This paper focuses on the pathogen *Salmonella enterica*, the nonsporulating, non-capsulated, anaerobic, gram negative, flagellated bacteria with variants responsible for typhoid and paratyphoid fevers. Aspects of the pathogen and the febrile disease(s) associated with variants of this species that are covered in this paper include microbial ecology, routes of transmission, epidemiology of infection, risk demographics and an assessment of the current global public health burden of typhoid and paratyphoid fevers. This paper also discusses vaccine development and efficacy, current research directions and intervention and prevention strategies to limit the spread of *S. enterica* and resultant enteric fever.

S. enterica subspecies *enterica* serovar[1] Typhi (*Salmonella* Typhi) is the bacterium responsible for the enteric disease typhoid fever, while *S. enterica* serovar Paratyphi A, B and C (*Salmonella* Paratyphi A, B and C) are the bacteria responsible for paratyphoid fever (GBD 2017). Collectively, these serovars of *S. enterica* are considered the causative agents for the more generally identified enteric fever. The overall global incidence of typhoid and paratyphoid fevers appears highest in children, with ~56% of the 2017 global burden occurring in children younger than 15 years of age (GBD, 2017). Of significance in consideration of preventive immunizations (discussed further below), approximately 13% of the global typhoid fever burden occurs in children < 5 years of age, an age demographic that can be challenging to diagnose and complicated to treat. In terms of absolute numbers, between 11 – 18 million cases of enteric fever are estimated to occur annually worldwide (Mogasale et al., 2014; GBD, 2017).

[1] A serovar or serotype is a sub-species level identification of bacteria based on structures on cell surfaces. Different cell surface structures can create different levels of immune response following exposure. The result of serovar differences can be differing levels of pathogenicity for strains of the same bacterial species.

Serovars of *S. enterica* causing enteric fevers are restricted to humans as a host reservoir although the molecular basis of this host specificity, as well as the factors and cellular pathways responsible for pathogenicity, remain incompletely understood (Spano, 2016). Within the human body, the bacteria are carried within blood and lymph (Kaur and Jain, 2012). Following exposure from contaminated water or food, the bacillus spreads from the intestines to the liver, spleen, gall bladder and bone marrow where the bacillus can persist for life (Kaur and Jain, 2012). Research does suggest, however, that previous exposure to the flagella of *Salmonella sp.* creates some level of immunity toward subsequent enteric infections, with immunity appearing as reduced inflammatory response and decreased febrile severity rather than a lower overall risk of re-contracting the disease (Kaur and Jain, 2012). Treatment for typhoid and paratyphoid fevers is via antibiotics, with the fluoroquinolone antibiotic, ciprofloxacin, as one of the most commonly and effectively used.

In terms of transmission routes from person to person, serovars of *S. enterica* are shed or excreted by human hosts predominantly through feces, thus transmission of enteric fever commonly, although not always, occurs via exposure to fecal-contaminated water and/or food (fecal-oral route) (Crump 2019). As well as shedding via feces, *S. enterica* can be shed in urine and infection with the bacterium has also been documented to occur as the result of anal sex (anal-oral route) (Reller et al. 2003). *Salmonella* Typhi and Paratyphi are not thought to reproduce efficiently outside of human hosts, although the bacteria may persist both in the human body and in the environment for periods of time. Extended or ‘chronic’ carrying of *S. enterica* by humans has been defined as ongoing shedding of the bacteria for > 1 year after onset of acute enteric fever (Crump 2019). Individuals in this ‘chronic’ or ‘carrier’ state with respect to

Salmonella Typhi, may be largely asymptomatic following the acute phase of febrile illness and may continue to shed the bacterium in feces for many years (Watson and Edmunds, 2015). It is estimated that between 3-5% of individuals infected with *Salmonella* Typhi become chronic carriers of the bacterium (Douesnard-Malo and Daigle, 2011).

With respect to the broader scale transmission of *S. enterica*, two general scenarios are commonly considered: short-cycle and long-cycle transmission (Crump, 2019). In short-cycle transmission, water and/or food become contaminated through local fecal shedding and transmission occurs in the immediate area as the result of poor sanitation and hygiene. In long-cycle transmission, pollution occurs on a wider scale through inadequate treatment of contaminated wastewater and/or use of contaminated wastewater in crop irrigation. Regarding typhoid fever, while either transmission scenario can occur in both low-incidence and high-incidence countries, chronic carriers of *Salmonella* Typhi likely play a significant role in the short cycle transmission of the bacterium and resultant local outbreaks of enteric fevers in low incidence countries (Crump, 2019). Extended persistence of the bacterium in the environment (days to weeks) may be facilitated by a free-living protozoan (*Acanthamoeba castellanii*) that either/both provides the bacillus with nutrients and/or increases the survivability of the bacillus in passage through the gastric acid barrier of the human gut (Douesnard-Malo and Daigle, 2011; Kaur and Jain, 2012). The specific mechanism of this facilitation remains uncertain, although recent studies have documented that 20+ species of pathogenic bacteria appear to associate with and/or survive within *A. castellanii* (Liu et al. 2018) suggesting some generalizability to the mechanism in question.

Regarding infection, it has been estimated that ~ 100,000 organisms are required for human host infection via *S. enterica* (Douesnard-Malo and Daigle, 2011), and both the risk for infection and the incubation period for enteric fever are sensitive to the magnitude of the ingested bacterial dose (Crump, 2019). The incubation period for enteric fever is between 5-21 days and acute enteric fever can result in significant gastrointestinal illness (e.g., diarrhea, nausea, vomiting) as well as febrile symptoms (e.g., fever chills) malaise, weakness and severe muscle aches (Kaur and Jain, 2012). If untreated, typhoid and paratyphoid fevers can result in long-standing intestinal/abdominal as well as neuropsychiatric concerns, the former potentially resulting in intestinal tissue lesions, perforations, sepsis and liver or gallbladder cancers, the latter likely as the result of the sustained high fever (reaching 104 F) that can last for weeks accompanying infection (Crump, 2019). Sepsis as defined for severe typhoid fever is mediated by lipopolysaccharide (LPS) / endotoxin molecules found in abundance on the surface of *Salmonella* Typhi cells (Kaur and Jain, 2012). *Salmonella* Typhi entry into host cells is effected by proteins secreted by the bacterium to disrupt the arrangement of host cell membranes (Kaur and Jain, 2012). This mechanism, known as 'membrane ruffling,' allows the bacillus to be internalized within host cells; once internalized, the bacterium is protected intra-cellularly by a membrane-bound vacuole (Salmonella-containing vacuole [SCV]) that contributes significantly to survivability within the human host (Kaur and Jain, 2012). In general, understanding mechanisms of pathogenic intra-cellular transport and protection (such as within vacuoles) is important in consideration and development of new treatments and, potentially, immunizations. In terms of facilitated bacterium survival in the environment via association with the protozoan *A. castellanii* (discussed above), it is not currently know whether this association is mediated specifically by 'membrane ruffling' and protection within SCVs.

Mogasale et al. (2018) conducted a pooled meta-analysis of published studies assessing the risk of contracting typhoid fever from consuming unimproved or unsafe water. Unimproved water was defined as per WHO/UNICEF and included water from unprotected springs, dug wells or surface water bodies, as well as water delivered by tanker truck (Mogasale et al., 2018). Of the 779 publications reviewed for pooled meta-analysis, 12 met inclusion criteria as case-control studies that evaluated typhoid fever risk in the context of water quality *and* with specification of whether the study has been conducted as an assessment of enteric fever risk in typhoid-endemic areas. Of the 12 studies that met inclusion criteria, 11 were conducted in Asia (with the majority in South or Southeast Asia); 9 were conducted in urban areas and 3 were conducted as a component of outbreak response. Results of the pooled meta-analysis indicated that exposure to unimproved water was higher among enteric fever cases (62.95%; n = 576/915) than controls (46.30%; n = 745/1609) and the case-weighted mean pooled odds ratio of having contracted typhoid fever while consuming unimproved water was greater than 1 (OR = 2.44 [95% CI: 1.65 – 3.59]) (Mogasale et al., 2018).

Additional risk factors for typhoid fever identified from the pooled studies included socioeconomic status, food consumption, water handling and personal hygiene practices, living conditions and awareness of the causes of typhoid fever (Mogasale et al., 2018). Other published surveys assessing community understanding of the causes of typhoid fever have similarly reported a high level of understanding (up to 77% of respondents) of the role that contamination of water and/or food as well as poor hand hygiene play in the contracting of the disease (Imanishi et al., 2014). As detailed by Imanishi et al. (2014), however, although studies may

present overall high numbers with respect to local understanding of transmission routes, the translation of this understanding into preventative action varies with rural versus urban status as well as with the *perceived* cleanliness of a family's water supply (e.g., surface water perceived as unclean versus water from boreholes or wells perceived as clean). These sociological factors appear to play significant roles in the extent to which families actively practice household water treatment, such as with chlorination via either tablets or drops.

Lee et al. (2013) assessed factors most likely to have been responsible for the documented decrease in typhoid fever incidence in Korea during the 2nd half of the 20th century, focusing specifically on the availability of clean tap water and access to pharmacy medications. Regarding clean tap water, the researchers determined that the decline in typhoid fever incidence within communities in Korea decreased proportional to the increase in available tap water and required (at least in the population-level data that they assessed) approximately 35% of the population have access to a sufficient daily quantity of clean water. Lee et al. (2013) defined a sufficient daily quantity of clean water as ~173 L (45 gallons), a volume that likely says more about the data sources evaluated in their study than the absolute magnitude of clean water needs for typhoid fever prevention. The World Health Organization (WHO), as example, recommends an individual daily minimum of 20 L for personal and food-related hygiene,[2] a volume that inarguably brackets the low end of the adequate water quantity range for individual and community health, and is likely significantly more reflective of water availability limitations in regions of the world lacking adequate water provision infrastructure.

[2] https://www.who.int/water_sanitation_health/emergencies/qa/emergencies_qa5/en/ [Accessed: 11/1/20]

With regard to individual and community health in high-incidence areas, a significant concern regarding the impact of endemic enteric fevers is the role that the magnitude or force of infection can play in shifting the exposure age demographic downward toward infants and toddlers (Crump 2019). That is, in low incidence countries, infants, and to some extent, toddlers, are protected from short cycle exposure to *S. enterica* serovars through both maternal antibody protection and the role that breastfeeding can play in limiting ingestion/exposure to fecal-contaminated water and food. As the typhoid fever incidence rate increases in an area, however, the age range of exposures expands and the potential severity of the illness in the youngest cohorts increases (Watson and Edmunds, 2015). Based on these observations of increasing enteric fever rates among infants and toddlers in high incidence areas, WHO is currently recommending the administration of typhoid conjugate vaccines (TCV) for infants > 6 months in locations in which there is a high burden of enteric disease (Crump 2019). Global regions in which there is a significant risk of endemic enteric fever include Central Africa (68% of the population at high risk of typhoid fever); East Africa (58%); the Caribbean (40%); South America (27%); Southeast Asia (25%); and North Africa (25%) (Mogasale et al., 2014). In terms of overall numbers of individuals, the population at high risk of typhoid fever is approximately 1.6 billion people, or 29% of the overall population (5.6 billion people) in typhoid endemic areas (Mogasale et al., 2014). Demographically, as presented in Mogasale et al. (2014), high risk populations in these global regions are generally more rural in Africa, more peri-urban in Latin America and the Caribbean and approximately evenly distributed between rural areas and peri-urban areas in Asia. In areas in Southeast Asia and Africa where typhoid fever is considered highly endemic, case rates can exceed 100 infected individuals per 100,000 people (Lo et al. 2018), or 0.1% of the population.

While *S. enterica* can be treated effectively with antibiotics, strains of drug resistant *S. enterica* are increasingly being documented (Lo et al. 2018). As example, the Centers for Disease Control and Prevention (CDC) note that over the years 2015-2017, greater than 70% of *S. enterica* Typhi strains were no longer treatable with one of the front line fluoroquinolone drugs, ciprofloxacin.[3] The implications of increasing drug resistance in *S. enterica* treatment is of significant concern, based both on current annual estimates of the global enteric fever burden (between 11 – 18 million cases worldwide [Mogasale et al., 2014]), and on the difference in documented case fatality rates between those receiving antibiotics for enteric fever (case fatality rate < 1%) versus those whose enteric fever goes (or historically went) untreated (> 10%) (Lee et al., 2013; Crump, 2019). As noted by Lo et al. (2018), antibiotic resistance for treatment of typhoid fever is a global concern in that there is currently no worldwide public health strategy focused on enteric fever control via immunization, a concern that is particularly acute for resource-poor areas.

Watson and Edmunds (2015) evaluated typhoid transmission models and economic valuations to determine the current state-of-understanding of the role that anti-typhoid vaccines can play in effective enteric fever disease control. This evaluation was conducted to examine the balance of impacts (synergistic or otherwise) of programs focused on: (1) vaccinations; (2) water, sanitation and hygiene (WASH); and (3) health surveillance to identify chronic typhoid carriers. In the context of vaccine efficacy, identification and treatment of chronic typhoid carriage is important because a high background carrier rate of typhoid fever in an area will

[3] https://www.cdc.gov/drugresistance/pdf/threats-report/salmonella-typhi-508.pdf. Accessed 10/31/20

reduce the indirect protective effects of vaccination for those are not carrying *S. enterica* and remaining unvaccinated (Watson and Edmunds, 2015). That is, in a community with a high incidence of asymptomatic chronic carrier infection (and bacterial shedding), those who are both unvaccinated and not carrying *S. enterica* are still potentially significantly subject to infection via chronic carriers even if surrounded by a newly immunized population. Overall, the impact of this scenario – in which there is significant local carriage of typhoid fever in an area targeted for a vaccination campaign – would be a significant extension of the timeframe for elimination of the disease. Following review of the available literature, Watson and Edmunds (2015) concluded that there is a paucity of recent research on typhoid fever transmission modeling and a likewise limited number of economic valuation studies focused on possible synergies between prevention strategies for *S. enterica* infection and spread of typhoid fever.

With respect to the question of immunization as a prevention strategy, while vaccines do exist and have been used for decades in the context of enteric fever outbreaks or preventative travel medicine, vaccination has not been widely applied for endemic typhoid fever control. Currently, there are two typhoid fever vaccines available, a multidose live (attenuated) oral vaccine (Ty21a) and single dose injectable (Vi polysaccharide purified antigen) vaccine (Lo et al. 2018). Both vaccines are characterized by limited efficacy, only provide short-term protection and are recommended against use in children (< 2 years old for Vi polysaccharide and < 5 years old for Ty21a), thereby precluding their inclusion in infant and toddler immunization campaigns (Lo et al. 2018). Because infants who contract typhoid fever may present with atypical symptoms and it may be challenging to collect sufficient blood from an infant for the routine typhoid fever culture and determination, the development of a typhoid fever vaccination that is safe and

effective for children < 2 years old has been recognized as a significant public health need (Thiem et al. 2011). As detailed by Thiem et al. (2011), the new typhoid fever conjugate vaccine (Vi-eEPA) has been developed to improve upon the above listed shortcomings with only minimal side effects (e.g., low-grade fever) and appears to currently only lack the development of an effective delivery strategy focused on proactive (i.e., infant immunization) versus reactive (i.e., in response to an outbreak of enteric fever) implementation.

This data gap in determination of an effective delivery strategy for a typhoid fever vaccine should be viewed in the context of a significant number of other practical deficits in understanding regarding (as examples): (1) the ability to estimate prevalence – including subclinical infections - in areas in which enteric fevers are endemic; (2) the duration and extent of immunity following illness; (3) contact patterns as a function of age to improve understanding of transmission dynamics in short-cycle scenarios; (4) how best to combine vaccination and WASH-related programs to optimize transmission control; (5) the ability to predict efficacy and duration of vaccine-related disease prevention; and (6) the extent to which vaccinations effectively prevent (or, at least, minimize) shedding by chronic carriers (Watson and Edmunds, 2015; Crump, 2019). Taken together, these research needs – as well as other needs identified throughout this paper – highlight the significant science, public health and policy gaps that remain between current global public health practices and public health needs to combat enteric fevers. As detailed by Crump (2019), despite over a century of research on human host susceptibility and impacts of *Salmonella enterica* exposure, the physiological and demographic variability of the enteric fevers that result continue to create challenges in both quantifying and mitigating the overall global health burden of these diseases.

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