What Bizzozero never could imagine – *Helicobacter pylori* today and tomorrow

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Summary. With the observation of spiral organism in the stomach about one hundred years ago a long history of a bacterium started ending up in a worldwide research programs, studies and consensuses. With this bacterium rediscovered by Marshall and Warren and later named *Helicobacter pylori*, a milestone of research was laid nearly twenty years ago. *Helicobacter pylori* is now recognized as the main cause of most cases of gastritis and ulcer disease in the stomach and the duodenum. In the course of the *Helicobacter pylori* research, *Helicobacter pylori* was found to trigger neoplastic alterations on the ground of the inflammation in the stomach. At first, a large number of publications served to describe the connection between *Helicobacter pylori* and the low malignant B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) of the stomach. Furthermore, on the basis of numerous seroepidemiological studies, researchers succeeded in documenting the participation of *H. pylori*, at least as a co-factor, in the development of gastric carcinoma.

From large epidemiological studies made during the last twenty years as well as from microbiological research we have learned much more about this in the beginning nameless and mysterious bacterium.

**Yesterday**

Italian Giulio Bizzozero (1) was the first who observed and described spiral organisms in the stomach of dogs and though he did not realize what actually he had seen it seemed to be important enough to publish his observation in 1893 (Fig. 1). Just three years later Hugo Salomon from Germany confirmed Bizzozero work (2). In the beginning of the twentieth century it was another German, W. Krienitz, who detected spiral germs in the stomachs of patients with gastric carcinoma (3). Neither Bizzozero nor Salomon and Krienitz could suspect that this spiral-shaped bacterium will be the most widespread infectious agent of gastroduodenal diseases.

Despite the published papers it seemed to be undoubted that there is no bacterial life possible in the acidic environment of stomach and that bacterial infection is unlikely.

It was the internist B. Marshall and the Dr. J. Warren who broke this dogma in the early eighties of the last century. By this time both Warren and Marshall started to study the connection between the spiral organisms and various upper gastrointestinal symptoms. Their Lancet publications (4, 5) on unidentified curved flagellated bacilli in the stomach of patients with gastritis and peptic ulceration made this bacterium to step into the limelight of scientific interest. Barry Marshall had easily shown the first two Koch’s postulates, as the bacteria were present on the gastric epithelial tissue of patients having a disease (gastritis) and they could be grown in culture outside the body. In order to fulfill the third postulate, he drank a suspension of the bacterium and developed an acute dyspeptic illness. When bacteria from the biopsy were grown in culture, the fourth of Koch’s postulate was also fulfilled. This extraordinary finding induced an explosion of research on the role of *H. pylori*, formerly called *Campylobacter pyloridis* (6).

**Today**

Pathology

Based on the research over the last two decades the benefit of the routine work increased dramatically. The best diagnostic tool is still the histopathology. Though
most of the gastrointestinal pathologists were focusing mostly on tumor pathology, the pathology of inflammatory diseases of the gastrointestinal tract became more and more important, especially with regard to the gastritis. After reaching an agreement on a new gastritis classification, the so-called Sydney-Classification, on the World Congress of Gastroenterology in 1990 (7), the pure histopathological description in reporting gastritis diagnosis was now in the past. It was also a pioneering work to include an endoscopic section into the Sydney System. A consensus with the American and Japanese pathologists was reached on the gastritis-workshop in 1994 and the updated Sydney System was introduced (8). There are five path morphological criteria for gastritis induced by Helicobacter pylori:

1. Chronic inflammation – increase in lymphocytes and plasma cells in the lamina propria;
2. Activity of the inflammation – neutrophil polymorph infiltration of the lamina propria, pits or surface epithelium;
3. Atrophy – loss of glands from either antrum or corpus;
4. Intestinal metaplasia – goblet cells and absorptive cells in the foveolar or surface epithelium;
5. H. pylori – density of the bacterium overlaying epithelium (Fig. 2).

According to the Sydney System, the pathologists grade these five criteria (no inflammation, mild, moderate or severe) on a 0–3 scale.

The knowledge about the initial phase of the infection is still quite low and there is no need for a patient to see his GP, because the clinical symptoms are usually absent as well. After 2–4 weeks a chronic inflammation develops and histologically a mixture of polymorphs and lymphocytes as well as plasma cells can be observed (Fig. 3).
The intestinal metaplasia occurs in different types and is easy to diagnose by the presence of goblet cells in the foveolar or surface epithelium. The type III intestinal metaplasia, characterized by sulphomucin production in the columnar cells, is thought to be a precancerous step to dysplasia and carcinoma of the intestinal type according to Lauren’s classification (9). P. Correa included the type III-metaplasia in his hypothesis on the development of gastric cancer (10).

During a longer lasting inflammation lymphoid follicles and aggregates appear in the gastric mucosa as an immunological response to chronic *H. pylori* infection. There are evidences linking *H. pylori*-induced gastritis with development of a low-grade B-cell-lymphoma of the MALT of the stomach (11). Meanwhile it is known that this kind of lymphomas can be treated by eradication of *H. pylori*. It seemed to be a revolution to treat a malignant lesion by antibiotics; however, a long lasting remission was not seen in many cases.

Unfortunately a low-grade B-cell-lymphoma of the MALT type is not always easy to diagnose and sometimes confusing with a severe chronic gastritis. Today, molecular-biological methods may confirm a monoclonal B-cell proliferation or lymphoepithelial lesions are required to confirm the diagnosis of lymphoma.

While the link between *H. pylori* infection and MALT lymphoma is doubtless the role of the bacterium in the tumorogenesis of gastric carcinoma is still not clear. Long lasting follow-up studies are required to confirm the WHO statement on *H. pylori* infection as a class I carcinogen (12).

**Epidemiology**

Although over the past 20 years many important questions relating to the epidemiology of *H. pylori* have been defined, a number of issues, including the route of transmission of *H. pylori*, remain controversial.

A number of studies have proposed that acquisition of *H. pylori* occurs via a common environmental source. In particular water has been implicated as a potential source of infection and the presence of *H. pylori*-specific DNA in a water sources has been reported by a number of studies (13). But there are two important factors that must be considered: first, the detection of *H. pylori* DNA does not indicate viable cells and second, the specificity of PCR in environments where an undiscovered *Helicobacter spp.* may be present is unknown. However, some seroepidemiological studies from Far East could not reveal water as a reservoir of the microorganisms (14). Because culturing of *H. pylori* from water was unsuccessful it has been suggested that when *H. pylori* is exposed to adverse environmental conditions, the organism takes on a viable but non-culturable coccoid form (15).

Seroepidemiological studies examining the relationship between pet ownership and the prevalence of *H. pylori* have in general failed to support such a relationship (16).

In conclusion, no significant reservoirs outside the human stomach have been shown. This fact suggests that direct person-to-person contact is the most likely mode of transmission (14). The finding of identical microbial strains within one family supports an intrafamilial transmission (17). Goodman et al. (18) and Rothenbacher et al. (19) showed that family composition may also influence the transmission of *H. pylori*.

It is probably true that the most studied and certainly the most controversial area of *H. pylori* epidemiological research today is the route of transmission. However, whether *H. pylori* reaches the oral cavity via the gastrooal...
for the most part in childhood. The prevalence of 
H. pylori infection both within and between countries 
(23). Numerous studies revealed low socioeconomic 
status to be associated with an increased prevalence of 
H. pylori infection (24). It is therefore not surprisingly 
that the overall prevalence of H. pylori infection in 
developed countries is lower compared to developing 
countries (25). Educational level has been shown to be 
an important determinant of H. pylori prevalence as 
well (26).

The influence of living conditions on the prevalence 
of H. pylori infection is clearly illustrated in countries 
where socioeconomic conditions have significantly 
improved over the last few decades, such as Japan (27) 
or Korea (28). In both developed and developing 
countries high density of living has been consistently 
related to an increased prevalence (29).

Natural acquisition of H. pylori infection occurs 
for the most part in childhood. The prevalence of H. 
pylori infection in less than 10-year-old children resid- 
ing in developed countries is approximately 0 to 5% 
compared with 13 to 60% in children residing in deve- 
loping countries. Examination of the data for age-re- 
lated prevalence showed that this difference in 
prevalence of infection has been attributed to the rate 
of acquisition of H. pylori in childhood (30).

The decreased levels of H. pylori infection asso- 
ciated with younger age groups, particularly in deve- 
loped countries, are due to improvements in medical 
care, sanitation, and living conditions (22). In contrast 
to this view, a number of studies have argued that there 
is a continuous risk of acquisition of H. pylori of appro- 
ximately 1% per year in adulthood (31). Clarification 
of this issue will require large cohort studies.

In Lithuania, 41% of 10–15 year-old children in 
Šilainiai microdistrict of Kaunas city, 51.7% of 20– 
23-year-old undergraduate students of Kaunas 
University of Medicine and 78.5% of 18–60-year-old 
blood donors have been found to be infected with H. 
pylori. The prevalence of H. pylori infection in our 
country was found to be similar to other Central or 
Eastern European countries (32–34).

Epidemiological data on H. pylori infection are ext- 
remely helpful in preventing the spread of pathogenic 
agents and further studies with a long term follow up 
should be performed.

**Bacteriology**

When Barry Marshall asked his microbiologist 
Stewart Goodwin to culture the “unidentified curved 
bacilli” obtained from gastric biopsies he was probably 
optimistic to get a culture very soon. But the microbio-
logy failed. Just by accident, over the extended Easter 
holiday time in 1982 the forgotten biopsy was examined 
after 5 days and was found to have a growth of Campy-
lobacter like organisms.

In fact, H. pylori is fastidious both in its growth 
and transport requirements. Culture of H. pylori is 
becoming more and more important for routine diag-
nostic management, since antibiotic sensitivity testing 
becomes useful to guide the therapeutic management 
in cases of non-responders to a recommended therapy 
protocols. Otherwise culture of the microorganism 
should be restricted on research settings.

Meanwhile the microorganism has been cultured 
from various tissue samples, such as ectopic gastric 
mucosa in Meckel’s diverticulum, esophagus, rectum, 
urethral bladder, dental plaque and feces (35–40). Stu-
arts transport medium for up to 24 h at low temperature, 
Albimi broth with 20% glycerol or skim milk with 17% 
glycerol are used for transportation of biopsies. At 
room temperature there is a heavy decrease in H. pylori 
titer after 6h, whereas at 4°C H. pylori can survive for 
about one week and in liquid nitrogen, it will survive 
indefinitely. An inappropriate transportation can there- 
fore cause false negative results.

*H. pylori* is usually grown in jars with gas-generation 
kits for at least 10–14 days (41). Skirrows and 
Dents selective media seem to be the best available 
and widely used commercial media. The colonies are 
small, translucent to yellowish. In very young cultures 
*H. pylori* can be seen microscopically as almost straight 
rod. After 3–5 days of incubation the bacteria look 
pleomorphic. In old cultures, *H. pylori* may appear as 
coccoid forms that Gram stain poorly and which seems 
to be a “survival form” of microorganism. More recent 
studies suggest that coccoid forms are not viable 
dormant forms but represent early stages of bacterial 
death (15).

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the organism. The Rapid Urease Test (RUT) as a fast method to detect a *H. pylori* infection has a high accuracy. The sensitivity and specificity is in the range of 95% and 100%, respectively. But, RUT requires a high bacterial density, such that recent use of antibiotics, bismuth-containing compounds, or proton pump inhibitors may cause false-negative results. Examples of commercial RUT include the original CLO test and two second-generation tests: hpfast and PyloriTek. Another very elegant method, which uses urease as a marker of infection is the Urea Breath Test (UBT). The specificity and sensitivity of the $^{13}$C-UBT is excellent, providing reliable information about *H. pylori* status before or after therapy (41).

In addition to protecting enzymes the bacterium secretes cytotoxins.

The Vacuolating Toxin (VacA) – a protein and product of the gene vacA, which induces cytoplasmic vacuolization in epithelial host cells. The gene coding for the vacuolating cytotoxin, vacA, is present in all *H. pylori* strains whereas the protein is produced by only 50–60% of the strains. Despite its lower potential cytotoxic effects it has been shown to be associated with ulcer development. The cytotoxin associated gene A (cagA) is part of a 40-kilobase pathogenicity island (cag PAI) and is present in approximately 60% of *H. pylori* strains. CagA (the Cytotoxin-associated gene A protein) – a product of the cagA gene, is usually co-expressed with the vacuolating cytotoxin. Based on the presence of these two virulence determinants, *H. pylori* strains can be divided into two major groups (45):

- Type I *H. pylori* possess cagA and express both CagA and VacA;
- Type II *H. pylori* do not have cagA and do not produce CagA and VacA proteins;
- The mixed Type is related to type I but express either CagA or VacA.

It has been thought that infection with *H. pylori* strains expressing CagA constitutes a factor associated with increased risk of gastric cancer (46). Our results from Lithuania show a high prevalence of CagA+ seropositivity in gastric cancer patients (73.9%) and controls (60.9%) and suggest, that other factors than CagA predominate in gastric cancer pathogenesis in our country (47).

Several immunological techniques have been performed to detect antibodies against *H. pylori*. The initial serologic tests were patterned and consisted of a mixture of very crude antigens. Indeed, cross-reactivity with other bacterial antigens occurred. Second generation tests have used purified antigens such as the high-molecular-weight antigens, and some of the tests have proven accurate worldwide.

There are numerous methods developed for the detection of anti-*H. pylori* IgG in whole blood, serum, saliva and urine (48). ELISA-tests are widely available because of their low costs, rapidity, and reproducibility. Immunoblotting is highly sensitive and specific and mostly used to identify false-positive ELISA reactions and to analyze antibody reactivity to individual bacterial proteins (49) (Fig.4).

The detection of serum IgG against *H. pylori* typically indicates a current or prior infection. Antibody titers fall following a successful eradication of *H. pylori*. However, the decrease in titer after treatment is slow and unpredictable. One can therefore never expect serologic tests to provide as accurate results for the presence of an active *H. pylori* infection as histology, culture or UBT.

Recently there has been increased interest in identifying *H. pylori* protein antigens in stool as a marker of infection. Study results have shown that the sensitivity and specificity of stool antigen testing are comparable to histology and UBT (40). There are also exceptions reporting less satisfactory results (50).
Although the main histopathological and microbiological features have been described over the last twenty years, the story of H. pylori has not been finished. Until now the relationship between different H. pylori strains and different patterns of gastritis remains an unsettled issue. Furthermore the question whether there is a link between certain strains of the germ and the risk of developing malignant tumors in the stomach has to be answered.

Is H. pylori always a pathogen or is it sometimes a commensal? Is this depending on the strain or is there an influence coming from the host? Do we need to eradicate all infected patients? What new methods of eradication will we have?

Could a H. pylori infection be a benefit for patients, especially in the prevention of gastro-esophageal reflux? Do we need a routine characterization of H. pylori strains to distinguish the “good” ones from the “bad” ones? How can PCR as a modern tool be helpful in a quick and routine based characterization of H. pylori strains? Will there be other elegant testing systems for example stool tests for diagnosing H. pylori infection?

A lot of unanswered questions are left and they will keep H. pylori researchers and routine workers busy. Indeed, Bizzozero could not imagine that his observation of a small curved bacterium in a dog’s stomach will spark off an explosion of research, diagnostic tools, and developments of treatment protocols.

Ko Bizzozero niekada negalėjo įsivaizduoti – Helicobacter pylori šiandien ir rytoj

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