Overview

Overview of the impact of Typhoid and Paratyphoid fever. Utility of Ty21a vaccine (Vivotif®)

D. AMICIZIA, L. ARATA, F. ZANGRILLO, D. PANATTO, R. GASPARINI
Department of Health Sciences, University of Genoa, Italy

Keywords

Typhoid fever • Enteric fever • Ty21a vaccine • Vivotif® • *Salmonella typhi* • *Salmonella paratyphi*

Summary

Cases of diarrhoeal disease number from 1.7 to 5 billion per year worldwide. One of the main causes of diarrhoeal disease is typhoid fever, which is a potentially life-threatening multi-systemic illness. According to the most recent estimates, a total of 26.9 million typhoid fever episodes occurred in 2010. The geographical distribution of the disease differs widely; in developed countries, the incidence rate per 100,000 per year varies from < 0.1 to 0.3, and the disease mainly affects people who travel to endemic areas located in low- and middle-income countries. Low- and middle-income countries are mainly affected owing to the lack of clean water and proper sanitation. In the fight against this plague, prevention is fundamental, and vaccination against typhoid is an effective measure. Vivotif® is an oral live attenuated vaccine which contains a mutated strain of Salmonella (Ty21a) and reproduces the natural infection. The vaccine was first licensed in Europe in 1983 and in the US in 1989, and over the years it has proved efficacious and safe. It is indicated for adults and children from 5 years of age upwards. Specifically, in the most developed countries, vaccination is suggested for high-risk population groups and particularly for international travellers to destinations where the risk of contracting typhoid fever is high. It must also be borne in mind that international travel is increasing. Indeed, international tourist arrivals totalled 1,184 million in 2015 and, on the basis of current trends, international travel is expected to grow by 3-4% in 2017. Vivotif® appears to be a powerful means of disease prevention, the importance of which is highlighted by the spread of antibiotic-resistant strains of Salmonella typhy (S. typhi).

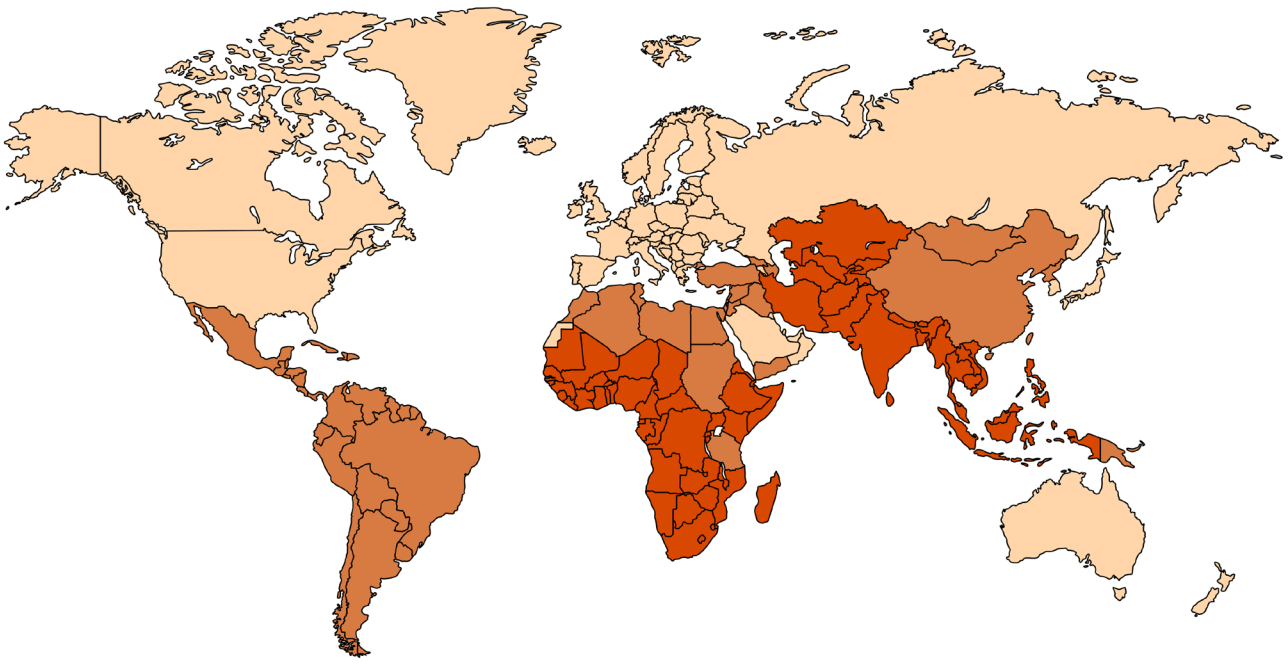
Introduction

Typhoid Fever (TF), also known as enteric fever, is a potentially life-threatening multi-systemic illness. It is mainly caused by *Salmonella enterica*, subspecies *enterica serovar typhi*, and to a lesser extent by serovars *paratyphi* A, B, and C, which are members of the family of *Enterobacteriaceae* [1]. The genus *Salmonella* is divided into serotypes, on the basis of surface antigens: O antigen based on the lipopolysaccharide component, and H antigen based on flagellar proteins. Moreover, pathogenic strains of *S. typhi* and *S. paratyphi* C present a Vi antigen polysaccharide component [2].
The burden of diarrhoeal disease is very high, accounting for 1.7 to 5 billion cases per year worldwide [3, 4]. The main risk factors of diarrhoeal disease are inadequate drinking water and inadequate sanitation; indeed, in 2014 the World Health Organization (WHO) attributed 502,000 deaths to inadequate drinking water and 280,000 to inadequate sanitation [5, 6]. Furthermore, according to WHO estimates, TF is one of the main causes of foodborne deaths and results in the greatest loss of Disability-Adjusted Life years (DALYs) worldwide [7]. Some authors have called TF "an old plague", asserting that "currently, despite major efforts in preventing and treating cases of enteric fever, millions of new infections (approximately 21 million new cases per year) of typhoid and paratyphoid fevers occur in many areas where poor sanitation and unsafe food and water access occurs frequently and among travellers to endemic areas" [8].
In the fight against this plague, preventive measures are fundamental. Vaccination against typhoid is an effective preventive intervention, especially when coupled with hand-washing, the treatment of household water, and the provision of adequate sanitation [9].
Currently, two well-tolerated and effective vaccines are available. One is based on the use of live attenuated bacteria and is administered orally; the other is based on Vi capsular polysaccharide (Vi-PS), and is administered intramuscularly or subcutaneously [9].
In the present overview, we investigated the epidemiological impact of typhoid and paratyphoid fever and assessed the utility of Ty21a vaccine (Vivotif®) and vaccination policies.

Epidemiology

As mentioned above, TF is one of the main causes of enteric disease worldwide [10]. The incidence of typhoid fever (overall population) is reported in Figure 1.
According to the most recent estimates, a total of 26.9 million typhoid fever episodes occurred in 2010 [11-14]. The distribution of the disease differs widely through-

Fig. 1. Typhoid fever incidence (from Mogasale, et al., mod. [63]).



out the world. In developed countries, the incidence rate per 100,000 per year varies from < 0.1 to 0.3, and the disease mainly affects people who travel to endemic areas located in low- and middle-income countries [11]. Low- and middle-income countries are mainly affected owing to the lack of clean water and of proper sanitation; indeed, the known risk factors for TF are: high population density, unsanitary living conditions, poor hygiene, low socio-economic status, and recent contact with a patient affected by TF [15]. TF has a heavy burden in Asia, with an overall incidence of 170.8 cases per 100,000 people per year, though this estimate varies across the continent [11]. Specifically, Buckle et al. estimated an annual incidence rate of 394.2 per 100,000 in southern Asia. With regard to Africa, the incidence is estimated to be 724.6 cases per 100,000 people per year; however, it is probably underestimated, owing to the lack of information and surveillance systems in the continent [11]. Moreover, Africa suffers many cases of invasive non-typhoid salmonellosis, which are additional confounding factors in estimating the typhoid fever burden [15]. Typhoid fever also affects countries in Latin America, the Caribbean and Oceania, although to a lesser extent, with a median incidence rate of 22.3 cases per 100,000 people per year [14].

The 5-15 year-old age-group is considered the main target of the disease. Notably, even when properly treated, children have a high case fatality rate.

The median FT mortality rate varies from region to region: high-income countries such as North America, Europe, Australia and New Zealand register less than 0.1 death per 100,000 people per year, while the mortality rate is higher in Sub-Saharan Africa (7.2) and Southern Asia (3.9) [11].

S. paratyphi causes paratyphoid fever. *S. paratyphi* is thought to cause milder disease than *S. typhi*, with symptoms being predominantly gastrointestinal [16]. While this is probably true of *S. paratyphi* B infection, there are insufficient data to draw conclusions regarding *S. paratyphi* A [17]. In 2000, 5.4 million cases of paratyphoid fever were estimated to have occurred, with incidence rates ranging from 8 cases per 100,000 people per year in high-income countries to 77.4 in low-income countries. Specifically, in Eastern and Southern Asia, the annual incidence is 17.9 cases per 100,000 people. Moreover, *S. paratyphi* A has been found to be responsible for a considerable, and increasing, proportion of cases of enteric fever in some Asian regions [13].

The impact of *S. typhi* and *S. paratyphi* disease is probably underestimated because of inadequate surveillance systems in the most severely affected areas, the low sensitivity of diagnostic tools and healthcare inequalities resulting in low health-seeking behaviour among populations at the highest risk [8].

Typhoid fever disease

S. typhi is restricted to human hosts, and chronic carriers constitute the reservoir of infection.

The disease is mainly transmitted through the consumption of food, drink or water that have been contaminated by the faeces or urine of subjects excreting bacteria (sick or convalescent people or chronic asymptomatic carriers). After *S. typhi* has been ingested, it reaches the intestinal epithelium, where it colonizes macrophages and dendritic cells in the lamina propria; but these fail to destroy the bacterium [9]. Subsequently, bacteria invade the bloodstream, multiply and spread to the lymph nodes, spleen and liver, causing multi-systemic disease [14]. The main manifestations of the disease are fever, which can reach 38°-40°C, and abdominal symptoms (such as diarrhoea or constipation). Nonspecific symptoms, such as weakness, anorexia, headache and dizziness, may precede the fever. Moreover, rose-coloured spots may appear on the trunk, and patients may also experience neuropsychiatric manifestations, hepatomegaly and splenomegaly. The most severe complications are gastrointestinal bleeding, intestinal perforation and typhoid encephalopathy, which occur in 10-15% of patients, generally in the third and fourth weeks of infection [18, 19]. The duration of infection is a major determinant of the risk of severe complications, and a delay in administering appropriate antibiotic treatment may have serious consequences.

Isolation of *S. typhi* from blood is the most common method of diagnosis, though the bacterium can also be isolated from bone marrow, faeces and duodenal fluid. Blood culture displays suboptimal sensitivity, generally being positive in only about 50% of cases. Blood culture also has several limitations, including the volume of blood needed, the need for prompt transport to the laboratory, interference due to prior antibiotic use, limited laboratory expertise and equipment, and expense [13]. Bone marrow culture increases the diagnostic yield to approximately 80% of cases. Stool culture is not usually positive during the earliest phase of the disease [20]. Multiple cultures increase sensitivity and may be required in order to reach a diagnosis.

Although the Widal test is unreliable, it is widely used in developing countries because of its low cost. Newer serologic assays for *S. typhi* infection are occasionally used in outbreak situations, and are somewhat more sensitive and specific than the Widal test; however, they are not an adequate substitute for blood, stool, or bone marrow culture [1].

Early diagnosis and the prompt institution of appropriate antibiotic treatment are essential for the optimal management of TF, especially in children. Ciprofloxacin is commonly used as an empiric treatment, as fluoroquinolones are recommended. However, as fluoroquinolone-resistant or multidrug-resistant strains are spreading, third-generation cephalosporins are used when the possibility of resistance is high [18, 19].

Preventive measures

In the late 19th and early 20th centuries, typhoid was endemic in all countries, including Europe and the Americas. Subsequently, the widespread use of chlorination, sand filtration, and other means of water treatment drastically reduced the incidence of TF, despite the high prevalence of chronic carriers [14]. Today, TF still places a devastating burden on many low- and middle-income countries; in high-income countries, the impact of the disease is mainly linked to travel to endemic disease areas [16].

Prevention by vaccines

Currently, 2 typhoid vaccines are internationally available, and both have been shown to be safe and efficacious [9].

The first is an oral vaccine based on a live attenuated *S. typhi* Ty21a strain (Vivotif®), which has been developed in two formulations: enteric coated capsules and a liquid formulation. On the market Vivotif® is available in enteric coated capsules.

The second is a Vi capsular polysaccharide (Vi-PS) vaccine, which is injectable. Furthermore, typhoid conjugate vaccines (TCVs) have been developed, one of which is based on Vi conjugated to rEPA, a recombinant exoprotein A from *Pseudomonas aeruginosa* [22]. Two Vi-tetanus toxoid conjugate vaccines have recently been licensed in India [22].

Ty21a vaccine (Vivotif®)

Vivotif® is a vaccine which contains a mutated strain of Salmonella (Ty21a) and reproduces the natural infection. The Ty21a strain is a mutant of Ty2 strains lacking Uridine-diphosphate-galactose (UDP-Gal)-4-epimerase. It was obtained in the early 1970s by chemically inactivating the galE gene. Inactivation of the galE gene generates a complete lack of Uridine-diphosphate-galactose (UDP-Gal)-4-epimerase, which is responsible for the conversion of UDP glucose into UDP galactose. As galactose is incorporated into lipopolysaccharide (LPS) as UDP galactose, the lack of galE produces incomplete development of LPS; this results in LPS without the O antigen, which is the chief surface antigen; in this phase, the mutant strain is not immunogenic. However, when the Ty21a strain is alimented by galactose, bacteria are able to generate UDP galactose in an alternative way, expressing a complete and immunogenic LPS. Moreover, owing to the lack of UDP Gal 4-Epimerase, galactose cannot be metabolised and is accumulated in the cytoplasm, resulting in lysis of bacteria and thus eliminating the virulence of the vaccine strain [23-26].

The vaccine was first licensed in Europe in 1983 and in the US in 1989.

The vaccine is administered orally through the ingestion of gastro-resistant capsules (one capsule on days 1, 3 and 5) [27]. It is indicated for adults and children from 5 years of age upwards.

The availability of an oral vaccine constitutes a major step forward in the prevention of typhoid fever, as oral

administration efficaciously stimulates the mechanisms of local, cell-mediated and systemic antibody immunity. Parenteral vaccines lack this triple action.
In countries where the risk of contracting the disease is high, vaccination is recommended every 3 years.
Revaccination every year is recommended if the subject travels from a non-endemic area to an endemic area.
The vaccine can be administered simultaneously with other vaccines and with antimalarial prophylaxis [28].
Vivotif® can be given to HIV-positive subjects who have a CD4 count above 200/mm^3.

Immunogenicity

The immunogenicity of Ty21a vaccine has been evaluated in children and adults in several studies. The assessment of immunogenicity is an important proxy in evaluating efficacy/effectiveness with regard to both the specific strain and paratyphoid fever pathogens.
The vaccine has proved to elicit a good local production of IgA against the O antigen and to induce good humoral and cell-mediated immunogenicity against the O antigen in adult male subjects [29].
The immunogenicity of the Ty21a vaccine was evaluated in 634 Thai children who underwent a three-dose immunization schedule [30]. A seroconversion rate of 60% in 3-year-old and 91% in 6-year-old vaccinated children ($p < 0.005$) was found; this was higher than the seroconversion rate in unvaccinated age-matched children. Seroconversion rates displayed an increasing trend with age in vaccinated children.
Gilman et al. [31] showed that 155 adult males vaccinated with Ty21a vaccine had very good rates of seroconversion of antibodies against the O antigen, resulting in protection from disease.
A study carried out in young Chilean adults (15-19 years old) revealed that serum Ig O antibodies, as assessed by means of ELISA, increased when several doses of the vaccine were administered within a week [32]. In another study, the Ty21a vaccine elicited a strong systemic CD4$^+$ T-helper type 1 response; booster doses induced a significant increase in levels of IgG and IgA anti-LPS in healthy adults [33].
Recently, a study involving volunteers was performed in order to evaluate the immune response against *S. typhi, paratyphyi* A, B and C (cross-reactive). Evaluating specific plasmablasts expressing homing receptors for intestine ($\alpha4\beta7$) demonstrated that the response was great for *S. typhi*, intermediate for *S. paratyphi* B and low for *S. paratyphi* A [34]. In a recent study, Wahid et al. investigated the Ty21a-elicited antibodies which mediate the opsonophagocytosis and intracellular killing of *S. typhi*, *S. parathypi* A and B. The authors found that, after immunization with Ty21a vaccine, opsonophagocytosis increased against *S. typhi* and, in to a lesser degree, also against *S. paratyphi* A and B [35].

Efficacy and effectiveness

The basic evidence that the Ty21a vaccine protects naïve subjects stems from an experimental study in which adult subjects were challenged with wild *S. typhi* 5-9 weeks after vaccination. Subjects who had received the attenuated strain had a lower attack rate than control subjects (87% efficacy, $p = 0.0002$) [36].
In pre-licensure studies, 4 formulations of the vaccine were evaluated, namely: a lyophilized/bicarbonate formulation "Alexandria formulation", a gelatine capsule/bicarbonate formulation, an enteric-coated capsule formulation, and a sachet "liquid" formulation. Three doses (48 hours apart) of the first formulation were administered to 16,486 Egyptian children (aged 6-7 years) while 3 doses of placebo were administered to 14,557 control group children. Subjects were followed up for three years, during which time 22 cases of typhoid fever were bacteriologically confirmed in the placebo group and one only case was confirmed in vaccine recipients (efficacy 95.6%; CI: 77-99%, $p = 0,001$]) [37]. The gelatine capsule/bicarbonate formulation proved to confer poor protection, and its development was soon abandoned [38].
In a clinical trial performed in Chile on the enteric-coated capsule formulation, a total of 43,759 schoolchildren (aged 6-17 years) received three doses of vaccine. Volunteers were randomly assigned to two groups, one of which received the three capsules within 48 hours (22,179 subjects), and the other within 21 days (21,598). In addition, children (21,906) who received placebo served as the comparator group. Over the 3-year follow-up period, 68 cases of typhoid fever were bacteriologically confirmed in the placebo group, 23 cases were observed in subjects on the short-interval regimen (efficacy 67%; CI: 47-79%, $p < 0.0001$), and 34 cases were confirmed in the long-interval group (efficacy 49%; CI: 24-66%, $p = 0.0006$) [38].
Although the clinical trials of the enteric-coated capsule formulation allowed the Ty21a vaccine to be authorized in several countries, another formulation was tested with the aim of achieving greater efficacy. This formulation, named "sachet liquid formulation", consisted of a vaccine sachet containing lyophilized Ty21a bacteria and another sachet containing a buffer. To formulate the liquid suspension, the contents of the two sachets were mixed in 100 ml of water. In order to study the efficacy of this formulation, two large clinical trials were organized [39, 40]. The first was implemented in Chile and the second in Indonesia. In Santiago, 36,623 schoolchildren were recruited for the administration of three doses of the vaccine, while a comparable group of 10,302 children constituted the placebo control group. Over a 3-year period of follow-up, a significant difference was observed in the incidence of bacteriologically confirmed typhoid fever, which yielded a vaccine efficacy rate of 77% (CI: 60-87%, $p < 0.0001$).
The two above-mentioned formulations of the Ty21a vaccine (enteric-coated capsule and sachet liquid formulation) were compared in a large randomized double-blind trial in Indonesia, in which 20,543 subjects (aged 3-44 years) received 3 doses of either placebo or Ty21a vaccine in enteric-coated capsules or in liquid formulation. All subjects were observed for a follow-up period of 30 months; the rates of blood-culture-positive

typhoid fever recorded were: 810 per 100,000 per year among controls, 379 in the liquid-formulation group, and 468 in the coated-capsule group. Vaccine efficacy was assessed as 53% for the liquid formulation and 42% for capsules [40].

In 2007, Fraser et al. carried out a systematic review and meta-analysis of randomised controlled trials on Typhoid vaccines. Concerning the Ty21a vaccine (3-dose regimen), efficacy was 49% (95% CI: 16-70%), 60% (95% CI: 44-71%), 59% (95% CI: 32-75%), 78% (95% CI: 35-93%) and 47% (95% CI: 24-78%) after 1, 2, 3, 4, and 5 years of follow-up, respectively [41].

With regard to effectiveness, an enlarged study was conducted in Chile on 222,998 subjects, the aims being to verify the possibility of introducing school-based mass vaccination and to ascertain the best management of the vaccination. This investigation demonstrated the good effectiveness of the vaccine and revealed that the regimen of three doses within seven days was the best schedule for the routine vaccination of the target population [42].

The effectiveness studies carried out in Chile provided information on herd immunity effect of Ty21a vaccine; large-scale vaccination appeared to elicit a herd-immunity effect. Two plausible explanations were formulated: first, the excretion of *S. typhi* significantly decreased in vaccinated subjects, causing less environmental contamination; second, the smaller number of temporary carriers reduced *S. typhi* transmission, thereby extending protection to unvaccinated subjects [24].

Safety and tolerability

The manifold mutations of the Ty21a vaccine make it genetically very stable; indeed, reversion to virulence has not been observed either *in vitro* or *in vivo* [31].

In a study conducted in children (6-7 years) in Alexandria, Wahadan et al. demonstrated the very good safety and tolerability of the Ty21a vaccine [36]. In their controlled trial, the authors recruited 32,388 children (16,486 received the vaccine, 15,902 received oral placebo, and 25,625 did not receive either) and observed that, out of 92,675 doses administered, there were 49 cases of vomiting among vaccinees, versus 21 in the placebo group; 1 case of fever after the vaccine and 3 cases in the control group, and finally 14 cases of abdominal pain in the vaccinated group, versus 2 cases in the placebo group. Obviously, the conclusion was that the vaccine was stable and safe. Furthermore, in the pilot phase of this trial, the Ty21a strain was never detected in the stool for two weeks after vaccine administration [41].

Subsequently, other studies [43-45] demonstrated the good safety of the Ty21a vaccine. Indeed, the study conducted on adults in Chile recorded the following adverse reactions in 385 vaccinees: diarrhoea 1.8%, vomiting 0.5%, fever 0.5%, and rash 0.5%, while in 367 placebo group subjects diarrhoea was found in 1.1%, vomiting in 0.3%, and fever in 0.6% of the subjects. In the study carried out in Indonesia on volunteers of all ages (311 vaccinees with enteric-coated capsule vaccine versus 291 placebo subjects): 3.9% of vaccinees versus 3.1% of placebo subjects had diarrhoea, 1.0% of vaccinees versus 1.7% of placebo subjects suffered from vomiting, 1.7% of vaccinees versus 4.8% of placebo subjects had fever, and 0.3% of vaccinees versus 1.2% of placebo subjects had a rash.

Furthermore, in an extensive field study conducted with the Ty 21a vaccine on 555,000 schoolchildren in Chile [46], passive surveillance did not find vaccine-related adverse effects. Thus, it is now accepted that the Ty21a vaccine is very safe and very well tolerated, and that adverse reactions are rare and self-limiting and consist of: abdominal discomfort, nausea, vomiting, fever, headache, and rash or urticaria [47, 48].

Indeed, in the period 1990-2000, more than 38 million persons received the Ty21a vaccine, with only 743 spontaneous reports of adverse effects, i.e. an incidence of 0.002% [49]. The most common adverse reactions were mild, and mostly temporary, gastrointestinal disorders, followed by general reactions such as fever.

A post-marketing surveillance report published in 2001 mentioned only minor and rare adverse reactions related with the Ty21a vaccine [47].

Cross-protection against paratyphoid fever

Studies aimed at evaluating the cross-protection of the Ty21a vaccine against paratyphoid fever have been carried out. A study carried out in Area Norte and Occidente of Santiago, Chile, demonstrated protection against paratyphoid B fever (efficacy 49%; 95% CI: 8-73%, $p = 0.019$) [50-54].

There is also some evidence of protection against *S. paratyphi* A infections. Tagliabue et al. found that the Ty21a vaccine induced cellular immunity against *S. paratyphi* A and B, and supposed that the mechanism involved in cellular immunity would be that of antibody-dependent cellular cytotoxicity [51]. A study by Pakkanen et al. demonstrated that the Ty21a vaccine was able to induce the presence of *paratyphi* A plasmablasts in the blood of vaccinees [34]. Furthermore, in 2012, Wahid R. et al. reported that subjects who had received the Ty21a vaccine displayed a humoral immune response of the same magnitude against both *S. typhi* and *S. paratyphi* A [35]. They demonstrated cross-reactive IgA of Antibody Secreting Cells (ASC) responses to *S. paratyphi* A and *S. paratyphi* B LPS following Ty21a vaccination. A subsequent investigation by the same researchers showed that, although the opsonophagocytic antibodies elicited by vaccination were not able to kill *S. paratyphi* A inside the macrophages, phagocytosis of *S. paratyphi* A bacterial cells was increased owing to opsonisation, albeit to a lesser degree than that of B bacterial cells [53]. Subsequently, Wahid R et al. investigated the activity of the Ty 21a vaccine in cross-reactive multifunctional CD8+ T cell responses against *S. typhi*, *S. paratyphi* A and *S. paratyphi* B in humans [54]. They demonstrated that the oral vaccine elicited specific cell-mediated immune responses against *S. typhi* and *S. paratyphi* B, and post-vaccination increases in specific CD8+ T cell responses were observed against all three Salmonella-infected targets. This increase was seen predominantly in the T

Effector/Memory (TEM) cells and in the CD8+CD45RA+ TEM (TEMRA subsets) cells. In another study, these researchers confirmed that live oral typhoid vaccine induced multifunctional *S. typhi*-specific CD4+ T cell responses that cross-react with *S. paratyphi* A and *S. paratyphi* B [54].
These results suggest that the oral live attenuated vaccine elicits protection against *S. paratyphi* A and *S. paratyphi* B. This, albeit modest, degree of protection could, however, yield a milder course of the disease, and may reduce contagiousness. This view is theoretically supported by comparative whole-genome analysis, which shows a high degree of homology among *S. typhi, S. paratyphi* A and *S. paratyphi* B [55, 56].

Importance of vaccination in the light of *S. typhi* antibiotic resistance

As the antibiotic resistance of *S. typhi* continues to increase, the immunization of subjects at risk appears crucial to containing the spread of typhoid fever. Indeed, since 2001, over the complete genome sequence of Multiple Drug Resistant (MDR) *S. typhi*, the genes of resistance to antibiotics commonly used in the treatment of typhoid fever, and especially to fluoroquinolones, have been identified. Parkill et al. demonstrated that the genes of antibiotic resistance were located in pHCM1 and pHCM2 plasmids and were: the dhfr1b (trimethoprim), su/II (sulphonamide), catI (cholarmphenicol), bla (TEM-1; ampicillin) and strAB (streptomycin) genes [57]. More recently, a study conducted on African isolates suggests that there are at least 3 important MDR lineages (namely: A [haplotypes H56 and, rarely, H42], B [haplotype H55], and C [haplotype H77] [58]. These can be added to the well-known H58 haplotype. The H58 haplotype acquired plasmid-encoding resistance to ampicillin, chloramphenicol and co-trimoxazole, and later acquired resistance to ciprofloxacin because of a chromosomal point mutation [59].
Infection caused by the MDR strains has been documented to be associated with more severe illness and higher rates of complications and death, and with a higher rate of prolonged asymptomatic carrier status [60].

Conclusions

Enteric fever is a major public health challenge. As the spread of MDR strains of *S. typhi* is increasing, global strategies for combating *S. typhi* infections need to be improved. In this perspective, the most effective way to fight typhoid fever and its severe complications is to improve sanitation, ensure safe supplies of food and water, identify and treat chronic carriers, and implement vaccination. The typhoid vaccine appears to be a powerful means of prevention, and the WHO recommends that countries should consider the programmatic use of typhoid vaccines in order to control endemic disease [60]. A study carried out by Watson et al. has shown that an efficient vaccination programme against typhoid fever can be cost-saving to health services in countries where the disease is endemic; moreover, targeting vaccination to the most seriously affected age-groups would improve cost-effectiveness [61].
The WHO recommends the vaccination of school-age and/or preschool-age children in areas where typhoid fever in these age-groups is known to be a significant public health problem, particularly where antibiotic-resistant *S. typhi* is prevalent, and during outbreaks [60]. In the most developed countries, vaccination is suggested for high-risk population groups (such as persons with intimate exposure to chronic carriers, microbiologists and other laboratory workers), and particularly for international travellers to destinations where the risk of typhoid fever is high and/or in locations where antibiotic-resistant strains of *S. typhi* are prevalent. It must also be borne in mind that international travel is increasing. Indeed, in 2010, the number of international tourist arrivals worldwide reached 949 million, and a total of 1,184 million was registered in 2015, according to the latest UNWTO World Tourism Barometer [62]. On the basis of current trends, international travel is expected to grow by 3-4% in 2017.
In conclusion, Vivotif® appears to be a powerful means of preventing enteric fever. Indeed, over the years it has proved efficacious, eliciting a triple immunologic response. Furthermore, clinical studies have also demonstrated its partial cross-protection against *S. paratyphi* and large-scale vaccination has appeared to elicit a herd-immunity effect. The vaccine is very safe and well tolerated, and its oral administration ensures very good compliance.

Acknowledgments

The University of Genoa received a grant by PaxVax Italy to conduct this overview.
The authors declare no conflict of interest.
The authors thank Dr. Bernard Patrick for revising the manuscript.

Authors' contribution

RG and DP conceived and designed the overview. DA, LA and FZ performed a search of the literature on epide-miology of typhoid and paratyphoid disease. RG carried out a search of literature on the immunogenicity, effi-cacy and safety of the oral live attenuated vaccine. All authors contributed to the draft of the article. RG, DA and DP revised critically the manuscript. RG supervised the manuscript. All authors read and approved the final version of the manuscript.

References

[1] CDC. Newton Ae, Routh JA, Mahon BE. *Infectious diseases related travel*. Chapter 3. *Typhoid & Paratyphoid fever.* Available at https://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/typhoid-paratyphoid-fever [Accessed on 20/01/2017].

[2] Popoff MY, Bockemühl J, Gheesling LL. *Supplement 2002 (no. 46) to the Kauffmann-White scheme*. Res Microbiol 2004;155(7):568-70.

[3] World Health Organization (WHO). *Diarrhoeal disease.* Available at: http://www.who.int/mediacentre/factsheets/fs330/en/ [Accessed on 20/01/2017].

[4] GBD 2013 Mortality and Causes of Death Collaborators. *Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet 2015;385(9963):117-71. doi: 10.1016/S0140-6736(14)61682-2. [Accessed on 20/01/2017].

[5] World Health Organization (WHO). *Sanitation*. Available at http://www.who.int/mediacentre/factsheets/fs392/en/ [Accessed on 20/01/2017].

[6] Prüss-Ustün A, Bartram J, Clasen T, Colford JM Jr, Cumming O, Curtis V, Bonjour S, Dangour AD, De France J, Fewtrell L, Freeman MC, Gordon B, Hunter PR, Johnston RB, Mathers C, Mäusezahl D, Medlicott K, Neira M, Stocks M, Wolf J, Cairncross S. *Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries.* Trop Med Int Health 2014;19(8):894-905. doi: 10.1111/tmi.12329.

[7] World Health Organization (WHO). *WHO estimates of the global burden of foodborne diseases.* Available at: http://apps.who.int/iris/bitstream/10665/199350/1/9789241565165_eng.pdf?ua=1 [Accessed on 23/12/2016].

[8] Franco-Paredes C, Khan MI, Gonzalez-Diaz E, Santos-Preciado JI, Rodriguez-Morales AJ, Gotuzzo E. *Enteric fever: a slow response to an old plague*. PLoS Negl Trop Dis 2016;10(5):e0004597. doi: 10.1371/journal.pntd.0004597.

[9] World Health Organization (WHO). *Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines*. Available at: http://www.who.int/biologicals/areas/vaccines/TYPHOID_BS2215_doc_v1.14_WEB_VERSION.pdf?ua=1&ua=1 [Accessed on 23/12/2016].

[10] Steele AD, Hay Burgess DC, Diaz Z, Carey ME, Zaidi AK. *Challenges and opportunities for typhoid fever control: a call for coordinated action.* Clin Infect Dis 2016;62(Suppl 1):S4-8. doi: 10.1093/cid/civ976.

[11] Buckle GC, Walker CL, Black RE. *Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010*. J Glob Health 2012;2(1):010401. doi: 10.7189/jogh.02.010401.

[12] Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleesschauwer B, Döpfer D, Fazil A, Fischer-Walker CL, Hald T, Hall AJ, Keddy KH, Lake RJ, Lanata CF, Torgerson PR, Havelaar AH, Angulo FJ. *World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis.* PLoS Med 2015;12(12):e1001921. doi: 10.1371/journal.pmed.1001921. eCollection 2015.

[13] Crump JA, Mintz ED. *Global trends in typhoid and paratyphoid fever*. Clin Infect Dis 2010;50(2):241-6. doi: 10.1086/649541.

[14] Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. *Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections.* Clin Microbiol Rev 2015;28(4):901-37. doi: 10.1128/CMR.00002-15.

[15] Wain J, Hendriksen RS, Mikoleit ML, Keddy KH, Ochiai RL. *Typhoid fever*. Lancet 2015;385(9973):1136-45. doi: 10.1016/S0140-6736(13)62708-7.

[16] Bhan MK, Bahl R, Bhatnagar S. *Typhoid and paratyphoid fever*. Lancet 2005;366(9487):749-62.

[17] Maskey AP, Day JN, Tuan PQ, 2 Thwaites GE, Campbell JI, Zimmerman M, Farrar J, Basnyat B. *Salmonella enterica serovar paratyphi A and S. enterica serovar typhi cause indistinguishable clinical syndromes in Kathmandu, Nepal*. Clinical Infectious Diseases 2006;42:1247-53.

[18] Anwar E, Goldberg E, Fraser A, Acosta CJ, Paul M, Leibovici L. *Vaccines for preventing typhoid fever.* Cochrane Database Syst Rev 2014;(1):CD001261. doi: 10.1002/14651858.CD001261.pub3.

[19] CDC. *Health information for international travel.* The yellow book 2016. Oxford University Press. Available at: https://wwwnc.cdc.gov/travel/page/yellowbook-home-2014/ [Accessed on 20/01/2017].

[20] World Health Organization. *The diagnosis, treatment and prevention of typhoid fever*. WHO/V&B/03.17. Geneva, Switzerland: WHO, 2003.

[21] World Health Organization. *Typhoid*. Available at: http://www.who.int/immunization/diseases/typhoid/en/ [Accessed on 20/1/2017].

[22] Szu SC. *Development of Vi conjugate – a new generation of typhoid vaccine*. Expert Rev Vaccines 2013;12(11):1273-86. doi: 10.1586/14760584.2013.845529.

[23] Lopalco PL, Prato R, Germinario C. *Typhoid fever: from parenteral to oral vaccines*. Ann Ig 2002;14(Suppl 3):27-32.

[24] Gentschev I, Spreng S, Sieber H, Ures J, Mollet F, Collioud A, Pearman J, Griot-Wenk ME, Fensterle J, Rapp UR, Goebel W, Rothen SA, Dietrich G. *Vivotif® – a 'magic shield' for protection against typhoid fever and delivery of heterologous antigens.* Chemotherapy 2007;53(3):177-80.

[25] Ivanoff B, Levine MM, Lambert PH. *Vaccination against typhoid fever: present status*. Bull World Health Organ 1994;72:957-71.

[26] Germanier R, Furer E. *Immunity in experimental salmonellosis. II. Basis for the avirulence and protective capacity of galE mutants of Salmonella Typhimurium*. Infect Immun 1971;4:663-73.

[27] Vivotif. Available at: https://www.medicines.org.uk/emc/history/30294 [Accessed on 10/01/2017].

[28] Faucher JF, Binder R, Missinou MA, Matsiegui PB, Gruss H, Neubauer R, Lell B, Que JU, Miller GB, Kremsner PG. *Efficacy of atovaquone/proguanil for malaria prophylaxis in children and its effect on the immunogenicity of live oral typhoid and cholera vaccines*. Clin Infect Dis 2002;35:1147-54.

[29] Nisini R, Biselli R, Matricardi PM, Fattorossi A, D'Amelio R. *Clinical and immunological response to typhoid vaccination with parenteral or oral vaccines in two groups of 30 recruits*. Vaccine 1993;11(5):582-6.

[30] Cryz SJ Jr, Vanprapar N, Thisyakorn U, Olanratmanee T, Losonsky G, Levine MM, Chearskul S. *Safety and immunogenicity of Salmonella typhi Ty21a vaccine in young Thai children.* Infection Immunity 1993;61(3):1149-51.

[31] Gilman RH, Hornick RB, Woodard WE, DuPont HL, Snyder MJ, Levine MM, Libonati JP. *Evaluation of a UDP-glucose-4-epimeraseless mutant of Salmonella typhi as a liver oral vaccine*. J Infect Dis 1977;136(6):717-23.

[32] Levine MM, Ferreccio C, Black RE, Tacket CO, Germanier R. *Progress in vaccines against typhoid fever.* Rev Infect Dis 1989;11(Suppl 3):S552-67.

[33] Viret JF, Favre D, Wegmüller B, Herzog C, Que JU, Cryz SJ Jr, Lang AB. *Mucosal and systemic immune responses in humans after primary and booster immunizations with orally administered invasive and noninvasive live attenuated bacteria.* Infect Immun 1999;67(7):3680-5.

[34] Pakkanen SH, Kantele JM, Kantele A. *Cross-reactive gut-directed immune response against Salmonella enterica serovar Paratyphi A and B in typhoid fever and after oral Ty21a typhoid vaccination.* Vaccine 2012;30(42):6047-53.

[35] Wahid R, Simon R, Zafar SJ, Levine MM, Sztein MB. *Live oral typhoid vaccine Ty21a induces cross-reactive humoral immune responses against Salmonella enterica serovar paratyphi A and S. paratyphi B in humans.* Clin Vaccine Immunol 2012;19:825-34.

[36] Wahdan MH, Serie C, Germanier R, Lackany A, Cerisier Y, Guerin N, Sallam S, Geoffroy P, el Tantawi AS, Guesry P. *A controlled field trial of live oral typhoid vaccine Ty21a.* Bulletin of the World Health Organization 1980;58:469-74.

[37] Wahdan MH, Sérié C, Cerisier Y, Sallam S, Germanier R. *A controlled field trial of live Salmonella typhi strain Ty21a oral vaccine against typhoid: three year results*. J Infectious Dis 1982;145:292-6.

[38] Levine MM, Ferreccio C, Black RE, Germanier R. *Large-scale field trial of Ty21a live oral typhoid vaccine in enteric-coated capsule formulation*. Lancet 1987;1(8541):1049-52.

[39] Levine MM, Ferreccio C, Cryz S, Ortiz E. *Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in randomised controlled field trial.* Lancet 1990;336(8720):891-4.

[40] Simanjuntak CH, Paleologo FP, Punjabi NH, Darmowigoto R, Soeprawoto, Totosudirjo H, Haryanto P, Suprijanto E, Witham ND, Hoffman SL. *Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine.* Lancet 1991;338(8774):1055-9.

[41] Fraser A, Paul M, Goldberg E, Acosta CJ, Leibovici L. *Typhoid fever vaccines: systematic review and meta-analysis of randomised controlled trials.* Vaccine 2007;25(45):7848-57.

[42] Ferreccio C, Levine MM, Rodriguez H, Contreras R. *Comparative efficacy of two, three, or four doses of TY21a live oral typhoid vaccine in enteric-coated capsules: a field trial in an endemic area.* J Infect Dis 1989;159(4):766-9.

[43] Levine MM, Black RE, Ferreccio C, et al. *The efficacy of attenuated Salmonella typhi oral vaccine strain Ty 21a evaluated in controlled field trials*. In: Holmgren J, Lindberg A, Molly K. (Eds.) *Development of vaccines and drugs against diarrhea.* Lund, Sweden: Studentlitteratur 1986, pp. 90-101.

[44] Black RE, Levine MM, Young C, ooney J, Levine S, Clements ML, O'Donnell S, Hugues T, Germanier R. *Immunogenicity of Ty21a attenuated Salmonella typhi given with bicarbonate or in enteric-coated capsules*. Dev Biol Stand 1983;53:9-14.

[45] Simanjuntak CH, Paleologo FP, Punjabi NH, Darmowigoto R, Soeprawoto, Totosudirjo H, Haryanto P, Suprijanto E, Witham ND, Hoffman SL. *Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine.* Lancet 1991;338(8774):1055-9.

[46] Black RE, Levine MM, Ferreccio C, Clements ML, Lanata C, Rooney J, Germanier R. *Efficacy of one or two doses of Ty21a Salmonella typhi vaccine in enteric-coated capsules in a controlled field trial. Chilean Typhoid Committee*. Vaccine 1990;8(1):81-4.

[47] Griot-Wenk ME, Hartmann K, Herzog C, Ackermann J, Maspes B. *Excellent long-term safety data established in a recent postmarketing surveillance for the oral typhoid fever vaccine, VIVOTIF®.* Ital J Trop Med 2001;6:104-5.

[48] Begier EM, Burwen DR, Haber P, Ball R; Vaccine Adverse Event Reporting System Working Group. *Postmarketing safety surveillance for typhoid fever vaccines from the Vaccine Adverse Event Reporting System, July 1990 through June 2002*. Clin Infect Dis 2004;38(6):771-9.

[49] Guzman CA, Borsutzky S, Griot-Wenk M, Metcalfe IC, Pearman J, Collioud A, Favre D, Dietrich G. *Vaccines against typhoid fever.* Vaccine 2006;24(18):3804-11.

[50] Levine MM, Ferreccio C, Black RE, Lagos R, San Martin O, Blackwelder WC. *Ty21a live oral typhoid vaccine and prevention of paratyphoid fever caused by Salmonella enterica serovar paratyphi B.* Clin Infect Dis 2007;45(Suppl 1):S24-8.

[51] Tagliabue A, Villa L, De Magistris MT, Romano M, Silvestri S, Boraschi D, Nencioni L. *IgA-driven T cell-mediated anti-bacterial immunity in man after live oral Ty 21a vaccine*. J Immunol 1986;137(5):1504-10.

[52] Wahid R, Fresnay S, Levine MM, Sztein MB. *Immunization with Ty21a live oral typhoid vaccine elicits crossreactive multifunctional CD8+ T-cell responses against Salmonella enterica serovar typhi, S. paratyphi A, and S. paratyphi B in humans*. Nature 2015;8:1349-59.

[53] Wahid R, Zafar SJ, McArthur MA, Pasetti MF, Levine MM, Sztein MB. *Live oral Salmonella enterica serovar Typhi vaccines Ty21a and CVD 909 induce opsonophagocytic functional antibodies in humans that cross-react with S. Paratyphi A and S. Paratyphi B.* Clin Vaccine Immunol 2014;21(3):427-3.

[54] Wahid R, Fresnay S, Levine MM, Sztein MB. *Cross-reactive multifunctional CD4+ T cell responses against Salmonella enterica serovars typhi, paratyphi a and paratyphi b in humans following immunization with live oral typhoid vaccine Ty21a.* Clin Immunol 2016;173:87-95. doi: 10.1016/j.clim.2016.09.006.

[55] Holt KE, Thomson NR, Wain J, Langridge GC, Hasan R, Bhutta ZA, Quail MA, Norbertczak H, Walker D, Simmonds M, White B, Bason N, Mungall K, Dougan G, Parkhill J. 2009. *Pseudogene accumulation in the evolutionary histories of Salmonella enterica serovars paratyphi A and typhi.* BMC Genomics 2009;10:36. doi: 10.1186/1471-2164-10-36.

[56] McClelland M, Sanderson KE, Clifton SW, Latreille P, Porwollik S, Sabo A, Meyer R, Bieri T, Ozersky P, McLellan M, Harkins CR, Wang C, Nguyen C, Berghoff A, Elliott G, Kohlberg S, Strong C, Du F, Carter J, Kremizki C, Layman D, Leonard S, Sun H, Fulton L, Nash W, Miner T, Minx P, Delehaunty K, Fronick C, Magrini V, Nhan M, Warren W, Florea L, Spieth J, Wilson RK. *Comparison of genome degradation in paratyphi A and typhi, human-restricted serovars of Salmonella enterica that cause typhoid.* Nat Genet 2004;36:1268-74.

[57] Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebaihia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG. *Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18*. Nature 2001;413(6858):848-52.

[58] Baltazar M, Ngandjio A, Holt KE, Lepillet E, Pardos de la Gandara M, Collard JM, Bercion R, Nzouankeu A, Le Hello S, Dougan G, Fonkoua MC, Weill FX. *Multidrug-resistant Salmonella enterica serotype Typhi, Gulf of Guinea Region, Africa.* Emerg Infect Dis 2015;21(4):655-9.

[59] Roumagnac P, Weill FX, Dolecek C, Baker S, Brisse S, Chinh NT. *Evolutionary history of Salmonella typhi.* Science 2006;314:1301-4.

[60] WHO. *Typhoid vaccines: WHO position paper.* Wkly Epidemiol Rec 2008;83(6):49-59.

[61] Watson CH, Edmunds WJ. *A review of typhoid fever transmission dynamic models and economic evaluations of vaccination*. Vaccine 2015 Jun 19;33(Suppl 3):C42-54. doi: 10.1016/j.vaccine.2015.04.013.

[62] World Tourism Organization Tourism Market Trends UNWTO. *UNWTO World Tourism Barometer*. Available at: http://mkt.unwto.org/barometer [Accessed on 20/01/2017].

[63] Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale VV, Ramani E, Kim YE, Park JK, Wierzba TF. *Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment*. Lancet Glob Health 2014;2(10):e570-80. doi: 10.1016/S2214-109X(14)70301-8.

■ Received on December 15, 2016. Accepted on February 20, 2017.

■ Correspondence: Lucia Arata, Department of Health Sciences, University of Genoa, Italy - Tel. +39 010 3538394 - E-mail: aratalucia@gmail.com