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# Identification of *Helicobacter pylori* and the evolution of an efficacious childhood vaccine to protect against gastritis and peptic ulcer disease

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Establishment of *Helicobacter pylori* infection as an etiologic agent of peptic ulcer disease and other gastric pathologies marked a revolution in gastroenterology which spurred an enormous interest in gastric physiology and immunology research. The association was soon also demonstrated in children as well. Application of antimicrobial therapies have proven remarkably efficacious in eradicating *H. pylori* and curing pediatric patients of duodenal ulcers as well as gastritis, negating a lifetime of ineffective therapy and life-threatening disease. Countries with high *H. pylori* prevalence and where *H. pylori* associated gastric cancer remains a primary cause of death due to cancer however would benefit from childhood vaccination. Studies in rodents and humans utilizing oral vaccination with bacterial exotoxin adjuvants demonstrated potential for limiting *H. pylori* colonization in the stomach. Almost 25 y of vaccine research recently culminated in a phase III clinical trial of over 4,000 children aged 6–15 y old to test an oral vaccine consisting of the *H. pylori* urease B subunit genetically fused to the *E. coli* heat labile toxin. Vaccination was demonstrated to have an efficacy of over 70%. Vaccination may now serve as an effective strategy to significantly reduce *H. pylori* associated disease in children throughout the world.

## *HELICOBACTER PYLORI* AND THE ETIOLOGY OF GASTRIC DISEASES

*Helicobacter pylori* is a Gram negative bacterium that infects the stomach of greater than half of the Earth's population and is a primary cause of gastric pathologies including peptic ulcer disease, dyspepsia, and gastric cancer (1–5). Prevalence varies widely from 10 to >70% (6,7). Infections are primarily but not exclusively acquired in early childhood and spread through fecal–oral and oral–oral transmission. Infection lasts for the life of the host and while most infected individuals remain asymptomatic, 10–20% develops peptic ulcer disease, 1% develops gastric adenocarcinoma, and < 1% will develop mucosa-associated lymphoid tissue lymphoma. The World Health Organization has classified *H. pylori* as a definite class I carcinogen (5). Gastric cancer remains the second leading cause of death due to cancer worldwide and large geographic regions including South America, Eastern Europe, and the Far East these levels range from 20 to 40 per 100,000 (8).

The association between *H. pylori* and gastric diseases was not readily accepted by the medical community. Spiral bacteria had been described in human gastric tissue biopsies by microscopy as early as 1906 and periodically throughout the next 70 y (9–12). There were studies however that failed to identify bacteria in gastric biopsy specimens (13). Additionally, there was a general acceptance in the medical community that bacteria could not survive in the acidic stomach and that such observations might be artifacts or evidence of bacterial contamination. The cause of gastritis and peptic ulcer disease was predominantly attributed to stress and were treated by neutralizing acid and with surgery. The debate was renewed in 1983 when Warren and Marshall published their works documenting the presence of spiral bacteria on the gastric epithelium and predominantly associated with active chronic gastritis (14). Importantly, they were also able to culture the bacteria from fresh clinical biopsies with prolonged microaerobic conditions. The bacteria, which would eventually be called *H. pylori* were termed Campylobacter-like organisms. These two investigators subsequently published a similar study linking the bacteria to peptic ulcers as well (2). The gastroenterology community however, largely continued to dismiss the link until, in separate studies, Barry Marshall and Arthur Morris fulfilled Koch's postulates by ingesting cultures of *H. pylori* and demonstrating an associated gastritis and epigastric pain (15,16). Barry Marshall and Robin Warren would receive the Nobel Prize in Physiology or Medicine in 2005 for their work which fundamentally changed our understanding of gastric disease and its treatments.

## PREVALENCE OF *H. PYLORI* INFECTION AND ASSOCIATED DISEASES IN CHILDREN

Diseases associated with *H. pylori* infection such as peptic ulcer disease and gastric cancer, and even symptomatic gastritis are manifested predominantly in adults. These diseases create a heavy burden on health care systems world-wide because of the prevalence of *H. pylori*. The US Department of Health and Human Services estimate that in the year 2004, 20 y after the identification of *H. pylori*, direct costs associated with peptic ulcer disease reached $2.6 billion in the United States alone

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(17). Indirect costs associated with loss of work productivity were estimated to be an additional $518 million. Gastric cancer costs were ~$487 million plus an additional $1.4 billion in indirect costs. Although these diseases are primarily observed in adults, a vaccine administered in early childhood would still be the most practical given the early age of *H. pylori* acquisition. It is also important to note that *H. pylori* are often present when children are assessed for abdominal pain or dyspepsia.

The first reports of a potential association between *H. pylori* and gastric health in children were all made in 1986. Three independent teams of physicians investigated biopsies for the presence of *Campylobacter pyloridis*, or *Campylobacter*-like organisms as *H. pylori* were termed at the time (18–20). Each study examined small numbers of patients including Czinn *et al*. who performed a detailed histologic analysis of gastric antral biopsies on five patients (19). Endoscopic evaluation revealed small ulcer, antral nodularity, and histologic gastritis. Histologic evaluation revealed diffuse chronic gastritis including well-formed lymphoid follicles in two patients. Higher magnification revealed the presence of spiral bacteria at the epithelium in all five patients.

These studies were followed by a larger scale prospective study by Drumm *et al*. in which 67 patients undergoing upper endoscopy and biopsy for gastrointestinal symptoms were examined (21). The study is notable for several reasons. First, there was a high degree of association between *H. pylori* and unexplained histologic gastritis. Eighteen of the 67 patients were diagnosed with gastritis by histologic examination. However, eight of those children had gastritis associated with Crohn's disease, eosinophilic gastroenteritis, or were receiving medication with a known association to increased incidence of gastritis. *H. pylori* were not detected in any of these cases. *H. pylori* were detected however in 7 of the 10 remaining patients presenting with histologic gastritis. Second, five of the eight patients with unexplained gastritis and positive for *H. pylori* were diagnosed with duodenal ulcers by endoscopy. Although *H. pylori* could be identified in the antrum but not the duodenum, duodenal ulcers were not detected in any of the other 62 patients. Finally, as noted above, *H. pylori* were not present in any of the biopsies from patients with histologic gastritis due to an underlying cause. Importantly, *H. pylori* were not detected in any of the 49 patients presenting without any evidence of gross or histologic gastritis. This type of control was lacking from many adult studies at the time and provided strong evidence for *H. pylori's* unique association with gastric disease in children.

## ESTABLISHING PRINCIPLES FOR A *H. PYLORI* VACCINE

*H. pylori* infection can be treated by established antimicrobial therapies. These therapies can vary but in general consist of combination therapies including two antibiotics and a proton pump inhibitor (3). The most recent consensus recommendation supports quadruple therapy and a course of 14 d (22). Eradication rates as high as 90% can be achieved in compliant patients. Unfortunately, as these therapies are taken multiple times per day for a minimum of 7–14 d, patient compliance is often poor. They are also often accompanied by side effects including diarrhea and nausea. In addition, it is not fiscally or practically possible to treat one half of the world's population with antimicrobial agents in an effort to cure peptic ulcer disease or prevent gastric cancer. In the United States, if only 10% of young people are infected, more than one million pediatric patients would be at risk to receive eradication therapy. The cost is prohibitive and it would also lead to the development of antibiotic resistance in both *H. pylori* and other human pathogens. Therefore, a concerted effort has been made toward vaccine development.

It should be noted that chronic *H. pylori* infection is associated with several significant health benefits, particularly in the West where a steady decline in the prevalence of *H. pylori* has coincided with increased esophageal pathologies. An inverse correlation has been described between *H. pylori* infection and Barrett's esophagus (23–25), Barrett's metaplasia (26), esophageal adenocarcinoma (24), and esophageal eosinophilia (27). A meta-analysis on published studies also suggests that *H. pylori* may provide protection from Inflammatory Bowel Disease (28). More significantly with respect to children, an inverse correlation has been described between *H. pylori* infection and allergic asthma (29–31). The protective function of *H. pylori* against allergic asthma has been confirmed in an experimental mouse model and demonstrated to be due to the overriding effects of *H. pylori* induced Treg cells which are able to suppress immune responses against unrelated antigens (32,33). The decision to apply widespread vaccination against *H. pylori* must be considered against the potential increase in other chronic diseases, but in populations where gastric cancer is more prevalent and associated with high morbidity and mortality vaccination would provide an overall advantage.

The nature of *H. pylori* infection has made development of a vaccine technically challenging. Since *H. pylori* resides at the surface of the gastric epithelium and does not invade the tissue, it is able to avoid many aspects of the host immune response. Although neutrophils can cross the epithelium and form crypt abscesses, host defenses against the motile *H. pylori* are limited to secreted antibodies and antimicrobial peptides. It was generally accepted by immunologists that systemic immunizations were ineffective at generating resistance to mucosal pathogens. Additionally, vaccines targeting mucosal tissues also induce weak immunity. The lack of safe and efficacious adjuvants to strengthen mucosal immunogenicity is largely responsible for the paucity of vaccines against venereal diseases and gastrointestinal infections.

One class of mucosal adjuvants is the bacterial exotoxins such as cholera toxin (CT) and *E. coli* heat labile toxin (LT). The toxins consist of a pentameric ring of B subunits that bind to GM1 gangliosides present on epithelial cells and an A subunit that has enzymatic activity (34). Upon endocytosis of the toxin, the A subunit activates a G protein which ultimately leads to continuous production of cAMP resulting in the efflux of ions and water. In small doses however, these exotoxins are not only immunogenic but when mixed in solution with an unrelated protein antigen confer potent immunogenicity to

that protein when applied by mucosal immunization (35–37). This technique had been employed with great success in mice to study host immunity to Sendai virus as a model for human influenza by the laboratory of John Nedrud (37). Steven Czinn, a pediatric gastroenterologist and early investigator of *H. pylori* pathogenesis collaborated with Nedrud to adopt this immunization strategy to demonstrate oral immunization could be used to generate a host immune response to *H. pylori* (38).

Mice were given four weekly doses of 1 mg *H. pylori* whole cell lysate antigen in combination with 10 μg CT adjuvant by oral gavage. They achieved a fivefold increase in anti-*H. pylori* serum IgA titers and a 16-fold increase in intestinal IgA compared with mice immunized without CT. Significant increases in IgG were also noted. Similar results were achieved when immunizing ferrets with 7 mg *H. pylori* lysate and 60 μg CT doses. Ferrets were selected because their stomachs become naturally colonized by the closely related species *H. mustelae* (39). This was the first demonstration that it was possible to induce significant levels of anti-*H. pylori* mucosal immune responses through the use of oral immunization combined with experimental exotoxin adjuvants.

These studies were performed prior to the development of a mouse model for *H. pylori* infection that could utilize immunocompetent mice. However, the use of the cat isolate *H. felis* was demonstrated to readily infect mice and to induce histologic gastritis similar to that observed in *H. pylori* infected humans within several weeks of infection (40). The development of this animal model made it possible to test the new oral vaccine protocol using a Helicobacter challenge model. Mice were orally immunized with weekly doses of *H. felis* lysate antigen in combination with CT adjuvant and then challenged 7–10 d after the final immunization (41). Mice were then examined 7 d following challenge and assessed for immunity. Similar to their prior study, Czinn *et al.* observed significant increases in serum and mucosal anti-H. felis antibody titers including a fourfold increase in gastric IgA, and an eightfold increase in intestinal IgA. Most importantly, 76% of immunized and challenged mice were determined to be protected from infection whereas only 22% of control mice were bacteria free. Similar protection was also observed by Chen *et al.* employing the same mouse model and closely related immunization protocol (42). These studies, combined with new animal models for *H. pylori* infection, served as the foundation for over two decades of studies by numerous laboratories to better characterize *H. pylori* pathogenesis, identify the immune mechanisms that contribute to protective immunity, and to test multiple variations and new strategies for vaccinating against *H. pylori* (43). Protecting mice from *H. pylori* could be accomplished with many candidate protein antigens, by multiple routes of immunization, and with many alternative adjuvants and delivery mechanisms. These results, however, while highly reproducible among laboratories were not so easily translatable in clinical trials.

## LIMITED SUCCESS IN EARLY CLINICAL TRIALS

The first and perhaps most well-known clinical trial to test a *H. pylori* vaccine for efficacy utilized the strategy developed by Czinn and Nedrud in experimental animal models. Given the practical considerations of performing an infectious challenge, and based on studies in mice and ferrets demonstrating success when applying a Helicobacter vaccine therapeutically (44–46), the investigators screened for *H. pylori* infected adults to receive the therapeutic vaccine (47). Groups of 4–6 subjects testing positive for *H. pylori* based on the [$^{13}$C]urea breath test and by serology were immunized orally with either 180, 60, or 20 mg recombinant *H. pylori* urease plus 5 μg LT. Urease was selected based on its extensive use in many immunization experiments which began with the first demonstration of induced protective immunity using a subunit vaccine instead of bacterial lysate antigen (48). They received four weekly doses and then gastric biopsies were collected 1 and 6 mo after the final immunization to evaluate the host immune response and bacterial load. The results were compared with subjects receiving either LT without antigen, or neither LT or antigen.

The study was disappointing to many as *H. pylori* were not eradicated by any of the tested doses. Additionally, the investigators reported significant patient complaints attributable to the vaccine formulation with 62.5% of subjects developing diarrhea. It occurred within 12 h of receiving the first dose and lasted for up to 24 h. These episodes decreased with each successive dose, and occurrence was independent of urease dose. It is notable that a subgroup of 12 subjects was initiated with 10 μg doses of LT and half of them experienced diarrhea that was significant enough to interfere with daily activities or lasted more than 1 d, prompting the investigators to switch these subjects to 5 μg LT in subsequent doses. The results highlight the problems inherent in utilizing exotoxin adjuvants for mucosal immunization. In follow up studies these investigators tested a range of lower LT doses and alternative mucosal routes of immunization to improve safety but were unable to derive a satisfactory protocol to attempt another clinical vaccine trial (49,50).

These results drew attention away from some of the positive aspects of the study. The investigators evaluated induced immunity through the measurement of antiurease antibody titers as well as enumerating the number of circulating urease specific B cells. They achieved significant increases in urease-specific serum IgA in subjects receiving either 180 or 60 mg urease antigen compared with control subjects, and all doses induced significantly greater numbers of urease specific IgA positive antibody secreting cells. More importantly, a significant reduction in bacterial load was achieved in subjects receiving the 20 mg dose of urease and when all subjects receiving urease were combined. While no sterilizing immunity was achieved, this study demonstrated that oral immunization could be used to positively impact mucosal immunity at the gastric mucosa as demonstrated by the reduced bacterial load. Given the predicted difficulties in immunizing against a mucosal pathogen, such a result could be categorized as encouraging despite the lack of efficacy compared with studies in the mouse model.

Three subsequent clinical trials performed by other investigators however were less encouraging. These included another therapeutic vaccine trial employing the use of killed whole cell

*H. pylori* in combination with a nontoxic LT variant containing an amino acid substitution (51), a prophylactic vaccine consisting of attenuated Salmonella strains expressing *H. pylori* proteins (52), and a prophylactic trivalent protein vaccine mixed with alum given intramuscularly (53). Although clearance of bacteria was observed in some subjects, the numbers were not greater than that observed for control subjects. It is difficult to define why these subsequent studies failed to achieve the limited success reported by Michetti *et al.* (47) but the use of the LT exotoxin seems to provide an advantage over other immunization strategies. The primary obstacle therefore involved gaining the benefit of LT adjuvanticity while eliminating its toxicity.

## LARGE SCALE CLINICAL SUCCESS WITH AN ORAL CHILDHOOD VACCINE

The problems associated with previous clinical trials may now have been overcome. The results of a large scale phase III clinical study on a *H. pylori* subunit vaccine were recently reported and demonstrated to have a vaccine efficacy of 71.8% (95% confidence interval 48.2–85.6) by the end of the first year (54). The follow up period postimmunization was performed at 3 y and efficacy had fallen to 65% (95% confidence interval 46.4 – 77.7). The overall utility of this vaccine will require additional longer term analysis but these results are highly significant for the long term goal of immunizing against childhood infection of *H. pylori*. The study employed a number of strategies that distinguish it from previous *H. pylori* clinical vaccine trials, although it is difficult to identify which of these factors or combination of factors is primarily responsible for achieving such improved efficacy over previous trials.

First, the study involved children aged 6–15 y old, a departure from all previous clinical trials which were performed exclusively in adults. In this light, it is worth considering whether vaccination might have been even more efficacious if delivered within the first several years of life on a schedule consistent with most childhood vaccines. Epidemiologic data show that in many societies *H. pylori* infection occurs within the first 5 y of life and often before the age of 2 y (55). However to the extent infection can continue to occur throughout the life, a vaccine would optimally be useful when applied to teenagers and adults as well. Additionally, should this vaccine possess therapeutic potential it would increase effectiveness in that the infected pool of hosts within a given population could be reduced, thereby limiting opportunity for spread. More information will be needed to determine if the age of application is important for vaccine efficacy and to determine the utility of vaccination at differing stages of life.

Second, the vaccine was given as a prophylactic. While two of the four previous clinical trials were also prophylactic vaccines, each of those trials relied upon an experimental challenge with well characterized strains of *H. pylori* (52,53,56). Zeng *et al.* tested the efficacy of their vaccine against naturally acquired *H. pylori* infection. It is likely the bacterial loads encountered naturally are lower than what investigators have used experimentally. Third, the scale of this most recent study was over two orders of magnitude higher than the average of the previous four studies. More than 5,700 participants were assessed for eligibility with 2,232 eligible subjects each eventually assigned to vaccine and placebo groups. Participants successfully completing the three dose vaccination schedule included 2,199 receiving vaccine and 2,204 receiving the placebo.

The nature of the vaccine was a combination of tested and new technology with regard to clinical trials for *H. pylori* immunizations. The vaccine was administered orally on days 0, 14, and 28. The antigen consisted of a fusion protein generated from a recombinant gene encoding the *H. pylori* urease B subunit and the exotoxin B subunit of *E. coli* heat labile protein. The investigators therefore selected an antigen widely used in both animal and clinical trials fused to a mucosal adjuvant subunit. Many *H. pylori* protein antigens have been tested for vaccine efficacy in animal models including those attributable to pathogenesis including urease, CagA, VacA, catalase, and others (43). No single protein has been identified to be superior to the others. There may be some benefit to using a multivalent vaccine as demonstrated in mice (57), and the inclusion of additional proteins may improve the efficacy of the vaccine tested in China. Never the less, the selection of urease is supported by previous studies. In many respects the strategy employed by Zeng *et al.* is remarkably similar to that originally described by Czinn and Nedrud (38,41). It consisted of an oral vaccine administered several times over the course of 28 d and included a protein antigen combined with a bacterial exotoxin mucosal adjuvant. The nature of their mucosal adjuvant construct however makes their results all the more surprising.

As discussed above, bacterial exotoxins derive their mucosal adjuvanticity from the enzymatic activity of the A subunit. Unfortunately, the A subunit is also responsible for the toxicity associated with the exotoxin. Preparations of B subunit devoid of any contaminating A subunit are safe but lack adjuvanticity (58–61). It is for this reason that efforts have been made to construct a holotoxin with point mutations in the A subunit in an attempt to retain adjuvanticity while minimizing the toxic effects of the enterotoxin. The function of the B subunit is to bind to the epithelium. In the context of the construct employed by Zeng *et al.* therefore, the fusion protein would potentially bind to the gastrointestinal epithelium but would lack adjuvancity. This strategy has been tested in many experimental models to induce immunity but has also been used to induce immunologic tolerance to the fusion protein to treat autoimmune diseases in animal models (62–67). Zeng *et al.* demonstrated significant increases in urease B-specific serum IgG and salivary IgA titers by 1 mo postimmunization. By 36 mo serum IgG titers decreased but remained fourfold higher for immunized subjects compared with those receiving placebo. Although antibodies have not been determined to be required for protection against *H. pylori* (68,69) and are not correlated with immunity to *H. pylori*, increases in antigen specific antibodies can be used as a measure of vaccine immunogenicity. In the absence of a third group of subjects receiving

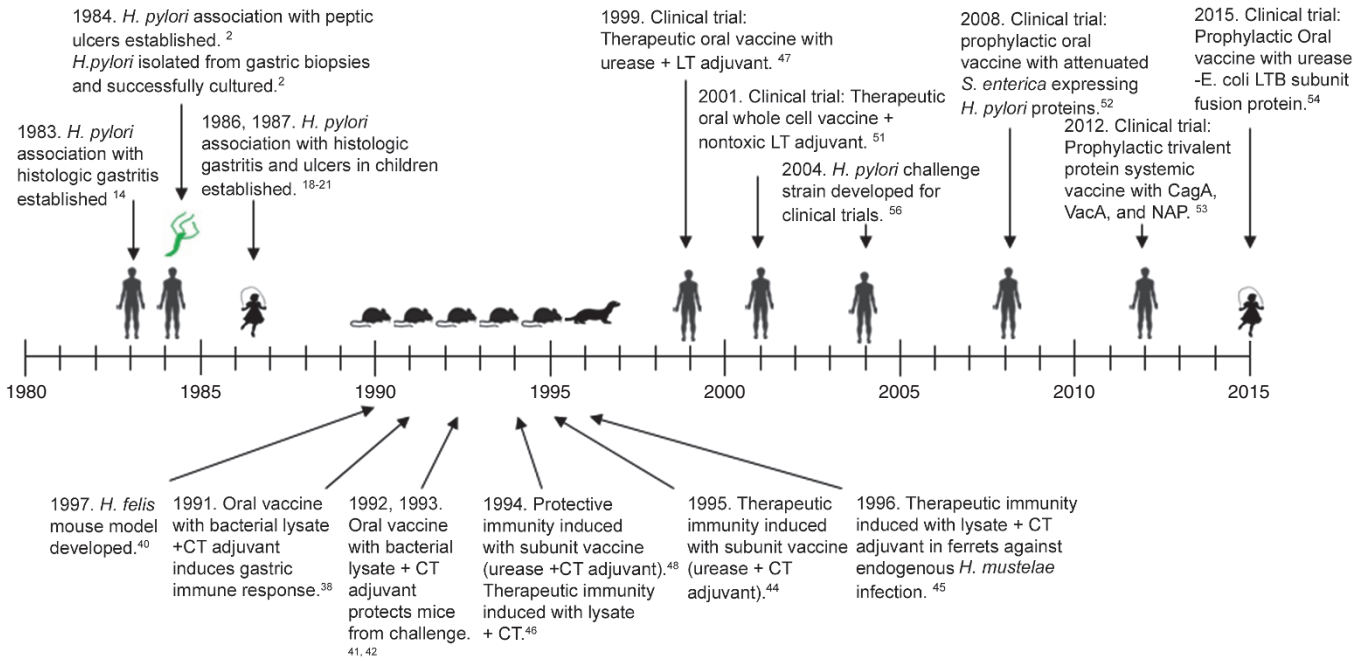


**Figure 1.** Milestones in the advancement of treating and preventing *H. pylori* associated diseases through vaccination.

urease B without adjuvant however, it is impossible to determine whether the antibody titers and protection achieved was due to the presence of the fused enterotoxin B subunit or would have been achieved by 15 mg doses of urease B protein alone.

It is also worth noting that the vaccine construct used by Zeng *et al.* was demonstrated to be safe for the participants. Although 7% of subjects receiving the vaccine experienced adverse reactions within the first few days of immunization, the numbers were equivalent for subjects receiving the placebo. The most common reaction observed was vomiting followed by fever and headache but in all cases conditions resolved within 24 hours. Subjects receiving the vaccine did experience bloating more than the placebo control group ($P = 0.0427$) but this condition was also mild and quickly resolved. All subjects were fasted for at least 2 h and then given a buffer solution consisting of sodium bicarbonate and sodium citrate prior to receiving 80 ml of vaccine or placebo.

Gastric health for pediatric as well as adult patients has benefitted greatly as a result of Warren and Marshall's identification of *H. pylori* and it's etiology with gastritis and peptic ulcer disease in the mid 1980's. Those previously diagnosed with a gastric or duodenal ulcer faced a lifetime of pain and suffering, a series of bromides of questionable efficacy, and a bland diet, to say nothing of surgical remedies. Today a diagnosis of peptic ulcer disease accompanied by *H. pylori* infection can be permanently cured in most cases with antimicrobial therapy. Although the etiology of symptomatic gastritis is multifaceted and complex, it is clear that when *H. pylori* infection is present, eradication of *H. pylori* often cures the disease. The development of a vaccine to protect against *H. pylori* has been slow to develop (Figure 1). Until recently, clinical trials offered little encouragement. The most recent large scale trial however is promising. A vaccine that demonstrates 65% efficacy after 3 y may require improvement, but there is no doubt their ureaseB-*E. coli* labile toxin B subunit fusion protein is capable of inducing protective immunity in the absence of untoward side effects. The study readily suggests a trend for continued research and trials including the inclusion of additional antigens fused to LTB, the testing of vaccination at earlier ages, and the testing of the vaccine when applied therapeutically to infected hosts. It will also be important to determine longevity of protection and the efficacy of vaccination when comparing populations at varying risks for reinfection. Future studies aimed at the improvement of this vaccine candidate, or which build upon its success may ultimately lead to an effective strategy for eliminating *H. pylori* from large population centers where *H. pylori* is endemic and *H. pylori*-associated diseases are prevalent.

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