

During March this year, a congress was held in Perth (Western Australia) to mark the 20th anniversary of the isolation in 1982 of the Gram-negative strongly ureolytic bacterium now known as *Helicobacter pylori*.

# Birthday Bug

## *Helicobacter pylori*

### twenty years on

How Australian pathologist Robin Warren and his gastroenterologist colleague Barry Marshall observed and successfully cultured a campylobacter-like organism from gastric biopsies of ulcer patients is now legend. **Bob Owen** reviews the current understanding and future challenges presented by this fascinating organism.

*Electron microscope Images of Helicobacter by kind courtesy of Dr Robert Owen FRCPATH of PHLS Helicobacter Reference Unit*

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he findings of Barry Marshall and Robin Warren reported in *The Lancet* in 1983 revolutionized the

management of peptic ulcer disease worldwide. Identifying the cause and means of curing peptic ulcers, a lifelong and life-threatening disease, dramatically changed the lives of many thousands of people and must be viewed as one of the major developments in medicine in general, and in gastroenterology in particular, of the latter half of the 20th century.

A unique aspect of the story was how it so much depended on chance as the organism had eluded culture until the blood agar plates, normally discarded at 48h, were left by mistake in the incubator for four to five days over the extended Easter vacation! The *Helicobacter* story is now well-documented and at the recent Congress in Perth, Barry Marshall launched a new book called *Helicobacter Pioneers* which contains first hand accounts of the many scientists who discovered Helicobacters between 1892 and 1982.

### Disease associations.

The discovery in 1982 was to be of enormous clinical importance as the new bacterium was not only proved to cause chronic superficial gastritis but was also established to be a key factor in the development of peptic ulcer disease. When the bacterium was eradicated with antibiotics, the ulcers healed. The discovery eventually revolutionized the management of peptic ulcers by outdating gastric surgery and offered a permanent cure based on a 7-day therapy. Evidence that infection with *H. pylori* was a risk factor in development of cancer of the gastric antrum and corpus has ensured a continuing high level of interest in the organism.

Adenocarcinoma of the stomach is the second most prevalent of all human malignancies worldwide and associated with infection, at an early age, with *H. pylori* - chronic atrophic gastritis is known to be a precursor of gastric cancer. Furthermore, there has been a growing interest in possible links between *H. pylori* and allied species and a variety of extragastric disorders such as cardiovascular disease, liver and gall bladder disease, idiopathic parkinsonism, skin disease, aphthous ulcers and even infertility. *H. pylori* was first isolated from the liver of a patient in 2001, although causality in the pathology of such extragastric conditions remains unproven. The role of other helicobacters in liver and biliary tract disease is also controversial as their presence is based mainly on DNA or antigen

tests. However, the role of infections with bile tolerant species such as *H. pullorum* with possible malignant transformations is an exciting area of research.

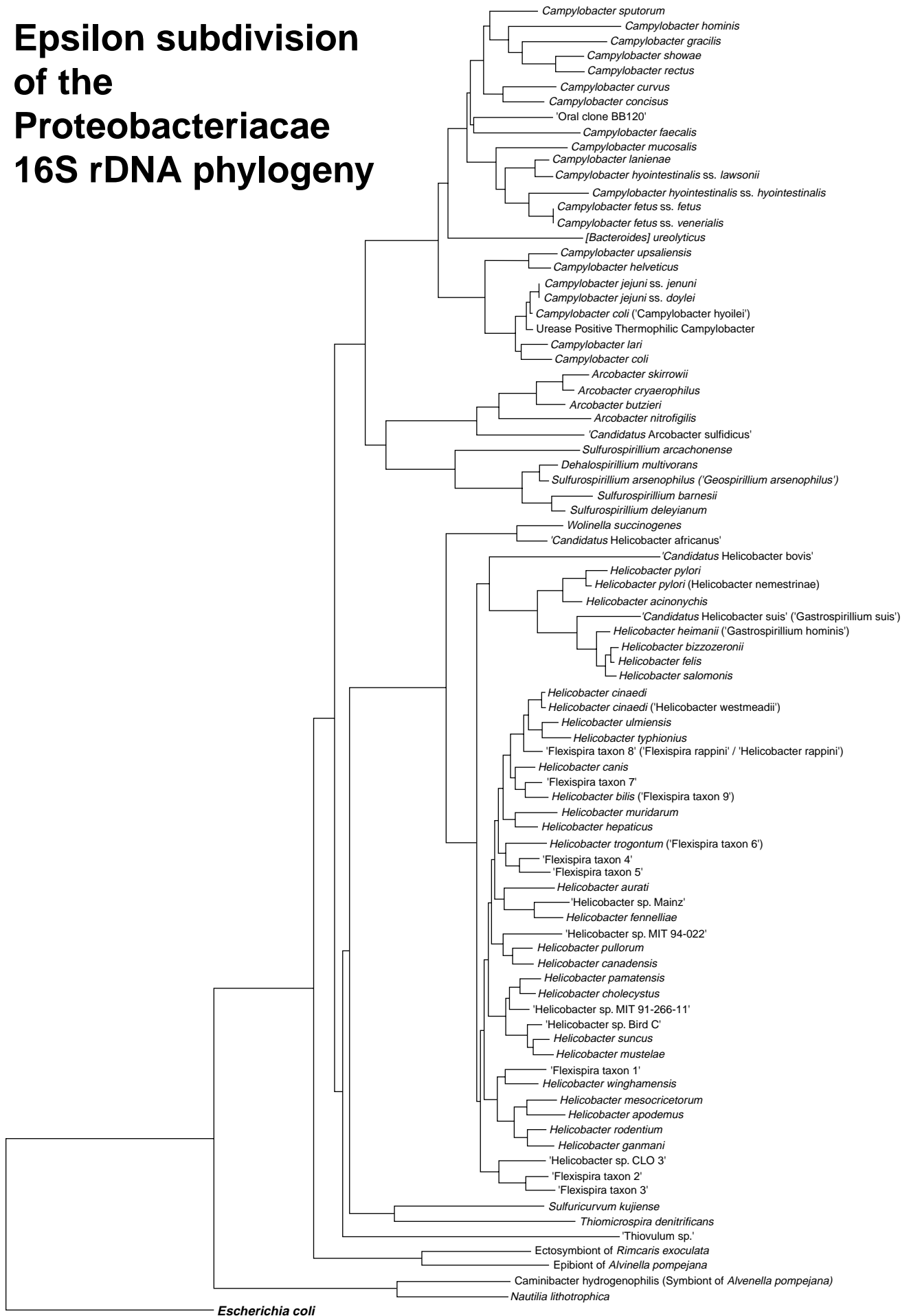
### An expanding genus.

From a general microbiology viewpoint, the discovery of *H. pylori*, provided the starting point for the growth of a new field of study that has rapidly expanded to include at least twenty formally named species, many unnamed strains identified by 16S rRNA phylogenetic analysis, and various *Candidatus* species identified by PCR and microscopy but so far uncultured. (see table on next page).

Marshall and Warren initially described their organism as '*Campylobacter pyloridis*', but subsequent phylogenetic analyses using 16S ribosomal RNA gene sequences showed it was taxonomically distinct and could justifiably be classified in a new genus of Gram-negative microaerobic bacteria within the epsilon subdivision of the Proteobacteriaceae. The novel name *Helicobacter* was proposed. Several species assigned to the new genus such as *H. cinaedi* and *H. fennelliae* were classified originally as *Campylobacter* but most species in the new genus were based on new isolates from the gastric mucosa or faeces of a variety of human, primate and non-primate mammals and bird hosts. (see table on next page).



# Epsilon subdivision of the Proteobacteriaceae 16S rDNA phylogeny



For convenience, species can be broadly categorized as gastric, intestinal and hepatobiliary. *H. pylori* is by far the most frequently isolated species from the gastric mucosa whereas *H. cinaedi* is the most commonly encountered of the intestinal species although isolation rates are relatively low. Interestingly, *H. nemestrinae*, which was isolated originally from a primate, is now considered to belong to *H. pylori* and so a rare example of the species outside man. Closest relatives are *H. acinonychis* isolated from the stomach of captive large cats (cheetahs) and an unnamed species from the dental plaque of dolphins. Natural infections with helicobacters are common in stomachs and faeces of cats and dogs but the extent companion animals act as a reservoir for human infection remains unclear. Most species of *Helicobacter* appear to have evolved specifically for infecting their natural host which suggests they have been part of the indigenous gastric biota for many thousands of years.

## The larger family.

One of the great surprises in the *Helicobacter* story was the discovery that *H. pylori* has a global distribution in the human stomach. It seems unbelievable how presence of the bacterium should have been overlooked until the 1980s. Current estimates based on seroprevalence surveys are that at least half the population of the world is infected and will remain so unless treated with appropriate antibiotics. Infection rates vary widely by geographic area, age, race and socioeconomic status. Generally, seroprevalence in developing countries may reach 70% or more compared with 40% or less in Western Europe, North American and other developed countries. In all populations the prevalence increases with age which is best explained by a birth cohort phenomenon with diminished acquisition during childhood as a result of socioeconomic development. Recent studies consequently indicate a decline in the prevalence of *H. pylori* infections in the Western world consistent with that phenomenon.

## The post-genomic era.

A milestone event in the history of *H. pylori* was the publication in 1997 of the genome sequence of strain 26695 originating from a UK patient with gastritis. Then in 1999, *H. pylori* became the first bacterial species to have genomes sequenced and compared from two independent isolates -the second strain J99 was isolated in the US from a patient with a duodenal ulcer. Interestingly, there are now plans to sequence a strain of *H. pylori*

from India. Both sequenced genomes had a total G+C content of 39% with sizes of about 1.6-Mb containing 1.50 and 1.59K predicted genes. Comparative analyses showed that 6 to 7% of the coding capacity of each strain contains genes not found in the other strain. An interesting feature of the strains was the relatively high nucleotide diversity within orthologous genes as it was rare that the same genes from the two strains would contain the same sequence. This is consistent with studies on other isolates from different geographical locations that the species essentially has a non-clonal population structure due to horizontal gene transfer and frequent recombination. Consequently DNA fingerprinting techniques, such as amplified fragment length polymorphism analysis, provide an excellent way of discriminating between strains in epidemiological studies especially as conventional methods of strain typing, such as biotyping, serotyping and phage typing, are not available for the species.

In 2001, the complete genome sequence performed by an international consortium was reported for *H. hepaticus* ATCC 51449, a species causing chronic active hepatitis and liver cancer in immunocompetent mice. This complete sequence will help us to understand how two closely related *Helicobacter* species colonise completely different ecological niches.

## Do virulent strains exist?

It still remains a puzzle why only a small group of *H. pylori* infected patients develop clinical symptoms and that most infected individuals remain largely asymptomatic. It has been suggested that humans have evolved with their indigenous microbiota over millions of years to reach a dynamic equilibrium. So when *H. pylori* colonisation was universal, peptic ulcers were apparently rare. The beginning of industrialisation in the nineteenth century saw a rise in peptic ulcer disease which suggests that social changes and improved nutrition caused a shift in the nature of *H. pylori* colonisation with a declining prevalence. Little is known about genetic predisposition of host to disease outcomes but while enormous effort has gone into the study of potential strain pathogenicity factors, no single factor provides a reliable predictor of likely disease severity. Vacuolating cytotoxin activity and the presence in strains of the *cag* pathogenicity island encoding for instance synthesis and secretion of the CagA protein, are two established virulence factors, often associated with increased host inflammatory response. These factors with other strain features such

as adherence as well as host factors need to be considered in combination.

## Future challenges.

A key aspect of direct clinical importance is the need to monitor drug resistance and risk factors for treatment failure. Widespread treatment in primary care of infections in individuals with non-ulcer dyspepsia using multidrug regimens remains a controversial topic, particularly as the benefits are marginal and there is the risk of increasing antibiotic resistance rates in the community. The relationship between *H. pylori* and gastroesophageal reflux disease (GORD) and its sequelae - adenocarcinoma of the lower oesophagus - is an area stimulating considerable debate in the light of findings suggesting presence of the organism has a protective role. There is growing interest in the possible sources and routes of infection. Isolation of *H. pylori* from municipal wastewater was reported for the first time this year - so water supplies contaminated with faecal material may be particularly relevant in developing countries. Until an effective vaccine is developed, improved sanitation and public education may be the most practical means of reducing infection rates.

We can expect to see increased application of DNA microarrays and proteomics to explore functional differences between strains in gene content and expression that can be applied in developing novel therapies, improved diagnostic assays and vaccines, and in understanding virulence mechanisms and host cofactors.

After many thousands of publications and numerous meetings over the past two decades, the picture emerging is that there appear to be both risks as well as benefits to the indiscriminate worldwide attempt to eliminate *H. pylori*.

## Useful Web sites

[www.phls.co.uk/facts/gastro/helicobacter](http://www.phls.co.uk/facts/gastro/helicobacter)

[www.helico.com](http://www.helico.com)

[www.digestivedisorders.org.uk/leaflets](http://www.digestivedisorders.org.uk/leaflets)

[www.cdc.gov/ulcer](http://www.cdc.gov/ulcer)

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