

## ***Campylobacter pylori*: clinical, histological, and serological studies**

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**SUMMARY** The presence of *Campylobacter pylori*, histologically diagnosed gastritis, and antibodies to *C pylori* were determined in a series of 113 patients undergoing endoscopy. Paired biopsy specimens from the fundus, body, and antrum were collected from 59 patients and from the antrum of 54 patients. The presence of *C pylori* was confirmed by either culture or silver stain in 30 of 59, 31 of 59, and 54 of 103 biopsy specimens from the fundus, body, and antrum, respectively. Of the specimens which contained *C pylori* 20 of 30 (66%) from the fundus, 25 of 31 (80%) from the body, and 54 (100%) from the antrum showed gastritis. *C pylori* and gastritis were shown in seven of nine (78.1%) of patients with gastric ulcers and in nine of 11 (82%) of patients with duodenal ulcers. Using an enzyme linked immunosorbent assay (ELISA) technique to detect IgG antibody to *C pylori*, all patients with histologically diagnosed gastritis and organisms present had titres of  $\geq 640$ ; eight of 39 (21%) of patients without gastritis and without organisms gave similar titres. Hence the presence of *C pylori* was associated with gastritis and with raised titres of IgG antibody.

Spiral organisms have been described in the stomach of man and other animals by several authors since the 1920s.<sup>1-5</sup> Following the reports of Warren<sup>6</sup> and Marshall<sup>7</sup> there has been an increase in interest in the organism which these authors named *C pyloridis* and which has since been redesignated *C pylori*.<sup>8</sup> These *Campylobacter*-like organisms and others<sup>9</sup> have been isolated from gastric biopsy specimens using techniques which were developed for the isolation of intestinal campylobacters.

Despite the profusion of short reports there have been few detailed substantive prospective studies, especially in Great Britain. We therefore present the results of our study in which 113 patients were investigated endoscopically, histologically, bacteriologically and serologically. Part of this study was designed to determine the distribution of *Campylobacter*-like organisms in infected or colonised stomachs, an aspect which has not clearly been established. The survey was also undertaken so that we could substantiate the association of these organisms with histologically diagnosed gastritis, gastric, and duodenal peptic ulceration. Furthermore, we inves-

tigated the value of an enzyme linked immunosorbent assay (ELISA) technique for detecting the antibody response in patients included in the survey.

### **Material and methods**

A series of 113 patients with a clinical indication for upper gastrointestinal endoscopy, originating from outpatient and inpatient referrals over three months in 1984 was studied. These patients comprised 71 men and 42 women with an age range of 19-91 years. The patients had a variety of symptoms relating to the upper gastrointestinal tract, most commonly, epigastric pain or dyspepsia, or both, and some presented with an acute gastrointestinal haemorrhage.

### **ENDOSCOPY AND SAMPLING PROCEDURES**

A 10 ml sample of blood was taken from each patient for serological tests, immediately before the endoscopy procedure. Biopsy specimens were obtained using biopsy forceps which had been disinfected in 2% glutaraldehyde and rinsed in distilled water. From the first 59 patients paired biopsy specimens from three sites were taken in the following order: gastric fundus, body, and antrum. In the remaining 54 patients paired biopsy specimens from the antrum only were

obtained. Examination of the duodenum was undertaken last to avoid contamination of the endoscope with intestinal bacteria.

#### HISTOLOGY

One biopsy fragment from each site was fixed immediately in 10% neutral buffered formalin, then processed routinely, and embedded in paraffin wax. Paired 5  $\mu$ m sections were cut and stained with haematoxylin and eosin and by a silver impregnation method (Warthin-Faulkner).<sup>10</sup>

Histological assessment of the stained sections was made independently by two histopathologists and included: (i) type and thickness of mucosa; (ii) presence or absence of chronic gastritis; and (a) whether quiescent or active in type, and (b) whether superficial or full thickness gastritis, using the guidelines of Whitehead<sup>11</sup>; (iii) glandular atrophy; (iv) intestinal metaplasia; (v) presence and distribution of Campylobacter-like organisms.

#### BACTERIOLOGY

The other biopsy specimen of any pair was placed into a dry sterile bijoux bottle and transported to the laboratory where it was cultured within two hours of collection. The biopsy specimens were bisected with sterile forceps and the freshly cut surface inoculated on to blood and chocolate agars. These plates were incubated microaerobically at 37°C for up to five days. Isolates were confirmed as *C. pylori* by colonial morphology, Gram stain morphology, electron microscopy, positive catalase, oxidase and urease reactions, negative nitrate and hippurate reactions, resistance to nalidixic acid (30  $\mu$ g disc) and sensitivity to cephalothin (30  $\mu$ g disc).

#### SEROLOGY

The antigen for the ELISA test was prepared from six strains of *C. pylori* grown on chocolate agar, incubated microaerobically at 37°C for three to four days. The growth was harvested into 10 ml of sterile saline, mixed for one minute on a vortex mixer, centrifuged at 3000 rpm for 15 minutes and the supernatant retained. Thiomersal was added to a final concentration of 0.01% and the antigen stored at 4°C. This antigen was diluted 1 in 400 and used to determine the titre of antibodies to *C. pylori* by a conventional ELISA technique.<sup>12</sup>

#### Results

##### HISTOLOGY

One hundred and eight of the 113 patients examined gave satisfactory biopsy specimens for analysis and these comprised 59 from whom multiple site specimens were taken and 49 from whom antral specimens only

Table 1 Histologically assessed inflammation in gastric biopsy specimens

| Type of gastritis        | Percentage of specimens showing gastritis |                  |                     |
|--------------------------|---|------------------|---------------------|
|                          | Fundus<br>(n = 59)                        | Body<br>(n = 59) | Antrum<br>(n = 103) |
| None                     | 63  | 52               | 40                  |
| Superficial gastritis    |   |                  |                     |
| Active                   | 7   | 5                | 13                  |
| Quiescent                | 20  | 29               | 12                  |
| Full thickness gastritis |   |                  |                     |
| Active                   | 7   | 5                | 20                  |
| Quiescent                | 3   | 7                | 10                  |
| Not assignable*          | 0   | 2                | 5                   |

\*Specimens showing gastritis but of insufficient thickness to subclassify further.

were taken. Five of the 59 patients in the first series had previously had a partial gastrectomy and therefore it was not possible to collect biopsy specimens from the antrum. The 108 patients studied yielded a total of 59 biopsy specimens from the fundus and body and 103 from the antrum.

Some specimens were of insufficient depth to differentiate between superficial and full thickness inflammation and were termed "not assignable". Full thickness gastritis was not always accompanied by glandular atrophy or intestinal metaplasia. Activity of inflammation implied high numbers of polymorphs in the lamina propria or within the epithelium. The results (table 1) showed an increase in the prevalence of gastritis, in particular full thickness gastritis and active gastritis, towards the antrum. In the first series 14 cases showed glandular atrophy and two cases showed intestinal metaplasia at one or more sites. In the second series three cases showed intestinal metaplasia.

Campylobacter-like organisms shown by silver stain were curved or spiral rods, and within any individual biopsy specimen the distribution of these

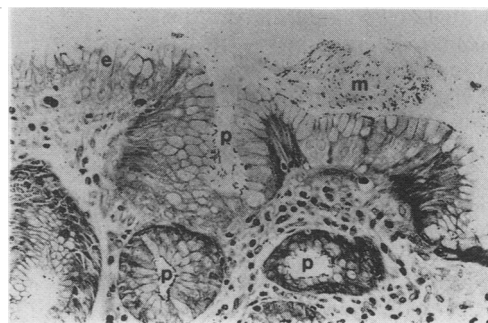


Fig 1 General view of superficial gastric mucosa. Campylobacter-like organisms are seen within surface mucus (m), distributed along the surface epithelium (e), and within lumen of gastric pits (p). (Warthin-Faulkner).

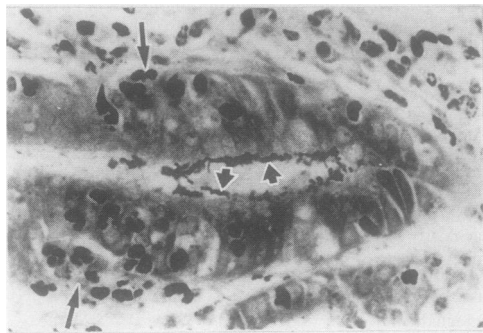


Fig 2 Demonstration of *Campylobacter*-like organisms within a gastric pit closely related to the surface of epithelial cells (short arrows). Neutrophil polymorphs (long arrows) are infiltrating the glandular epithelium. (Warthin-Faulkner).

organisms was patchy. They were often seen lying close to the surface epithelium, within and beneath surface mucus, and sometimes were seen around the intercellular margins of surface epithelial cells. They were most densely distributed within the lumen of the gastric pits (figs 1 and 2). Much less often they extended into the lumen of deeper glands. There were no convincing intracellular organisms, and none was seen in the lamina propria.

#### DETECTION OF CAMPYLOBACTER-LIKE ORGANISMS AND DENSITY OF COLONISATION

Detection of *Campylobacter*-like organisms in biopsy specimens was determined by their presence in silver stained sections or by culture (table 2). Silver stain alone or culture alone failed to detect *Campylobacter*-like organisms in a few cases, possibly reflecting patchy distribution of the organisms or poor quality of the biopsy specimen. When the results of silver staining and culture are combined then all or none of the multiple site biopsy specimens (first series) from an individual case contained organisms.

Qualitative assessment of *Campylobacter*-like organisms grown from biopsy specimens showed that there was a trend of increasing numbers of organisms towards the antrum (fig 3). Thirty per cent of culture positive biopsy specimens from the fundus produced a heavy (+++) or moderate (++) growth of *Campylobacter*-like organisms compared with 60% from the body and 78% from the antrum.

#### ASSOCIATION BETWEEN GASTRITIS AND PRESENCE OF CAMPYLOBACTER-LIKE ORGANISMS

Of those biopsy specimens from the fundus, body, and antrum which contained *Campylobacter*-like organisms 20 of 30, 25 of 31, and 54 of 54, respectively, showed gastritis (table 2). Antral biopsy specimens containing *Campylobacter*-like organisms were three

Table 2 Association between histological gastritis and presence of *Campylobacter*-like organisms

| Biopsy site and inflammation | Percentage of specimens containing <i>Campylobacter</i> -like-organisms |                     |                                 |                               |
|------------------------------|---|---------------------|---------------------------------|-------------------------------|
|                              | Detected by silver stain  | Detected by culture | Total detected by either method | Not detected by either method |
| <b>Fundus</b><br>(n = 59)    |   |                     |                                 |                               |
| Gastritis                    | 30  | 29                  | 34                              | 3                             |
| No gastritis                 | 14  | 15                  | 17                              | 46                            |
| <b>Body</b> (n = 59)         |   |                     |                                 |                               |
| Gastritis                    | 41  | 42                  | 42                              | 5                             |
| No gastritis                 | 10  | 9                   | 10                              | 43                            |
| <b>Antrum</b><br>(n = 103)   |   |                     |                                 |                               |
| Gastritis                    | 50  | 46*                 | 52†                             | 7‡                            |
| No gastritis                 | 0   | 0                   | 0                               | 41                            |

\*Ninety eight specimens cultured.

†Includes four specimens that were not cultured.

‡Includes one specimen that was not cultured.

times more likely to show full thickness gastritis than superficial gastritis and twice as likely to show active rather than quiescent inflammation. *Campylobacter*-like organisms were seen in the absence of gastritis in 10 of 37 fundal biopsy specimens and six of 31 body biopsy specimens. In none of the three sites was there any consistent relation between subjectively assessed degree of inflammation and density of colonisation by organisms. Gastritis in the absence of organisms was present in two of 59 (3%), three of 59 (5%), and seven of 103 (7%) of biopsy specimens from the gastric fundus, body, and antrum, respectively.

#### ASSOCIATION BETWEEN OTHER GASTRIC FINDINGS AND PRESENCE OF CAMPYLOBACTER-LIKE ORGANISMS

Biopsy specimens from the fundus which contained *Campylobacter*-like organisms were twice as likely to show glandular atrophy as those without organisms; the corresponding figure was one and a half times for

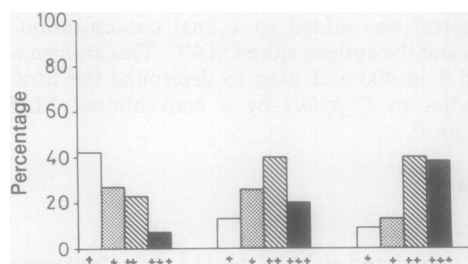


Fig 3 Qualitative growth estimates of *Campylobacter*-like organisms in biopsy specimens from the gastric fundus, body, and antrum.

Table 3 Comparison of endoscopy findings with the presence or absence of *Campylobacter*-like organisms in 108 patients

| Endoscopy findings          | Percentages of patients in each group         |   |
|-----------------------------|---|---|
|                             | <i>Campylobacter</i> -like organisms detected | <i>Campylobacter</i> -like organisms not detected |
| Normal (n = 29)             | 39  | 61  |
| Duodenitis (n = 21)         | 57  | 43  |
| Bile reflux (n = 22)        | 50  | 50  |
| Gastritis (n = 52)          | 50  | 50  |
| Partial gastrectomy (n = 5) | 80  | 10  |
| Gastric ulcer (n = 9)       | 78  | 22  |
| Duodenal ulcer (n = 11)     | 82  | 18  |

the body; no excess was seen in antral biopsy specimens. Intestinal metaplasia was present in six specimens (five subjects). All showed variable degrees of gastritis, and although one of these contained *Campylobacter*-like organisms, the organisms were absent from areas of intestinal metaplasia. Biopsy specimens taken from sites remote from gastric and duodenal ulcers contained *Campylobacter*-like organisms in seven of nine and nine of 11 cases, respectively, and these also showed gastritis at all or most sites. *Campylobacter*-like organisms were present in five (100%) of fundal biopsy specimens and in four of five (75%) of body biopsy specimens from cases of partial gastrectomy and were associated with variable degrees of gastritis, of no specific type.

#### ASSOCIATION BETWEEN ENDOSCOPY FINDINGS AND PRESENCE OF *CAMPYLOBACTER*-LIKE ORGANISMS

Of the 108 patients investigated, five had a previous partial gastrectomy, nine had a gastric ulcer, and 11 had a duodenal ulcer. In these patients there was a definite association between the endoscopy findings and the presence of *Campylobacter*-like organisms (table 3). Patients with endoscopically normal gastric mucosa were more likely to have specimens without *Campylobacter*-like organisms. Patients with bile reflux or endoscopically diagnosed gastritis were found equally likely to yield biopsy specimens with or without *Campylobacter*-like organisms.

#### SEROLOGY

IgG titres determined by an ELISA method are shown in table 4. All of the patients with histologically diagnosed gastritis and *Campylobacter*-like organisms detected had IgG titres of  $\geq 640$ . Only eight of thirty nine (21%) of patients without gastritis and without *Campylobacter*-like organisms gave similar titres. Similarly, all patients with duodenal or gastric

ulcers and *Campylobacter*-like organisms detected had IgG titres of  $\geq 640$  whereas only one of four patients with ulceration but without *Campylobacter*-like organisms gave a similar titre.

#### Discussion

This study differed from other prospective studies in that multiple biopsy specimens were taken from the three different areas of the stomach. There was excellent correlation between histological identification and isolation of organisms from paired specimens, a finding in accord with previous studies.<sup>13,14</sup> The few cases in which small numbers of organisms were seen histologically and were not cultured may represent patchy distribution as described by Goodwin *et al.*<sup>13</sup> The morphology and location of the organisms was similar to the descriptions by Warren<sup>6</sup> and Jones *et al.*<sup>14</sup> In agreement with these workers, intracellular organisms were not seen; nor were organisms definitely shown within the mucus of epithelial cells as reported by Meyrick-Thomas *et al.*<sup>15</sup> The distribution around the luminal margin of cells, however, was as shown by Phillips *et al.*<sup>16</sup>

The overall proportion of antral biopsy specimens (52%) positive for *Campylobacter*-like organisms, determined by cultural and histological techniques, was of the same order as most other reports which range between 42% and 66%, although in two larger series of 300 and 222 patients the respective identification rates were 39% and 35%.<sup>8,17</sup> In the present study and in common with other workers we have found that

Table 4 IgG serum antibody titres to *C pylori* in endoscopy patients

| Patient category   | Percentage of patients with ELISA IgG titres* |     |             |
|--|---|-----|-------------|
|  | $\leq 320$                                    | 640 | $\geq 1280$ |
| Gastritis†   |   |     |             |
| <i>Campylobacter</i> -like organisms detected (n = 59)     | 0   | 7   | 93          |
| <i>Campylobacter</i> -like organisms not detected (n = 9)  | 44  | 33  | 23          |
| Gastritis absent   |   |     |             |
| <i>Campylobacter</i> -like organisms detected (n = 1)      | 100   | 0   | 0           |
| <i>Campylobacter</i> -like organisms not detected (n = 39) | 79  | 8   | 13          |
| Duodenal or gastric ulcer                                  |   |     |             |
| <i>Campylobacter</i> -like organisms detected (n = 16)     | 0   | 12  | 88          |
| <i>Campylobacter</i> -like organisms not detected (n = 4)  | 75  | 0   | 25          |

\*Titres expressed as reciprocals

†Histologically diagnosed

there was a clear association between the presence of organisms and histological gastritis. In the antrum *Campylobacter*-like organisms were detected in 54 (88%) of the 61 biopsy specimens showing gastritis but were not detected in the 43 with normal morphology. This high correlation between gastritis and the presence of organisms did not extend to the body and fundus, where 16 of 59 (27%) of fundal and 11 of 59 (19%) of body biopsy specimens which were not inflamed contained *Campylobacter*-like organisms. In similar patients, however, Rathbone<sup>18</sup> and Goodwin *et al*<sup>19</sup> reported that gastritis was usually present somewhere in the stomach, and in our series this was so in all but two subjects. One of these had had a partial gastrectomy: in the other subject, who had no serological evidence of infection, there was a very small number of organisms in the biopsy specimens, perhaps representing contamination during collection or an early primary infection. In another series Rollason *et al*<sup>20</sup> were unable to find any association between the degree of colonisation by organisms and the severity of gastritis, and our results accord with that observation. Marshall and Warren<sup>21</sup> have linked activity of gastritis with colonisation by *Campylobacter*-like organisms but their series comprised mainly antral biopsy specimens. Our findings suggest that this limited sampling may have led to an overestimation of the prevalence of active inflammation in association with colonisation by *Campylobacter*-like organisms.

In our series a group of histological features, described by Dixon *et al*,<sup>22</sup> were seen in a few cases with endoscopically diagnosed bile reflux but only in the absence of *Campylobacter*-like organisms as reported by O'Connor *et al*.<sup>23</sup> There was, however, reflux associated with a very active or quiescent gastritis in almost equal numbers of cases with and without *Campylobacter*-like organisms present. Attention has been drawn to the absence of *Campylobacter*-like organisms in biopsy specimens showing intestinal metaplasia,<sup>13,19</sup> and in all but one of the six cases in the present study organisms were not detected. Only a small number of patients with peptic ulceration or partial gastrectomy for previous peptic ulceration were included in this series but at 80% the association of *Campylobacter*-like organisms and coexistent gastritis with peptic ulceration was of the same order as in other reports.<sup>21,24,25</sup>

In our study there was excellent correlation between the presence of *Campylobacter*-like organisms, gastritis, and raised titres of IgG antibody ( $\geq 640$  by ELISA). A similar finding has also been shown by Jones *et al*<sup>14</sup> and McNulty *et al*<sup>26</sup> using complement fixation tests, and this would suggest that the application of serodiagnosis to gastritis associated with *Campylobacter*-like organisms could result in a reduced need for endoscopy. In three of five patients

with IgG titres of  $\geq 640$  and gastritis but in whom *Campylobacter*-like organisms were not shown, multiple biopsy specimens had been collected, and in each subject gastritis was observed only at one site. These findings help to confirm the concept of patchy distribution of gastritis and probably also patchy distribution of organisms. In the present study IgG titres of  $\geq 640$  were found in all patients with *Campylobacter*-like organisms, gastric or duodenal ulcers, and gastritis at some location in the stomach, whereas in a similar study Marshall *et al*,<sup>27</sup> using a passive haemagglutination test, 93% of such patients had high titres of antibody.

Neither the symptoms of the patients investigated nor the endoscopic findings showed any correlation with the presence (or absence) of *C. pylori*. Although there is excellent correlation between the presence of *C. pylori*, gastritis, peptic ulceration and raised IgG antibody titres, the clinical importance of *Campylobacter*-like organisms in the aetiology of gastric illness has not been defined. This subject has, however, been widely discussed in many papers and extensively reviewed.<sup>19</sup> To date, the most persuasive evidence of an aetiological role is the attempt to fulfil Koch's postulates by ingesting a culture of *Campylobacter*-like organisms after first inducing hypochlorhydria.<sup>28</sup> Under physiological conditions, however, both gastritis and *Campylobacter*-like organism colonisation may be secondary to some other factor which is less operative at a distance from the antrum, thus accounting for the variable distribution and density of the colonisation and associated gastritis in other sites.

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