INFECTION WITH *HELICOBACTER PYLORI*

1. Exposure Data

1.1 Structure and biology of *Helicobacter pylori*

1.1.1 *Taxonomy*

The presence of spiral-shaped bacteria on human gastric mucosa was first recognized nearly one hundred years ago (Pel, 1899). These bacteria were isolated for the first time in 1982, in cultures of endoscopic biopsy specimens from patients with gastritis and peptic ulceration (Marshall, 1983; Warren, 1983). For phenotypic reasons, such as spiral shape, motility, growth under microaerophilic conditions and isolation from the alimentary tract, the organism was classified as a member of the genus *Campylobacter* and was called *Campylobacter pyloridis* (Marshall *et al.*, 1987), and then *C. pylori* (Marshall & Goodwin, 1987). It became clear, however, that *C. pylori* differed significantly from other members of the genus with respect to cellular fatty acids, lack of a methylated menaquinone, antimicrobial susceptibility and ribosomal ribonucleic acid sequences.

In 1989, a new genus, *Helicobacter*, was proposed, and *C. pylori* was renamed *Helicobacter pylori* (Goodwin *et al.*, 1989). The genus now includes a variety of 'gastric' and 'non-gastric' *Helicobacter* species. Classification of bacteria into the new genus was based mainly on a homology greater than 90% of the nucleotide sequence in the 16S ribosomal RNA molecule (Lee & O'Rourke, 1993). The gastric *Helicobacter* spp. are *H. pylori*, *H. mustelae* (ferrets; Fox *et al.*, 1986, 1988), *H. felis* (cats and dogs; Lee *et al.*, 1988, 1990, 1992), *H. nemestrinae* (macaque monkeys; Bronsdon *et al.*, 1991) and *H. acinonyx* (cheetahs; Eaton *et al.*, 1991a). One non-gastric *Helicobacter* sp. is *H. hepaticus* (mouse liver and intestine; Fox *et al.*, 1994). An additional spiral bacterium commonly found in the stomachs of cats, dogs and pigs and infrequently in those of humans, which has not yet been cultured and is known provisionally as '*Gastrospirillum hominis*' or '*H. heilmannii*', has been proposed for addition to the genus on the basis of morphological and RNA similarities (Solnick *et al.*, 1993).

1.1.2 *Biology*

(a) Morphology; ultrastructural features

H. pylori is a spiral or slightly curved gram-negative rod with two to six characteristic unipolar flagella. The bacterium has bluntly rounded ends and measures 2.5–4.0 μm in length and 0.5–1.0 μm in width. The cell wall is smooth and may be coated with a prominent

glycocalyx with a thickness up to 40 nm (Goodwin *et al.*, 1989); it is covered with ring-like subunits with a diameter of 12–15 nm. Occasionally, bacteria may contain bacteriophages. The flagella measure 2.5 μm in length and around 30 nm in thickness and have a distinctive terminal bulb (Goodwin & Worsley, 1993). Each flagellum consists of a central filament enveloped by a flagellar sheath. The filament consists mainly of a polymer of a 53-kDa [80 base-pair] flagellin protein (Geis *et al.*, 1989, 1993); it ends proximally in a basal body, which is associated with the cytoplasmic membrane. The sheath is formed by a lipid bilayer, which extends as a direct continuation from the bacterial outer membrane (Geis *et al.*, 1993). The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility (Hazell *et al.*, 1986; Suerbaum *et al.*, 1993). *H. pylori* may change from its normal morphological appearence into a range of coccoidal forms, especially *in vitro* after prolonged culture or after antibiotic treatment. It is not certain whether the coccoidal forms can resume the spiral, multiplying form. The viability of coccoidal organisms has been proven by means of acridine orange staining, bromodeoxyuridine incorporation and urease activity (Goodwin & Worsley, 1993; Nilius *et al.*, 1993).

(*b*) *DNA content; genome and plasmids*

The DNA of different *H. pylori* strains contains 34–38 mol % guanine and cytosine (Goodwin & Worsley, 1993). The genome varies in size from 1.6 to 1.73 megabases (Taylor *et al.*, 1992). About 35–50% of *H. pylori* strains contain plasmids, which have not been associated with any biological characteristic of the bacteria (Majewski & Goodwin, 1988; Penfold *et al.*, 1988; Simor *et al.*, 1990).

A number of specific genes have been cloned, including two structural urease genes which encode the subunits of the urease enzyme (Labigne *et al.*, 1991), two flagellin genes, called *fla*A (Leying *et al.*, 1992) and *fla*B (Suerbaum *et al.*, 1993), a cytotoxin production-associated gene, the *cag*A gene (Tummuru *et al.*, 1993), the cytotoxic *vac*A gene (Cover *et al.*, 1994) and a heat-shock protein encoding gene (Macchia *et al.*, 1993).

(*c*) *Growth conditions*

H. pylori can be cultured in both solid and liquid media. Basal solid media, such as Colombia blood agar base and brain–heart infusion agar supplemented with serum or charcoal, yield good results (Dent & McNulty, 1988; Goodwin & Worsley, 1993). Brain–heart infusion (or brucella) broth supplemented with charcoal, serum or cyclodextrins can also be used (Olivieri *et al.*, 1993). Microaerophilic culture conditions are essential, with optimal oxygen concentrations between 2 and 8%. Addition of extra carbon dioxide or 1–5% whole blood or serum may stimulate culture yields. Bacteria of the genus *Helicobacter* do not catabolize carbohydrates (Mégraud *et al.*, 1985; Goodwin & Worsley, 1993), but *H. pylori* can use glucose via the pentose phosphate pathway (Mendz *et al.*, 1993). Maximal growth occurs at 37 °C and neutral pH (Goodwin & Worsley, 1993). The bacterium is sensitive to almost all antibiotics *in vitro*, with the exception of nalidixic acid, trimethoprim, sulfonamides and vancomycin (Goodwin *et al.*, 1989; Goodwin & Worsley, 1993). Section 1.5 provides further information about the efficacy of antibiotics *in vivo*.

(*d*) *Enzymatic activity*

H. pylori is characterized by strong urease activity, with a Michaelis constant of 0.48 mmol/L for urea (Goodwin & Worsley, 1993). The hexameric enzyme has a relative molecular mass of about 600 kDa [909 base pairs] and is composed of six monomers, each with two protein subunits of 66 and 31 kDa [100 and 47 base pairs]. It is active at pH 4.0–10.0 and has an isoelectric point of 5.93 (Evans et al., 1992; Goodwin & Worsley, 1993; Mobley & Foxall, 1994). Of the total protein production of the bacterium, 6% consists of urease (Hu & Mobley, 1990). The urease molecule is associated with a 62-kDa [94-base-pair] heat-shock protein, the function of which has not been fully elucidated (Evans *et al.*, 1992).

H. pylori is oxidase-positive and produces large amounts of catalase (Goodwin *et al.*, 1989) and superoxide dismutase (Spiegelhalder *et al.*, 1993). The tetrameric catalase, with subunits of 50 kDa [76 base pairs], has an isoelectric point of 9.0–9.3. *H. pylori* also produces phospholipase A2 and C, γ-glutamyltranspeptidase, DNase, both acid and alkaline phosphatase, a mucus-degrading glycosulfatase (Mégraud *et al.*, 1985; Freland & Drugeon, 1988; Slomiany *et al.*, 1992; Ottlecz *et al.*, 1993), alcohol dehydrogenase (Salmela *et al.*, 1993) and leucine aminopeptidase (Mégraud *et al.*, 1985). It has significant alcohol dehydrogenase activity at both low and high concentrations of ethanol (Salmela *et al.*, 1993; Salaspuro, 1994). Hippurate hydrolysis and nitrate reduction do not occur (Goodwin & Worsley, 1993), nor does *H. pylori* contain indole or produce hydrogen sulfide (Mégraud *et al.*, 1985).

1.1.3 *Agent–host relationship*

(*a*) *Host and target tissues*

Natural infection with *H. pylori* has been demonstrated only in humans and in nonhuman primates. Oral challenge under laboratory conditions may lead to colonization in *Macaca* species, gnotobiotic piglets and dogs (Fox *et al.*, 1991). The reasons for this narrow host range are unknown but may be related to specific binding capacities for human mucosal antigens (Husson *et al.*, 1993). In infected humans, *H. pylori* specifically colonizes the gastric mucosa, as it is uniquely adapted to survive the acidic environment. Within the stomach, infection is usually greatest in the antrum (Dixon, 1991); colonization densities in the acid-producing corpus region of the stomach are lower. For unknown reasons, antral colonization may decrease and corpus colonization may increase under conditions of lower acid output (Louw *et al.*, 1993). Microscopically, the bacterium can usually be observed within the surface mucus layer, both on the surface epithelium and within the pits. Under the electron microscope, it is usually observed close to intercellular junctions of mucus-secreting cells (Hazell *et al.*, 1986; Caselli *et al.*, 1989). It is not found in areas of intestinal metaplasia (Correa *et al.*, 1989). Epithelial cell invasion is very rare (Caselli *et al.*, 1989). The specific affinity of *H. pylori* for gastric epithelium is exemplified by the occasional demonstration of these bacteria on metaplastic gastric mucosa in the oesophagus (Paull & Yardley, 1988), in the duodenum, in Meckel's diverticulum or in the rectum (Offerhaus *et al.*, 1990; Kestemberg *et al.*, 1993).

Interest in possible routes of transmission (see section 1.3) has focused research on the presence of *H. pylori* in the mouth and faeces of infected individuals. Although *H. pylori* has been detected in both dental plaque and faeces (Thomas *et al.*, 1992; Nguyen *et al.*, 1993), a limited number of successful isolations have been made, the number of cases studied is small,

and occasionally the cultured bacteria have been incompletely identified. The bacterium has been found only in the gastrointestinal tract.

(*b*) *Immune response of infected individuals*

The presence of *H. pylori* on the gastric mucosa elicits an inflammatory response in all infected individuals. This response is characterized by inflammatory cells in the mucosa (see sections 1.4 and 4.1) and by local and systemic humoral immune responses. The specific immunoglobulin (Ig)A response, both locally and systemically, consists mainly of the IgA1 subclass (van der Est *et al.*, 1992). The systemic IgG response involves all four subclasses. Different subclass responses have been noted in gastritis patients with and without duodenal ulcer; it is unknown whether this difference is related to the host or to the bacterial strain (Bontkes *et al.*, 1992). The IgG response diminishes within 6–12 months after the infection has been eradicated with antibiotics (Kosunen *et al.*, 1992). It also appears to diminish after histological disappearance of *H. pylori* due to the development of gastric mucosal atrophy, which is unfavourable to colonization; however, only retrospective evidence is available to substantiate this claim (Crabtree *et al.*, 1993a), and long-term follow-up studies have not yet been carried out (Kuipers *et al.*, 1994a).

(*c*) *Colonization factors*

A variety of factors play a role in the establishment and maintenance of *H. pylori* colonization in the strongly acidic stomach. Motility makes possible rapid transit through the acidic lumen and penetration into the viscous epithelial mucus layer, which protects against acid contact. The unipolar flagella are essential for this motility: aflagellated mutants have been shown to be immobile (Suerbaum *et al.*, 1993). Adherence to the gastric epithelium is the next important factor for virulence. Microscopic research has shown adherence to epithelial pedestals (Caselli *et al.*, 1989), and several investigators have shown specific binding capacities for both extracellular matrix proteins and cellular antigens (Borén *et al.*, 1993; Moran *et al.*, 1993). Binding to Lewis^b blood group antigens has been reported (Borén *et al.*, 1993).

The production of enzymes, especially urease, is a third factor of importance in *Helicobacter* colonization. In laboratory experiments, a mutant strain of *H. pylori* with only very weak urease activity was unable to colonize gnotobiotic piglets (Eaton *et al.*, 1991b). Urease inhibition does not, however, eradicate an established infection. *In vitro*, urease-positive bacteria do not survive at pH 1.5 in the absence of urea but can survive when urea is added (Marshall *et al.*, 1990; Ferrero & Lee, 1991). These observations led to the hypothesis that the potent urease is required to establish new infections; however, once the bacteria have reached a protected niche deep within the mucus layer, protection is no longer necessary and urease may be needed only for delivery of nitrogen.

(*d*) *Pathogenic mechanisms*

In the interaction between *H. pylori* and the gastric mucosa, a number of factors have been claimed to play a role in the chronic inflammatory reaction and epithelial cell damage which, in some cases, lead to overt clinical disease (see section 1.4). Firstly, the bacterium secretes several enzymes that can alter the integrity of both the mucus and epithelial cells. It

produces a glycosulfatase that causes loss of mucus viscosity and a diminished capacity to retard hydrogen ion diffusion (Slomiany *et al.*, 1992); mucus secretion is also diminished (Micots *et al.*, 1993). Ammonia produced by the potent urease enzyme is directly toxic to gastric epithelial cells both *in vivo* and *in vitro* (Mégraud *et al.*, 1992; Tsujii *et al.*, 1992). The phospholipase activity of the bacterium (Daw *et al.*, 1993) can cause degradation of membrane phospholipids, and its alcohol dehydrogenase activity leads to production of the toxic acetaldehyde in the presence of ethanol (Salmela *et al.*, 1993). The clinical importance of the latter finding is unknown.

Helicobacter also produce a variety of substances that may damage the infected host. Shedding of bacterial surface proteins in close proximity to the mucosa may have a chemotactic action on leukocytes (Mai *et al.*, 1992). About 50–60% of *H. pylori* strains can produce a cytotoxic protein that causes vacuolization of cultured epithelial cells (Cover *et al.*, 1990; Fox *et al.*, 1992).

1.2 Methods for detection of infection

1.2.1 *Methods based on gastric biopsy specimens*

Specimens collected before treatment from both the antrum and the corpus with standard forceps can be cultured after placing them in either saline (analysis within 4 h) or transport medium (analysis after up to 24 h) or freezing them at –70 °C or in liquid nitrogen (delayed analysis).

(*a*) *Rapid urease test*

The urease in *H. pylori* breaks down urea into carbon dioxide and ammonia; as ammonia raises the pH, a positive reaction can be read on a pH indicator within a few minutes (Langenberg *et al.*, 1984). Urease tests are agar-based, designed for use in hospital and give results in less than 1 h; their sensitivity has been reported to be 80–98% and their specificity close to 100% (Marshall *et al.*, 1987). Clinical experience indicates, however, that this test may not be specific enough to test the success of treatment. A reading at 24 h increases the sensitivity but decreases the specificity.

(*b*) *Histological examination*

Sections, which must include the superficial and foveolar epithelium, are fixed in formaldehyde or Bouin solution. They can be stained with the standard haematoxylin–eosin stain (Taylor *et al.*, 1987), also used in grading gastritis, but most researchers favour the modified Giemsa stain because better contrast with the background is obtained (Gray *et al.*, 1986). *H. pylori* is best seen under oil immersion. A positive result is expressed semi-quantitatively according to the histological subclassification of the Sydney system (see pp. 207–208) (Price, 1991)

The sensitivity and specificity of histological examination for detecting *H. pylori* depend on the observer's experience. Specificity can be impaired by the presence of other spiral bacteria or coccoidal bacteria, and interpretation may be difficult when only a small number of bacteria are present. Histological methods are best for detecting the non-culturable *Helicobacter*, *H. heilmannii* (Heilmann & Borchard, 1991).

(*c*) *Bacteriological tests*

Smears are prepared by scraping a biopsy specimen with the mucus side against the slide. Gram staining allows observation of curved and spiral gram-negative bacteria. This is a quick, simple and inexpensive test with a sensitivity of about 80% (Montgomery *et al.*, 1987).

Culture is the best means of identifying most infectious agents, because the presence of even one bacterium in the specimen can result in the growth of colonies, allowing precise identification of the organism. For optimal recovery of *H. pylori*, biopsy specimens should be ground, and fresh media containing blood, preferably of human origin, should be used (Westblom *et al.*, 1991). 2,3,5-Triphenyltetrazolium chloride can be included in the medium in order to detect early *H. pylori* colonies (Queiroz *et al.*, 1987). Both selective and non-selective media should be inoculated (Tee *et al.*, 1991), and the culture should be incubated in a microaerobic atmosphere at 37 °C for up to 10 days.

H. pylori colonies are identified by microscopic examination and biochemical tests (see above). Antimicrobial susceptibility tests and molecular fingerprinting can be undertaken in cultures. Since acquired resistance has been noted to four groups of agents used to eradicate *H. pylori*—nitroimidazoles, macrolides, fluoroquinolones and rifamycins, resistance—must be monitored in clinical trials (Glupczynski *et al.*, 1991).

(*d*) *Polymerase chain reaction*

The primers used for detection of *H. pylori* by the polymerase chain reaction (PCR) correspond to genes that encode urease (Labigne-Roussel *et al.*, 1989), 16S ribosomal RNA (Ho *et al.*, 1991), a specific 26-kDa [40-base-pair] protein (Hammar *et al.*, 1992) and an uncharacterized 1.9-kilobase-pair fragment of chromosomal DNA (Valentine *et al.*, 1991). No one pair of primers has proved to be superior to another, but the use of two pairs of primers from different genes may increase specificity. PCR can be used to detect specific genes of pathogenic relevance, such as the *cag*A gene (Figura & Crabtree, 1994).

1.2.2 *Methods based on gastric juice samples*

The techniques used for gastric biopsy specimens can also be used for gastric juice samples. PCR is equally reliable for gastric juice and biopsy specimens (Westblom *et al.*, 1993a). Culture is less sensitive when performed with gastric juice, probably because viable *H. pylori* are lost during prolonged contact with acid (Freland & Drugeon, 1988).

1.2.3 *Methods based on faecal specimens*

Techniques based on faecal specimens are still in an early stage of development. *H. pylori* has been cultured from faeces of infants in the Gambia (Thomas *et al.*, 1992) and has been detected by PCR in faeces (Mapstone *et al.*, 1993), although faecal inhibitors of the reaction remain a problem.

1.2.4 *Methods based on dental plaque and saliva samples*

H. pylori has also been cultured from dental plaque (Krajden *et al.*, 1989) and saliva (Ferguson *et al.*, 1993). Use of PCR has been reported, but these techniques cannot be used as diagnostic methods.

1.2.5 *Methods based on blood samples*

The systemic immune response present in 98% of infected individuals (Glupczynski *et al.*, 1992) can be used for the serological diagnosis of infection (Dooley *et al.*, 1989). Cross-reactions to *C. jejuni* may occur (Newell, 1987). After infection, IgG antibodies are detected within a few weeks. Where it has been validated, the sensitivity and specificity of an enzyme-linked immunosorbent assay (ELISA) with IgG are greater than 90%. Ideally, such tests should be standardized in the population under study; however, it may sometimes be difficult to identify a sufficient number of uninfected people as controls. When *H. pylori* has been eradicated, titres decrease consistently after six months (Kosunen *et al.*, 1992). Immunoblotting allows the detection of a *H. pylori*-specific 120–128-kDa [182–194-base-pair] cytotoxin-associated protein, the *cag*A gene product (Crabtree *et al.*, 1991; Tummuru *et al.*, 1993).

1.2.6 *Urea breath test*

Urea can be hydrolysed by the strong urease of *H. pylori*. In the urea breath test, urea labelled with $^{13}CO_2$ is absorbed and subsequently eliminated in the breath. Breath samples are collected before and 30 min after absorption of labelled urea and analysed by mass spectrometry (Graham *et al.*, 1987). A European protocol has been proposed for this test (Logan *et al.*, 1991). Similar tests involve the use of ^{14}C-urea, as $^{14}CO_2$ can be measured easily with a scintillation counter, but some concern has been expressed over the use of a radioactive isotope. Low-dose tests are being developed to overcome this problem (Bell *et al.*, 1987).

1.3 Epidemiology of infection

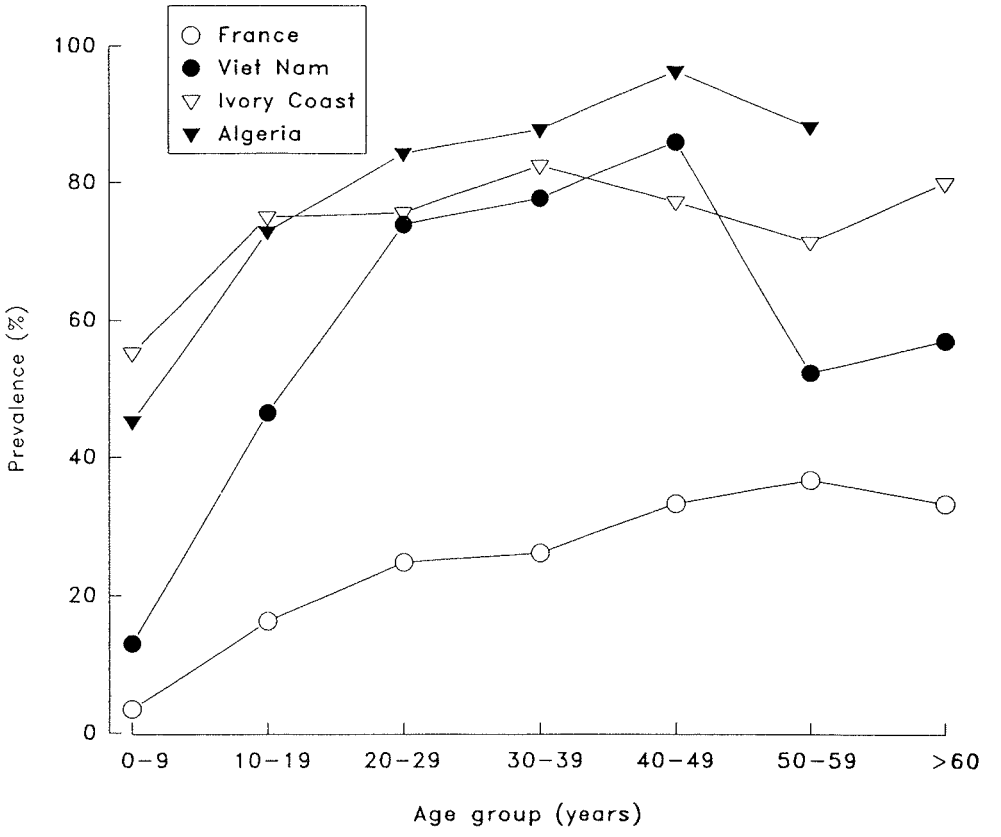
H. pylori infection is long-standing and only rarely resolves spontaneously; it may occasionally be influenced by concomitant antimicrobiological treatment. Thus, it is the prevalence of this infection rather than its incidence that is usually estimated in epidemiological studies (Langenberg *et al.*, 1988; Kuipers *et al.*, 1993a).

1.3.1 *Prevalence*

The prevalence of *H. pylori* infection has been estimated in all the continents on the basis of the results of serological tests on populations such as blood donors, individuals presenting themselves to health centres and volunteers recruited in different ways.

In developing countries, the prevalence of infection increases rapidly during childhood and adulthood and is usually 80–90%. The prevalence is substantially lower in developed countries, especially in childhood (see section 1.3.2). These findings are illustrated in a study in which the same ELISA technique was used in subjects from four countries with different geographical and socioeconomic status (Mégraud *et al.*, 1989) (Figure 1). Similar results were reported in the EuroGast study, in which defined populations of two age groups, 25–34 and 55–64 years, from 17 geographical areas, mainly European, were studied by the same protocol (EuroGast Study Group, 1993a,b; Table 1).

Figure 1. Distribution of seropositivity for *Helicobacter pylori* immunoglobulin G antibodies by age and country of origin



From Mégraud *et al.* (1989)

1.3.2 *Risk factors for infection*

The difference in prevalence between developed and developing countries seems to be linked to socioeconomic factors rather than to ethnicity. In most developed countries, the poorest people also have the highest prevalence. A low level of education and poor housing conditions have been associated with infection (Al Moagel *et al.*, 1990; Fiedorek *et al.*, 1991; Sitas *et al.*, 1991; EuroGast Study Group, 1993a).

No difference in seroprevalence has been found between men and women (Mégraud *et al.*, 1989), and no consistent association has been found with smoking or drinking habits (EuroGast Study Group, 1993a) or any particular diet (Hansson *et al.*, 1993a; Palli *et al.*, 1993). One study in Japan showed a positive association between eating salty food and infection with *H. pylori* (Tsugane *et al.*, 1994). No sexual transmission has been observed (Polish *et al.*, 1991). In cross-sectional studies, an association is always observed between prevalence of infection and age (see Figure 1 and Table 1). Two mechanisms may contribute to this age pattern of prevalence: an age effect, i.e. the progressive acquisition of infection throughout adult life (Graham *et al.*, 1991), and a cohort effect, i.e. a progressive reduction of the rate of infection early in life of people in successive birth cohorts. The extent to which

Table 1. Prevalence of seropositivity to *Helicobacter pylori* in 17 populations

Country	Centre	*H. pylori* seropositivity (%)				
		25–34 years		55–64 years		Total sample
		Male	Female	Male	Female	
Algeria	Algiers	42	44	49	69	200
Belgium	Ghent	20	17	60	47	208
Denmark	Copenhagen	23	5	34	27	157
Germany	Augsburg	14	22	57	65	187
	Deggendorf	40	40	74	76	198
	Mosbach	24	33	65	75	158
Greece	Crete	53	54	80	70	229
Iceland	S. Region	31	40	56	62	206
Italy	Florence	17	14	38	57	205
Japan	Miyagi	55	64	88	87	186
	Yokote	70	54	90	80	200
Poland	Adamowka	69	70	79	93	171
Portugal	Gaia	57	57	73	65	132
Slovenia	Ljubljana	51	27	71	70	201
United Kingdom	Oxford	8	8	49	42	158
	Stoke	27	10	49	41	200
USA	Minneapolis-St Paul	13	16	36	32	198

From EuroGast Study Group (1993b)
NA, not available

these two effects contribute to the cross-sectional association of age with prevalence of infection may vary between populations. The following observations indicate the relative importance of a cohort effect:

- Infection is more strongly correlated to risk factors present during childhood (crowding, size of the family, sharing a bed) than to current risk factors (Mendall *et al.*, 1992; Mitchell *et al.*, 1992; Whitaker *et al.*, 1993; Webb *et al.*, 1994).
- Among adults in developed countries, new cases of infection are uncommon (Mégraud *et al.*, 1989; Rautelin *et al.*, 1990).
- Crude rates of seroconversion from negative to positive have been estimated to be around 0.3–0.5% per year (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1993a); recurrence of infection after eradication therapy may reflect recrudescence of the treated infection rather than true reinfection.
- The cohort effect has been demonstrated in a cohort in the USA (Parsonnet *et al.*, 1992) and in a cohort in the Netherlands (Kuipers *et al.*, 1993a) as well as in a study performed in the United Kingdom. In the last study, sera collected from the same area in 1969, 1979 and 1989, tested for *H. pylori* antibodies by immunoblot and plotted by age group showed that at a given age the prevalence had decreased over the two decades (26% per decade) (Banatvala *et al.*, 1993).

In some populations, a decrease in seroprevalence has been observed in older people. This finding has been attributed to the disappearance of *H. pylori* from the gastric mucosa (loss of *H. pylori* infection) when atrophy develops as a result of long-standing gastritis. Such loss has been observed in some populations (Karnes *et al.*, 1991; Kuipers *et al.*, 1994b) but not in another (Guarner *et al.*, 1993). Furthermore, it is still unclear whether a gradual decrease in *H. pylori* colonization also leads to negative seroconversion. Negative seroconversion was claimed in one retrospective study (Crabtree *et al.*, 1993a) but not in two prospective studies (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1994a).

The prevalence of infection is consistently higher in institutionalized children than in control groups from the surrounding area (Berkowitz & Lee, 1987; Pérez-Pérez *et al.*, 1990).

For a long time, the stomach was thought to be sterile, and precautions such as the use of gloves were not taken in performing endoscopies. A higher prevalence of *H. pylori* infection has now been found among gastroenterologists who perform endoscopies than among other physicians or dentists (Mitchell *et al.*, 1989). In countries with a high prevalence of infection, endoscopists have, nevertheless, a lower prevalence than the general population, probably due to the fact that they come from the middle and upper classes (Matysiak-Budnik *et al.*, 1994).

1.3.3 *Routes of transmission*

Reservoirs of *H. pylori* are the digestive tracts of humans and some primates. Transmission from reservoirs is considered to be person-to-person. This assumption is supported by the finding of clustering of similar strains within families, as shown by molecular fingerprinting (Bamford *et al.*, 1993) and by the consistent demonstration of close interpersonal contact as a risk factor for infection. The *H. pylori* status of mothers of *H. pylori*-positive children is significantly different from that of mothers of *H. pylori*-negative children, indicating that the intimate contact between mother and child could be a cause of transmission (Drumm *et al.*, 1990). Transmission can exist between couples: 68% of spouses of *H. pylori*-infected people were infected, whereas 9% of spouses of uninfected people were infected (Malaty *et al.*, 1991). In another study, the association disappeared in a multiple logistic regression analysis (Pérez-Pérez *et al.*, 1991). Two modes of transmission have been proposed: oral–oral and faecal–oral transmission.

(*a*) *Evidence for faecal–oral transmission*

H. pylori is eliminated in faeces after turnover of the gastric mucosa. It has been detected by PCR (Mapstone *et al.*, 1993) and by culture (Thomas *et al.*, 1992). Consumption of raw vegetables fertilized with human faeces was found to be a risk factor for infection in Santiago, Chile (Hopkins *et al.*, 1993), and consumption of municipal water was found to be a risk factor in children in Lima, Peru (Klein *et al.*, 1991). *H. pylori* has been detected by PCR in sewage water in Peru (Westblom *et al.*, 1993b).

(*b*) *Evidence for oral–oral transmission*

H. pylori has been detected in the oral cavity (Mapstone *et al.*, 1993) and in the saliva of one person (Ferguson *et al.*, 1993). Several claims have been made of the detection of *H. pylori* by PCR in dental plaque (Krajden *et al.*, 1989; Majmudar *et al.*, 1990). When

gnotobiotic puppies infected with *H. felis* were put together with uninfected litter-mates in a germ-free isolator, with continual oral–oral contact, the agent was transmitted. Transmission did not occur between germ-free mice, which are coprophageous, under the same conditions (Lee *et al.*, 1991).

1.4 Clinical disease in humans (other than cancer)

1.4.1 *Gastritis*

H. pylori is a major cause of gastritis. This inference is based on the following observations: (i) ingestion of *H. pylori* led to acute gastritis in a small number of case studies (Marshall *et al.*, 1985a; Morris & Nicholson, 1987; Sobala *et al.*, 1991); (ii) *Helicobacter* colonization of the stomach is virtually always accompanied by inflammation of the mucosa (Dixon, 1991); (iii) *H. pylori* infection can be detected in more than 85% of patients with inflammation of the gastric mucosa (Dooley *et al.*, 1989); and (iv) this inflammation disappears completely within two to three years after eradication of the infection (Rauws *et al.*, 1988; Genta *et al.*, 1993a).

The infection disappears only as a result of antibiotic therapy, after the development of unfavourable gastric conditions such as mucosal atrophy or after partial gastrectomy with bile reflux (Karnes *et al.*, 1991; Kuipers *et al.*, 1993a). 'Spontaneous' clearance of infection is very rare and may in fact be due to unreported use of antibiotics (Kuipers *et al.*, 1993a). In some infected individuals, endoscopic signs of gastritis can be found. The gastritis affects predominantly the antrum (Tytgat *et al.*, 1993), although corpus involvement is observed histologically in most infected individuals (see also section 4).

1.4.2 *Duodenal ulcer disease*

H. pylori infection is the most significant risk factor for duodenal ulcer disease. After exclusion of a small subset of cases of duodenal ulcer with specific etiology, such as use of non-steroidal anti-inflammatory drugs, Crohn's disease or ischaemia, the remaining cases are caused by *H. pylori* (Mégraud & Lamouliatte, 1992). The main arguments for a causal relationship between *H. pylori* infection and duodenal ulcer disease are that the infection is seen to precede the disease (Sipponen *et al.*, 1990) and that the disease disappears after treatment of the infection. While ulcers have been shown in many studies to relapse within 12 months after symptomatic treatment in 50–100% of patients (Tytgat *et al.*, 1993), eradication of *H. pylori* almost totally prevents ulcer recurrence (Marshall *et al.*, 1988; Graham *et al.*, 1992; Tytgat *et al.*, 1993). It has been estimated that up to 10% of infected people will develop duodenal ulcer during life (Tytgat *et al.*, 1993).

1.4.3 *Gastric ulcer disease*

H. pylori infection is present in approximately 70% of patients with gastric ulcers (Labenz & Börsch, 1994). A variety of noxious agents such as non-steroidal anti-inflammatory drugs and bile reflux are risk factors for the development of gastric ulcers. After exclusion of patients with those risk factors, the bacterium is present in more than 95% of the remaining cases. Eradication of the infection significantly prevents ulcer recurrence (Graham *et al.*, 1992; Labenz & Börsch, 1994).

1.4.4 *Hypertrophic protein-losing gastritis*

Hypertrophic protein-losing gastritis is a rare clinical disorder characterized by chronic gastritis with giant folds, gastric protein loss and hypoalbuminaemia. The etiology of this disorder is unknown. Significant clinical improvement was seen after *H. pylori* eradication therapy in two studies (Lepore *et al.*, 1988; Meuwissen *et al.*, 1992).

1.4.5 *Childhood diseases*

In children in developing countries, *H. pylori* infection has been associated with chronic diarrhoea and malnutrition (Sullivan *et al.*, 1990). In developed countries, it has also been associated with chronic abdominal pain and growth retardation.

1.5 Treatment and control

1.5.1 *Antibiotics and acid suppressive therapy*

Since the introduction of H2-blockers and proton pump inhibitors, *H. pylori*-related disorders have been treated with moderate success (Susi *et al.*, 1994). The effects of acid suppressive medication on *H. pylori*-related gastritis have not been examined adequately; however, such medication does not cure the infection (Kuipers *et al.*, 1993b). The bacterium is sensitive to a wide range of antibiotics *in vitro*, but most are unsuccessful *in vivo*. Three strategies have been chosen to overcome this problem: (i) combination of multiple synergistic antibiotic drugs; (ii) prolongation of drug administration; and (iii) combination of antibiotics with acid suppressors. A large number of clinical trials have been carried out to find an effective treatment regimen. The current preference is for therapy lasting 14 days with either two antibiotics combined with a bismuth preparation or with one to two antibiotics combined with an acid inhibitor, usually omeprazole (Labenz *et al.*, 1993). With these regimens, eradication has been achieved in 60–95% of cases, depending upon the prevalence of antibiotic-resistant strains and patient compliance.

1.5.2 *Vaccination*

H. pylori infection is always accompanied by local and systemic immune responses, with no clearance of infection (Bontkes *et al.*, 1992). It is thus unclear whether immunization can prevent new infections. Successful oral immunization of mice with a sonicated preparation of *H. felis* plus adjuvant (cholera toxin) has been achieved (Chen *et al.*, 1993).