the prevalence of *H pylori* is much lower.[4] In all populations the prevalence of *H pylori* increases with age[5–9] which is best explained by a birth cohort phenomenon with diminished acquisition during childhood as socioeconomic development has occurred.[10 11] In a Finnish population, the prevalence of *H pylori* colonisation substantially decreased between 1973 and 1994.[12] Colonisation decreased markedly between 1973 and 1994 from 56% to 31% (p=0.001).

H pylori strains are either *cagA⁺* or *cagA⁻* based on whether or not they possess a 128 kDa protein that is a marker for the presence of the 35–40 kb *cag* island.[13 14] This is a fundamental dichotomy among *H pylori* strains, associated with important differences in clinical outcome in Western populations.[15–18] A serological response to the CagA protein is strongly predictive that a patient is carrying a *cagA⁺* strain.[16 17 19] In general, although individuals are usually colonised with either *cagA⁺* or *cagA⁻* strains, mixed populations may be present, particularly in developing countries.[20 21] Elements in the *cag* island are responsible for the type IV secretion system mediated injection of the CagA protein into epithelial cells (see for example Odenbreit and colleagues[22]), explaining at least in part why CagA is an immunodominant antigen. Using the stored serum specimens from the Finnish population studied earlier,[12] we determined whether the proportion of subjects colonised by *cagA⁺* or *cagA⁻ H pylori* strains has changed as the overall prevalence of *H pylori⁺* has declined.

MATERIALS AND METHODS
Serum samples
Sera had been collected from 911 healthy subjects randomly selected from the population over 14 years of age in Vammala,

Finland in 1973 (n=408) and 1994 (n=503). The two populations tested in 1973 and 1994 came from the same sample of 16 000 inhabitants of Vammala which had not had any marked shifts in socioeconomic status. The population in Vammala was approximately 16 000 in both years, and descriptions of the study design have been reported.[12] We also examined paired serum samples from 221 healthy subjects obtained in both 1973 and 1994 that had been collected as part of this study.[12]

Serological assays
IgG and IgA antibodies to *H pylori* had been measured previously by an ELISA using an acid-glycine extract as the antigen, as previously reported.[12] The sensitivity and specificity of these assays were 94% and 93% for IgG and 73% and 95% for IgA, respectively.[12] Antibodies to the *H pylori* CagA antigen were measured using a recombinant CagA fragment by ELISA, and an optical density ratio (ODR) of ≥0.35 was considered positive, as described previously.[16] The sensitivity and specificity of the assay were 94.4% and 92.5%, respectively, in a US population.[16] All assays were performed at least in duplicate.

Statistical analysis
H pylori positive status was defined as a positive result in any of the three serological assays whereas *H pylori* negative status was defined when all three assays were negative. In subjects from whom paired specimens were available from 1973 and 1994, seroconversion or reversion was defined on the basis of

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Abbreviations: ODR, optical density ratio; RGMT, reciprocal geometric mean titre.

IgG response to the glycine extracted *H pylori* antigens. Differences in frequencies of response to CagA antigen among groups were examined by χ^2 analysis. The magnitude of the responses in different groups was analysed using the Student's *t* test for independent samples.

RESULTS

Validation of CagA threshold in the tested population

As gastric biopsies were not obtained from these healthy subjects, we sought an alternative way to determine whether the threshold for positivity in the CagA assay was valid in this population. To address this question, we used specimens from 65 subjects who were positive in both years for both the IgG and CagA assays as a gold standard to define subjects persistently colonised by *cagA+ H pylori* strains. For these 130 specimens, the mean (SD) ODR in the CagA assay was 0.78 (0.22). By subtracting two intervals of SD, we defined a level that was 97.5% likely to represent truly positive CagA status. This value (0.34) was nearly identical to the a priori value (0.35) determined based on a group of *H pylori* negative healthy children in the USA; this threshold represented the mean value plus 3 SD for the negative children.[16] Thus the results of the two methods were nearly identical, validating the threshold for CagA positivity used for this study population.

Prevalence of CagA antibodies in the 1973 and 1994 populations sampled

The prevalence of CagA antibodies in the sampled population significantly ($p<0.001$) declined from 36.5% in 1973 to 20.4% in 1994, which paralleled the overall decline in *H pylori* seroprevalence in the community during that time.[12] The decline was substantially the same for males and females (data not shown). Among subjects 14–44 years old, the CagA seroprevalence rate declined precipitously (34.3% to 8.0%; $p<0.0001$) (fig 1). Among subjects over 45 years, the decline (40.7% to 31.6%) was less substantial. The proportion of subjects seropositive for *H pylori* but negative for CagA fell less markedly between 1973 and 1994 in those aged 14–44 years (11.6% to 5.9%; $p=0.03$) and over 45 years (30.7% to 24.1%; NS). Thus although the proportion of subjects who were *H pylori* seropositive fell in both age groups, and declined for both strain types (CagA+ or CagA−), the largest effect was the decrease in CagA positivity among subjects aged 14–44 years. As the number of older individuals differed significantly in the two years studied, with a higher proportion (52.5%) of the population of Vammala in 1994 older than 45 years than in 1973 (34.3%), we calculated the proportion of *cagA* seropositivity in the 60–80 year age group at both points in the study, and the results were almost identical (35% in 1973 *v* 32.5% in 1994). In contrast, the prevalence of *cagA* seropositivity in the 45–59 year age group was greater in 1973 (41.3%) than in 1994 (29.9%). This is also consistent with the decline in *cagA* seropositivity observed in younger individuals, and suggests that the decline has been ongoing for decades.

We found 14 (3.4%) subjects who were *cagA+* but *H pylori* negative in 1973. Similarly, we found 20 (3.9%) subjects who were *H pylori* negative but *cagA+* in 1994. The prevalence of *cagA+* in the absence of *H pylori* antibody response in both years was slightly lower than the usual prevalence reported in other studies.[23 24] Subjects carrying *H pylori* may have substantial immune responses to the group antigen or to individual antigens (for example, CagA), reflecting the heterogeneity in the host responses in our outbred human population.

Seroprevalence in the cohort sampled 21 years apart

Sera from each of 221 subjects obtained both in 1973 and 1994 were examined to determine whether *H pylori* or CagA serostatus had changed. Of the 663 assays performed on sera obtained in 1994, 53 (8.0%) showed a change from 1973. In 23
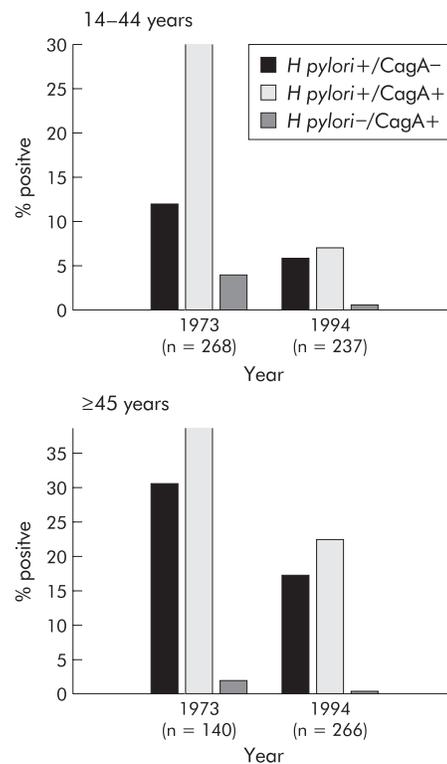

Figure 1 Change in seroprevalence of *Helicobacter pylori* carriage between 1973 and 1994 by age and *cagA* status (defined by CagA seropositivity). Both *H pylori* and *cagA* status were determined, and the 911 study subjects were stratified by age (14–44 and ≥45 years). Data are shown for specimens obtained in 1973 (n=408) and 1994 (n=503). The decline in seroprevalence of *H pylori cagA+* strains in subjects 14–44 years old was significantly ($p<0.001$) greater than that for *H pylori cagA−* strains.

(10.4%) subjects whose initial *H pylori* status was positive, one or more assays showed a change in status (in nine cases from positive to negative and in 14 cases from negative to positive) but the other assays did not change. Thus overall *H pylori* status for that subject remained positive. In total, only 12 (5.4%) subjects had a change in overall status over the 21 years (table 1). Of 115 subjects who were seronegative in 1973, nine (7.8%) seroconverted in one or more (mean 2.7 (0.7)) assays over the next 21 years, giving a crude seroconversion rate of 0.4% per year. Of the nine seroconverters, seven also became positive in the IgA assay, and eight also became positive in the CagA assay. The mean (SD) ages in 1973 of persistently seronegative subjects (31.7 (10.9) years) and seroconvertors (28.6 (7.9) years) were very similar, as were sex distributions. Of the 106 subjects who were defined as seropositive in 1973, only three (2.8%) seroreverted; each of these subjects had only been positive for *H pylori* IgG in 1973. The three seroreverters were not significantly different in age or sex distribution from the 103 serofast subjects. The crude seroreversion rate was 0.13% per year.

Stability of antibody responses

We next sought to determine the degree of fluctuation in the level of antibodies present among *H pylori+* subjects over the 21 year period (table 2). For the 95 subjects who were IgG+ for *H pylori* antibodies in 1973, 87 (91.6%) remained positive in 1994 and the overall reciprocal geometric mean titre (RGMT) decreased from 3179 to 3009 (5.3%) by 1994. Excluding the three subjects who seroreverted in 1994 and the five other subjects who only reverted in IgG but *H pylori* status did not change, for the remaining 87 subjects the RGMT changed from 3203 to 3411 (6.1% increase; NS), representing a mean

Table 1 Changes in status in *Helicobacter pylori* assays among 221 subjects in Vammala, Finland, examined in both 1973 and 1994

| Serological status | Total No of subjects affected | Assay that changed in status | | | | Mean (SD) No of assays with changes |
		IgG	IgA	CagA	Any	
Negative→positive*	9	9	7	8	24	2.7 (0.7)
Positive→negative†	3	3	0	0	3	1.0 (0.0)
Total (%)‡	12 (5.4)	12 (5.4)	7 (3.2)	8 (3.6)	27 (4.1)	2.3 (1.0)

*This change in status was defined as seroconversion.
†This change in status was defined as seroreversion.
‡Numbers in parentheses indicate the proportion of the total population of 221 represented by the subjects who showed a change in status in the specified assay.

Table 2 Stability of antibody responses to *Helicobacter pylori* antigens over 21 years in 95 subjects* who were IgG seropositive in 1973

| Assay | No of subjects positive in that assay | | Antibody level | |
	1973	1994	1973	1994
H pylori IgG†	95	87	3179	3009
H pylori IgA†	59	54	180	201
CagA IgG‡	69	69	0.75 (0.24)	0.80 (0.21)

*In 95 subjects (mean age 35.0 (12.0) years) who were IgG seropositive in 1973.
†Shown as reciprocal geometric mean titres (RGMT).
‡Shown as mean optical density ratio units (SD) .

increase of 0.29% in antibody level per year. Among the 59 subjects who also were IgA⁺ in 1973, 54 (91.5%) remained positive in 1994, and the overall RGMT also changed little (table 2), representing a mean annual increase of 0.6%. All 69 subjects who were CagA⁺ in 1973 remained positive in 1994, with a mean annual increase of 0.3% in measurable antibody levels (table 2).

DISCUSSION

The major observation of this study is that over the 21 year period, the seroprevalence of *cagA*⁺ *H pylori* strains declined faster than that for *cagA*⁻ *H pylori* strains, especially among subjects less than 45 years of age. This difference may be related to differential acquisition and loss rates in later birth cohorts. That among the 106 seropositive subjects in 1973 there were very few seroreversions (2.8%) by 1994 suggests that the overall decline in *H pylori* seroprevalence in Vammala between 1973 and 1994 predominantly reflected decreased acquisition of *H pylori* colonisation. However, as the subjects we tested were all over 14 years, our findings did not directly examine *H pylori* loss during early childhood. In many developed countries, the intensity of antibiotic exposure during childhood has been increasing markedly.[25][26] Even with the minimal success of antimicrobial monotherapy against *H pylori*,[27][28] the effects of repeated antibiotic regimens over the course of childhood may be substantial.[29] The accumulating evidence that *cagA*⁺ strains are more susceptible than *cagA*⁻ strains to eradication treatment[30][31] is consistent with the trends (fig 1) we observed.

Addition of the CagA serological assay to those for antibodies to *H pylori* acid-glycine extracted antigens increases the sensitivity of detecting *H pylori* carriage as there are subjects with strong responses to the immunodominant CagA antigen who do not meet the criteria for positivity in the assays using the acid extracted antigens.[32] Despite differences in the primary structure of *cagA* related to the geographic origin of strains,[33][34] host response to CagA is well preserved, as

described previously,[19] and as further validated in this study. The nearly identical serum antibody levels to CagA (and to the acid-glycine extracted antigens) in the same individuals 21 years apart (table 2) confirms both the stability of these responses and the longstanding equilibrium between hosts and their specific *H pylori* populations.[35] These results also indicate that there are no substantial age related changes among adults in serological responses to CagA antigens that could explain the declining prevalences observed between 1973 and 1994. These data confirm that *H pylori* acquisition chiefly occurs during childhood[2][3][5] but continues to occur during adulthood, albeit at low rates, in developed countries.[10][12][25][36]

An alternate but not mutually exclusive hypothesis is that with socioeconomic development, transmission of *cagA*⁺ strains has become proportionally less efficient than for *cagA*⁻ strains in this community. However, the observation that among the nine subjects who seroconverted nearly all were CagA positive contradicts this hypothesis. However, we have not examined a prior time period, and it is possible that transmission of *cagA*⁺ strains during adulthood was even more efficient previously than during the period 1973 to 1994. Another possible explanation is that as socioeconomic status increases, children become less important as a source of infection of adults (and adult to adult transmission thus becomes relatively more important). As such the few adult seroconversions that occur mirror existing adult infections (from early cohorts) their present childhood infections.

There has been a differential decline in transmission of the various *Shigella* species with improving hygienic conditions during the 20th century in which the most virulent strains (*S dysenteriae*) have disappeared most quickly.[37] Among populations in developing countries, in which nearly all adults are *H pylori* ⁺, most are also CagA seropositive.[38–40] In contrast, in developed countries in which *H pylori* seroprevalence is lower, higher proportions of strains are *cagA*⁻.[38][41][42] Our present data suggest that this change in proportion has been a dynamic process that occurred during socioeconomic development, probably as a birth cohort phenomenon, resulting from differential acquisition and/or loss rates between *cagA*⁺ and *cagA*⁻ strains. Since this study is the first to report the decline in *cagA*⁺ strains in developed countries, it will be important to seek independent confirmation. As *cagA*⁺ strains have more intense interactions with human epithelial cells than *cagA*⁻ strains,[15][43][44] and are associated with increased risk of both duodenal ulceration[17][42] and gastric cancer,[15][45] and with a decreased risk of oesophageal diseases,[23][46] the consequences of enhanced loss of *cagA*⁺ strains for human health may be profound.

ACKNOWLEDGMENTS

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Conflict of interest: Dr Blaser has a potential royalty interest in CagA diagnostics (licensed by Vanderbilt University) based on his discovery of CagA.

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