

Survival of *Campylobacter pylori* in artificially contaminated milk

The mode of transmission of *Campylobacter pylori* is unknown. Serological studies have shown that antibodies to *C pylori* increase with age.¹ Higher titres of antibodies to *C pylori* have been noted in occupational groups associated with domestic animals,² but there has been no report of isolation of *C pylori* from domestic animals, except from gnotobiotic piglets following experimental infection. (Proceedings of the IV International Workshop on Campylobacter contamination, 1987.) With the level of infection at 70-95% in some parts of the world (Workshop on gastroduodenal pathology and *C pylori*, 1988) the possibility of faecal-oral transmission remains. If the organism is transmitted by this route then it will have to survive in the external environment for a considerable period of time. We decided to look at the survival capacity of *C pylori* in milk, which is often ingested unheated, consumed universally, and where contamination after pasteurisation is possible.

Sterile ultra heat treated milk was used for the experiment, as unpasteurised and pasteurised milk often contained contaminating organisms. The milk was distributed in 5 ml volumes in bijoux bottles under sterile conditions. A three day old culture of *C pylori* on blood agar medium was suspended in 2 ml of sterile saline, and 20 µl aliquots of this suspension were added to four of the bijoux bottles of milk. Two bottles were maintained in air at room temperature and two at 4° C.

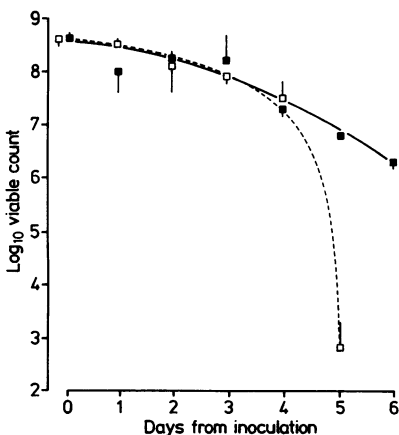


Figure Each square represents the mean of the three counts and the vertical bar represents the standard deviation of each set of observation. Survival curves of *C pylori* in artificially contaminated milk. ■—■ = 4° C; □ - - - □ = room temperature.

Just after adding the bacteria, a 0.1 ml aliquot from each of the inoculated samples of milk was transferred to 9.9 ml of sterile saline and cultured quantitatively by the Miles and Misra technique.³ This result was used as the value for inoculum added. Uninoculated milk was plated out to check sterility. On each of the following eight days, 0.1 ml milk was transferred from these bottles and cultured quantitatively as detailed above. These experiments were repeated on three occasions and the mean results are shown in the figure.

Our results show that *C pylori* did not multiply in milk, but survived with a steady decline by one log in four days at both temperatures. At room temperature the colony count fell by four logs on the fifth day, and colonies were undetectable from six days onwards on each occasion. In contrast, at 4° C, 10⁶ colony forming units/ml could be recovered up to six days on all occasions.

Pasteurisation will kill *C pylori*, therefore it is unlikely that milk will act as the usual vehicle of transmission. Survival of *C pylori* in water, food, and environmental samples requires further investigation.

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Ultrastructural localisation of urease in outer membrane and periplasm of *Campylobacter pylori*

It has been suggested that *Campylobacter pylori* might interfere with the integrity of the gastroduodenal mucosal barrier and may have a causal role in the pathogenesis of gastroduodenal diseases.¹ In 20% of *C pylori* positive cases viable organisms are isolated from the gastric juice. The ability of the bacterium to survive the bactericidal acidity present in the stomach is mostly attributable

to a high bacterial urease activity. A considerable concentration of urea is also usually found in the stomach in patients with active chronic gastritis.^{2,3} Urea and urease seem to be important prerequisites for the survival and colonisation of *C pylori* in the mammalian stomach. The hydrolysis of urea molecules surrounding the bacteria will produce ammonia which acts as an acceptor for H⁺ ions, thus leading to ammonium H⁻ ions with a consequent rise in local pH.^{1,2} To show such a mechanism, it could be postulated that the urease activity is localised at the outer membrane of the bacterium. To test this hypothesis we tried to localise the urease activity by an electron microscopic technique.

C pylori strains were isolated from gastric biopsy specimens taken by endoscopy from patients with active chronic gastritis. Specimens were cultured on blood agar (Wilken-Chalgren) supplemented after Skirrow (polymyxin B 1250 IU/l; trimethoprim-lactate 2.5 mg/l; vancomycin 5 mg/l). Cultures were incubated for four to five days at 37° C (5-12% carbondioxide and 5-15% oxygen). *C pylori* were identified by their characteristic enzyme profile—that is, catalase, oxidase, and urease and by light and electron microscopic techniques. The ultrastructural urease localisation was mainly done according to the method of McLean *et al.*⁴ Cells were suspended in a buffer solution containing 0.85% (w/v) N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) adjusted to pH 7.0. They were spun down (5000 rpm) in HEPES buffer for 10 minutes. After repeating this procedure cells were resuspended in the reaction solution (5mM sodium tetraphenylboron, 1 mM EDTA, 0.1 M urea dissolved in HEPES buffer), or control solution (without urea) for four hours. Cells were then spun down again and washed in HEPES buffer and resuspended in a silver replacement solution (2%-AgNO₃ dissolved in buffer) and left overnight at room temperature in the dark. Cells were then fixed in 5% HEPES buffered glutaraldehyde for 12 hours and washed several times before processing for transmission electron microscopy, omitting further staining with heavy metals. Thin sections were partly stained with 1% aqueous uranyl-lactate.

C pylori cells incubated with urea in the presence of sodium tetraphenylboron showed silver precipitates, which are predominately associated with the periplasm and outer membranes of the bacteria. These precipitates were not observed in control preparations incubated in the absence of urea (data not shown).

Letters to the Editor

The results accord with the suggestion that *C pylori* is able to neutralise bactericidal gastric acid very rapidly by buffering H⁺ ions. The rapid alkalisation of the microenvironment around *C pylori* seems likely to be mediated by the great amount of active urease available in the outer membrane of the bacterium. Thus urease of *C pylori* is a highly active enzyme that may be associated with virulence by different means: first, the urease activity protects the bacterium from the gastric acid; and secondly, the generated ammonia may then cause cytopathic effects like cell damage and possibly inflammation.⁵

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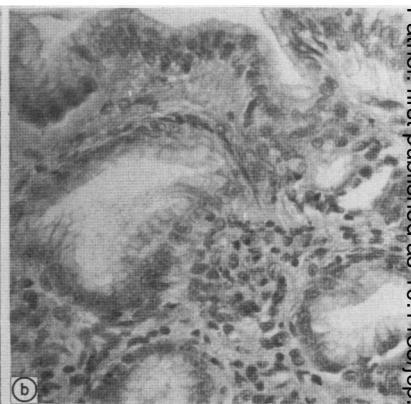
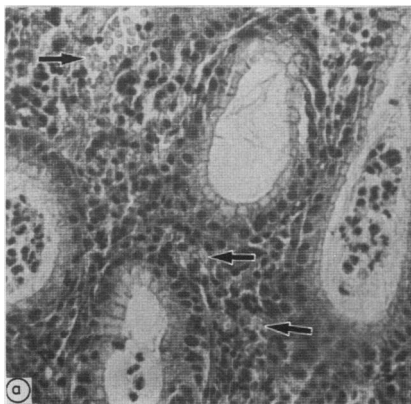


Figure (a) Antral mucosa before treatment. Presence of a dense, diffuse inflammatory infiltrate, mainly composed of polymorphonuclear leucocytes in the lamina propria and inside glands. Congested vessels are present (arrows). (b) Antral mucosa after treatment. Presence of a few mononuclear cells in the lamina propria. (Haematoxylin and eosin.)

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Campylobacter pylori associated acute gastritis in a child

The frequent association between *Campylobacter pylori* and chronic gastritis is well documented.¹ Marshall *et al* showed that this organism can cause acute gastritis in volunteers.² Marshall *et al* suggested that chronic gastritis associated with *C pylori* is preceded by an acute phase which is usually undiagnosed.² We could find only one such case.³ In this case the micro-organism was not cultured and it was only shown by histological techniques. We report a case of *C pylori*-associated acute gastritis occurring in a child, in whom the diagnosis was confirmed by histological and microbiological methods.

An 8 year old boy was admitted to hospital with a three week history of epigastric pain

and vomiting. The child was malnourished and there was no history of drug intake. A gastroduodenoscopy showed multiple erosions surrounded by red and friable mucosa in the antrum and body of the stomach. Biopsy specimens were taken from duodenum, stomach (fundus, body, and antrum), and oesophagus for histological assessment, immunoperoxidase staining,⁴ and bacteriology.⁵

Histologically, the antral mucosa showed oedema, hyperaemia, and an intense polymorphonuclear neutrophil leucocyte infiltration in the lamina propria, mucus layer, and inside the glands (fig 1a). The oxyntic mucosa showed mononuclear inflammatory cells in the lamina propria. Immunoperoxidase staining showed *C pylori* as dark brown, curved, or spiralled bodies within the gastric mucus, adherent to the epithelial cell surface and inside the antral glands. Brush smears of antral gastric mucosa showed curved and spiral Gram negative micro-organisms. Preformed urease tests were positive in biopsy specimens from fundus, body and antrum, and negative from oesophagus and duodenum. Culture of the biopsy specimens from gastric fundus, body and antrum showed a heavy growth of *C pylori*. The micro-organism had in vitro sensitivity to amoxycillin. No growth was observed in culture of specimens from oesophagus and duodenum. The patient was treated with oral amoxycillin (750 mg/day as three equal portions every eight hours, for one month). Within one week of treatment the symptoms disappeared. Biopsy specimens after treatment showed antral mild residual gastritis (fig 1b). *C pylori* was not shown by immunoperoxidase technique,

Gram stain, preformed urease test, or by culture.

The patient presented a clinical, endoscopic, and histological picture of acute gastritis that was associated with *C pylori*. We believe that *C pylori* might be a more common causative agent of acute gastritis than previously thought.

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