Typhoid fever : aspects of environment, host and pathogen interaction

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Summary & General Discussion

This thesis describes the contribution of environmental and host genetic factors, respectively, and characteristics of the pathogen as interactive determinants of (acquisition of) typhoid and paratyphoid fever. In the words of the epidemiologist J.R. Paul (Clinical Epidemiology, 1966) who, paraphrasing Matthias 13:3-8, designated the cause of infectious diseases to the triad 'the seed, the soil and the climate', herein *Salmonella typhi* and *Salmonella paratyphi* being "the seed", and the host and its environment "the soil" and "the climate", respectively, with the three together determining the ability of microbial pathogens to flourish in the population. *S. typhi* and *S. paratyphi* cause a severe systemic disease that may take weeks to recover and as such are special pathogens within the *Salmonellae* that generally cause a self-limiting gastro-enteritis, or bacteremia and/or focal infection striking immuno-compromized individuals in particular. Moreover, *S. typhi* is unique in being strictly confined to one host, man, whereas *S. paratyphi* may infect livestock as well. Also, a small percentage of typhoid patients become chronic carriers, i.e., they excrete the bacterium in the feces for years apparently without any adverse effect. This chronic carriership and temporary excretion by patients constitute the only reservoir for the man-adapted *S. typhi*. Although much is known about potential fecal-oral transmission chains of *S. typhi* and *S. paratyphi*, the weak link that in a particular situation becomes the critical element of disease transmission is usually not known. Detailed knowledge of such determinants of disease, however, is of pivotal importance in designing effective public health interventions. Therefore, the above-mentioned perspective on typhoid and paratyphoid fever was kept in mind in the present thesis that investigated these diseases in Jatinegara, a crowded area in the megacity Jakarta and provided empirical evidence on aspects of possible control of typhoid and paratyphoid fever.

Environmental factors and typhoid and paratyphoid fever in Jakarta

Most of the fastest-growing cities are in developing countries, and Jakarta with a population of over 12 million is placed within the top ten of the world's largest urban areas. In the next decade, its population is predicted to grow by one third and will surpass that in megacities like Calcutta and Buenos Aires. The study area, Jatinegara district, is located in East-Jakarta and has a population of about 0.3 million living in an area of only 10.6 km^2. This urban district was chosen because parts of it, for instance the Kampung Melayu, are typical of slum settlements with poor infrastructure that line rivers in the city. In these areas, health problems are related to poverty, lack of human waste disposal and safe water, and poor infrastructure. Poor waterways and the fact that part of Jatinegara lies below sea level cause annual flooding of the overpopulated riverbanks. Other parts of Jatinegara are much better structured, are provided with waterworks and common sewage disposal systems and are inhabited by a middle class population. Thus, the environment and population of both the low and middle socio-economic class in Jatinegara well represent the full spectrum of

Jakarta's problems with safe water, disposal of sewage, poor infrastructure, flooding and crowding.

In many parts of Jatinegara, poor sanitation and filth surrounding the population remain a serious health issue. Unfortunately, few studies have addressed health problems to reveal what is going on. Of note, in the study of food- and water-borne typhoid and paratyphoid fever described in **Chapter Two** we found that most doctors over-diagnose these diseases by up to tenfold because they usually do not take blood cultures that could confirm or refute the diagnosis. Thus, insight will benefit from population surveillance, and individual clinical diagnoses will be improved as well.

In the area of Jatinegara, we found that the major risk factors for transmission of *S. typhi*, the cause of typhoid fever, are related to the household, i.e., a recent case of typhoid fever in the household and sharing food from the same plate, especially in families that do not use soap for hand washing and lack a proper toilet.

Due to the continuing hangover from the Asian economic crisis of 1997, more than before the urban population became dependent on inexpensive food obtained from street vendors. As described in **Chapter Two** and **Three** this phenomenon is contributing to the relative increase in transmission of *S. paratyphi*, the causative agent of paratyphoid fever, as compared with *S. typhi*. Another risk factor that was associated with acquiring paratyphoid fever was flooding of the household.

The distinct routes of transmission of *S. typhi* and *S. paratyphi* suggest that attempting one single public health measure to control the enteric fevers (i.e., typhoid fever and paratyphoid fever combined) is highly questionable. Our findings illustrate the need to improve overall disease surveillance and individual clinical diagnoses before attempting to initiate expensive campaigns to address public health problems. Solving Jakarta's environmental health problems will require commitment to pay for public health research to survey, monitor and characterize the problems, and spending on the infrastructure (i.e., safe drinking water, sanitation and sewage disposal) needed to solve them. Besides costly, complex infrastructural interventions that need the input of various institutions combined, also simple interventions in hygiene habits of inhabitants, i.e., promoting adequate hand washing and boiling of drinking water, should be emphasized and could have an immediate impact on the transmission of many infectious diseases including typhoid. For instance, as described in the medical literature and observed in our study, patients with typhoid and paratyphoid fever excrete the bacteria in their stools for several weeks to months after the acute episode. Obviously, the combination of relatively long-term bacterial excretion, inadequate hand washing before eating and cooking, and sharing of food from a single plate with household members, greatly facilitates intra-household transmission, but likely can be stopped by raising the level of personal and cooking hygiene. Of note, finally, the relative upsurge of cases of paratyphoid questions a population-wide immunization campaign with a vaccine that provides temporary protection only against *S. typhi*.

Consistent with their presumed role in the spreading of pathogens within the population, the street food vendors' study described in **Chapter Three** showed that at any point in time, one in every twenty-five vendors excreted *Salmonella* bacteria. We observed that street vendors had a very poor hand washing discipline compared with food vendors in restaurants and on the streets direct contact of fingers with food occurred frequently in food stalls and push cars. Analysis revealed that many food vendors were infested with bowel parasites, underscoring their overall lack of (food) hygiene. Moreover, we found that samples of dishwater, drinking water and ice cubes frequently contained coliforms, a well-established surrogate marker of fecal contamination. Thus, multiple factors related to personal and food hygiene may contribute to the transmission of food- and waterborne diseases including the indicator diseases we focused on, paratyphoid fever and typhoid fever.

Many of the above-mentioned risk factors for disease transmission could be stopped by relatively simple and low cost interventions focused on street food vendors, e.g., instruction on proper hand washing and basic food handling hygiene, frequent renewal of dishwater and the use of soap in dishwater. The major investment here concerns means to let them execute these measures. Attention should be given to the fact that all commercially available water, often obtained from leaks or illegal taps of the city water plants, is safe for drinking only after being boiled, and ice cubes should be made after cooling the boiled water, to reduce the risk of transmission of waterborne illness. Overall, in Jakarta there is a need for an extensive food and water quality control.

Host genetics factors and typhoid and paratyphoid fever in Jakarta

After entering the human host, through contaminated drinks or food, *S. typhi* and *S. paratyphi* must evade other microorganisms competing for food and mucosal adhesion sites, gain entry into the body, evade the host innate defense mechanisms, find their unique niche within the mononuclear phagocyte system and be able to persist and replicate, and, finally, exit the host and be transmitted to a new, susceptible host. Many of these functions rely on host proteins that can exist in multiple, polymorph forms in the population. Thus, based on their genetic background individuals may vary in many of these factors, much alike the variation in blood group types. In studying host genetic factors in infectious diseases, it is important to make the distinction between factors that may control acquisition of disease, e.g., related to susceptibility to typhoid and paratyphoid fever, and factors that, once the bacterium gained entry into the body and found its niche, determine whether or not an individual becomes severely ill, needs admission to hospital, or suffers only a light illness. In studying the first question, all cases within a certain population must be collected (hospitalized or not) and, for instance, variations in relevant candidate genes analyzed in comparison with a randomly collected community control group that did not suffer the

disease. For the second question, cases, either hospitalized or treated at outpatient clinics, may be analyzed in relation to disease severity scores.
The role of pro-inflammatory proteins like TNF-α, IFN-γ, IL-1α, IL-1β, IL-12, IL-18, TNFR1, IFN-γR1, IL-1R, CASP1 and CRP in induction of expression of a variety of genes and in the synthesis of several proteins that induce acute and chronic inflammatory changes is well established. Whereas several polymorphisms in these pro-inflammatory genes have been reported to be associated with various infectious diseases, we did not find (**Chapter Four**) an association of these polymorphisms with susceptibility to typhoid or paratyphoid. For instance, a previous report from Vietnam associated *TNFA-308*A* allele with susceptibility to typhoid fever. In Indonesia, the prevalence of the –238 and –308 polymorphisms in the promoter region of the gene encoding TNF-α in patients with typhoid and paratyphoid fever does not differ from those in randomly selected community controls. The use of typhoid fever patients admitted to a hospital in Vietnam as compared with consecutive typhoid and paratyphoid fever patients enrolled in the community-based surveillance study in Jakarta suggested that the *TNFA-308*A* allele in particular, may play a role not so much in the increase of susceptibility to acquisition of typhoid or paratyphoid fever but more likely in the determination of its course and severity of the disease requiring hospitalization of patients. Apparently, this gene variation plays a role in determining how severely ill one becomes after being infected, rather than determine whether or not one becomes ill after being exposed. Some of the other single nucleotide polymorphisms (SNPs) in the pro-inflammatory genes we studied had a very low frequency of the minor allele. For instance, two SNPs in *IL1R* were absent from the study population, cases and controls alike. Consequently, to gain statistical power to detect a possible association of those SNPs with typhoid fever, a much bigger population sample (both cases and controls) would be needed.

Apart from pro-inflammatory genes, other polymorphisms in host genes might be of interest to look for the association with susceptibility to or severity of typhoid fever. One gene complex of interest concerns *PARK2/PACRG*, described in **Chapter Five**. *PARK2/PACRG* polymorphisms had been associated with leprosy, another intracellular pathogen that shares many aspects of the host immune reaction with *Salmonella*. The gene product of both *PARK2/PACRG* plays a role in poly-ubiquitination and protein degradation by the proteasome. This pathway may deal with bacterial proteins that are toxic or disrupt normal cell function by breaking them down into harmless molecules. Of the four SNPs in the *PARK2/PACRG* region, *PARK2_e01(-2599)* was found to be associated with typhoid fever, whereas *PARK2_e01(-697)*, *rs1333955* and *rs1040079* were not. Similarly, *PARK2_e01(-2599)* was demonstrated to be the polymorphism with the strongest association with leprosy. It is not yet known whether the proteasome-ubiquitin pathway plays a role in *S. typhi* and *S. paratyphi* infection. However, some interesting hypotheses are raised by two *in vitro* models that linked this pathway

to intracellular evasion mechanisms of *Salmonella*. The first model found that *Salmonella* invasion of host epithelial cells requires the reversible activation of Cdc42 and Rac1 by the bacterially encoded SopE and SptP. Stabilization of SopE by proteasome inhibition prevents cellular recovery after bacterial infection and therefore allows the continuation of a permissive environment for the bacteria to replicate or evade host defences. A second study found that non-virulent *Salmonella* strains interact with human epithelial cells to reduce the synthesis of inflammatory effector molecules elicited by diverse pro-inflammatory stimuli. This reduction resulted from both IκB-α phosphorylation and a reduced poly-ubiquitination of IκB-α. These mechanisms are important in the interaction between *Salmonella* and its host cells and consequently, any slight modification of these mechanisms may well help explain the association of *PARK2/PACRG* polymorphisms with typhoid and paratyphoid fever. Future studies of larger groups of patients will have to elucidate the exact pathophysiological consequences of these *PARK2/PACRG* polymorphisms in *Salmonella typhi* and *Salmonella paratyphi* infection.

An interesting molecule in typhoid and paratyphoid infection is the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). CFTR is a chloride channel that is the affected protein (i.e., usually absent) in patients with cystic fibrosis. Of interest here is that *in vitro* experiments suggested that *S. typhi* makes use of the CFTR protein as a docking station in the gut, i.e., to gain attachment to gastrointestinal mucosal cells. Obviously, attachment preludes the invasion of mucosal cells, and the ability to attach or not may be crucial to initiate typhoid fever. This leads to an interesting and testable hypothesis, namely that typhoid fever may have been a driving force to keep the CFTR mutation in the population, by providing its heterozygous carrier (3% of the population) enhanced resistance to the potentially lethal childhood disease: carriers express only about half of the normal number of CFTR on their cell membrane (described in **Chapter Six**). On analysis of the most common CFTR mutation, ΔF508 (mutation encountered in 30-75% of the cystic fibrosis in patients in Western Europe), we observed that none of the cases, fever controls or random community controls carried this mutation. Further inquiry suggested that cystic fibrosis is a rare disease among Indonesians. Therefore, additional polymorphisms within the CFTR gene had to be identified and studied in relation to typhoid and paratyphoid fever. A polymorphism in the number of CA repeats of microsatellites IVS17bCA and IVS8CA in the intronic region did occur in the study population with sufficient frequency to enable a meaningful analysis. The IVS17bCA with 13 CA repeat was the predominant allele with a 94% prevalence, hence this marker was not suitable ('polymorphic enough') for the differentiation between groups. Of the second microsatellite studied, IVS8CA, two major alleles 181 (CA_{16}) and 183 (CA_{17}) were found to have a protective effect against acquiring typhoid fever, compared with the other alleles in this marker. This finding suggest that even

though the mutation ΔF508 could not be found in the study population and the hypothesis on the connection of typhoid fever and cystic fibrosis neither confirmed nor refuted, the CFTR protein indeed plays a role in *Salmonella typhi* infection. In follow-up studies the association between the alleles 181 (CA_{16}) and 183 (CA_{17}) and reduced risk of acquiring typhoid fever could be studied in vitro, by analysing the whole CFTR protein in these specific cases, both by sequencing and determination of functional impairments (e.g., measuring the adherence and entry of *S. typhi* into transfected $CFTR^{-/-}$ cells).

Considering the genetic data, it appears that an individuals' susceptibility to *S. typhi* is not so much related to a pro-inflammatory response to the bacterium, but rather to the outcome of the interaction of *S. typhi* with bowel content and mucosa cells and its internalization. One could hypothesize that thereafter, i.e., after entry into the body of an effective inoculum, the immune reaction (re-)acts within the boundaries of its pre-set program, and although likely related to disease severity, this inflammatory response does not determine whether one becomes febrile or not (i.e., has typhoid or not), although the time to showing fever may differ accordingly. Obviously, whether or not an effective inoculum is internalized may be determined by many factors, including the particular CFTR polymorphism and ability of epithelial cells to limit or promote the intracellular outgrowth and transmucosal trafficking of *S. typhi*, e.g., as suggested by the role of polymorphism of *PARK2_e01(-2599)* in *PARK2/PACRG* gene.

The reported very low prevalence of cystic fibrosis in Indonesia, and apparent absence of the ΔF508 mutation, underscores the potential differences in host factors among diverse ethnic groups. Genetic studies, especially on variation of specific polymorphisms in the Indonesian population are still very limited; most studies were conducted in Western countries. The frequencies of polymorphisms in those studies, however, appear markedly different from those in our target population in Indonesia, making further studies on variation of polymorphisms in the Indonesian population in relation to common infectious diseases even more interesting. Because several polymorphisms have a very low frequency, such studies should include a sufficiently large collection of cases and controls, required to elucidate with enough statistical power an association of genetic host factors with susceptibility to or clinical course of disease.

Bacterial factors and typhoid fever and paratyphoid fever in Jakarta

It had been estimated that the presently prevailing *S. typhi* is a recent clone of only about 50.000 years old. The finding that *S. paratyphi* A contains fewer pseudogenes than *S. typhi* suggests that *S. paratyphi* may have arisen even more recently, provided that the accumulation of pseudogenes has proceeded at a similar rate in both serovars. In **Chapter**

Seven we validated an AFLP method to study the relatedness of our *S. typhi* and *S. paratyphi* isolates. The analysis revealed remarkably little variation between the bacterial isolates collected over a period of two years. The AFLP profiles also corroborated a previous study showing that *Salmonella paratyphi* A is even more homogenous than *Salmonella typhi* (reflected in a level of clustering at 89% vs. 85%, respectively).
Besides determining their AFLP profile, the *S. typhi* and *S. paratyphi* A isolates were phenotypically characterized by studying their susceptibility to multiple, unrelated antibiotics and by biochemical profiling. Again, a cluster analysis of the strains on the basis of antibiotic inhibition zones as determined by disk diffusion and biochemical characteristics did not discriminate between the isolates, showing that not only AFLP analysis but also standard phenotyping of strains will be of little use to help in the analysis of disease outbreaks.

Multi-drug resistant *Salmonella typhi* is a serious health threat in other parts of Asia including Vietnam, Pakistan and India. We found only a few antibiotic-resistant strains. Therefore, in Indonesia antibiotic regimens to treat typhoid and paratyphoid fever are still ample and include inexpensive first-line antibiotics like ampicillin or amoxicillin. The absence of spread of multi-antibiotic resistant *S. typhi* to Indonesia could be related to its geographical isolation: an archipelago with mostly aquatic borders with the exception of Northern Borneo with East Malaysia, Eastern Papua with Papua New Guinea and Northern Timor with Timor Leste, all comprising inhabitable border areas. Also in East Malaysia and Papua New Guinea, multi-drug resistant *Salmonella* is still not an issue. There is no data so far about multi-drug resistant *Salmonella* in Timor Leste. The specific geographical characteristics of Indonesia might act as a barrier to the spread of multi-drug resistant *Salmonella* strains from countries like Vietnam, India or Pakistan to Indonesia, especially in an epidemiological setting in which the bacteria are transmitted within family members or by chronic carriers (who are not being exposed to antibiotics!). Air transportation to and from the high-risk countries is still an expensive mode of transport and therefore of limited use for spread of multidrug resistant strains. Also, the passing of *S. typhi* within households, from reconvalescent household members and chronic carriers rather than in a hospital setting or through large scale outbreaks could be relevant in this respect. Most of the people in study area go to the first line health center where it is a common practice to prescribe antibiotics without prior antibiotic susceptibility testing; the antibiotics prescribed are usually chloramphenicol or co-trimoxazole. Some people will not adhere to the full course of treatment due to financial constraints, and use only a short course of antibiotics. Given the many resemblances of health care in Jakarta with that in countries in which multi-antibiotic resistant *S. typhi* is common, and the fact that we do not understand why it remains largely absent in Indonesia, there remains a real threat that multi-drug resistant Salmonella (and other bacteria) may surface in the near future. Adequate treatment of typhoid and

paratyphoid fever cases with appropriate antibiotics and adherence to the regimen should be promoted by the physicians.
Overall, the isolated *Salmonella typhi* and *Salmonella paratyphi* A strains from Jatinegara were very homogenous. This homogeneity was demonstrated in the phenotypic characteristics of bacteria as shown in biochemical profiles and antibiotic susceptibility, and also in genotypic features as analyzed by AFLP profile. The homogeneity of *Salmonella typhi* and *Salmonella paratyphi* A may be a reflection of the high degree of host adaptation in these serovars, apparently allowing little variation without loss of fitness to infect humans, and confinement to a particular geographical space. Alternatively, the apparent lack of variation might point to less likely explanations, such as a common source infection (unlikely because of the two year sampling and scattering of cases over the area). However, a more realistic evaluation would be that the typing of *Salmonella typhi* and *Salmonella paratyphi* awaits new molecular methods of strain analysis that do allow distinction between isolates to be made. One recently developed promising method in this respect concerns the pulsed-field gel electrophoresis.

Concluding remarks

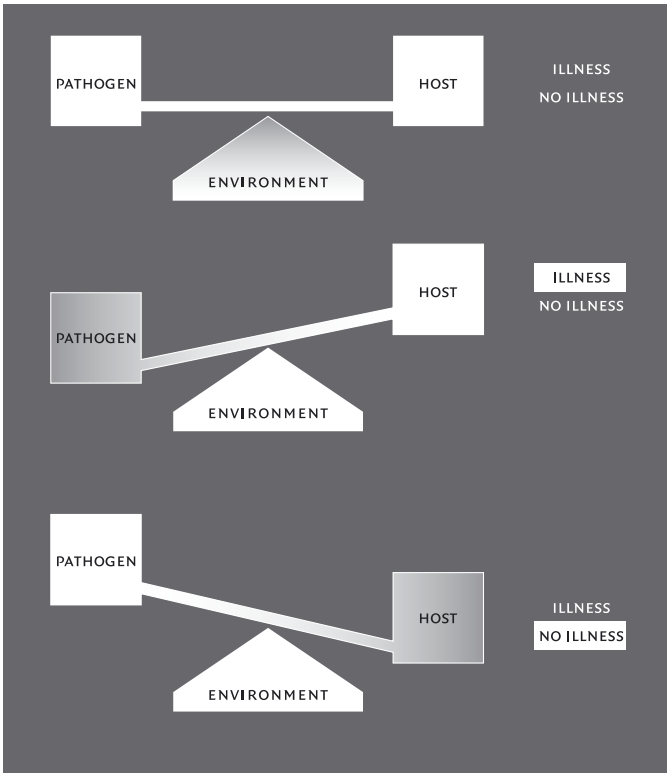
Salmonella has been characterized as “the great imposter of society”. In the developed Western countries, typhoid fever was most prevalent in the late nineteenth century during the late stages of industrialization and urbanization. Like typhus, typhoid was considered a disease typically related to lack of hygiene and to filth. The situation improved with the introduction of city sewage systems and a raise in awareness of personal and food hygiene, even before the introduction of vaccination and antibiotics. The construction of central water plants delivering drinking water to large parts of the cities paradoxically led to a succession of water-born epidemics of typhoid, because initially little precautions ware taken to prevent spillage of human waste into the water inlet, reservoirs and distribution pipes. Water filtering and chlorination improved this situation. Finally, the problem of chronic typhoid carriership was recognized and dealt with by outbreak investigations and targeted, individualized treatment. In the course of a century, these measures virtually eliminated typhoid fever as an endemic disease in Western countries. Of note, the introduction of antibiotic treatment of patients and of vaccination had little influence on this process. Interestingly, most of the factors mentioned above come together in present day Jakarta. The present finding of the relative increase in prevalence of paratyphoid as compared with typhoid fever, likely reflecting a changing socio-economic situation and increased consumption of cheap food of street vendors, underscores the notion that typhoid and paratyphoid fever prevalence reflect the developmental stage of society, in this case

of the megacity of Jakarta, with health problems related to poverty, pollution, flooding, lack of sewage and human waste disposal, and lack of safe water and hygiene. Likely, just like in Western societies a century ago, solving Jakarta's environmental health problems will require commitment to pay for disease surveillance, primary health care facilities for targeted individualized treatment and vaccination (e.g., of family members put at risk by faecal excretion of *S. typhi*), and health and hygiene education at schools, and large spending on the infrastructure needed to solve the problems in the future. The characteristics of the study area, e.g., its socio-economic level, living conditions and hygiene behavior of the population can be encountered in many other megacities in developing countries, with their massive urbanization problems.

At the individual level, host genetic factors likely determine background resistance against typhoid fever and may be unique to the population studied. Thus, although the cytokine genes seem to control the intensity of the host inflammatory reaction after entry of *S. typhi* and *S. paratyphi* into the body, and thereby severity of clinical manifestations of disease, they do not play a role in susceptibility to acquire the disease when an individual is exposed to the pathogens. Instead, the initial contact of the bacteria with the host mucosal cells may be decisive in the initialization of disease, and our finding that a polymorphism in the CFTR gene, encoding the main docking station of the bacteria in the gut, is associated with susceptibility to typhoid and paratyphoid fever attest to this notion. As a consequence an individual's susceptibility to acquire disease, or develop severe disease, may influence the likelihood of transmitting typhoid and paratyphoid fever to other members in the population, e.g., when more individuals are prone to develop into a chronic typhoid carriership, or shed large numbers of bacteria with the feces.
The particular *Salmonella* strain prevalent in the study might be unique. As reported from other countries, we observed a very high homogeneity of *S. typhi* and *S. paratyphi* strains. Of practical importance, a low prevalence of multidrug resistance was observed in the Jatinegara district -quite unlike those reported from studies in other developing countries- and this makes it possible to treat typhoid patients with antibiotics like cotrimoxazole, that by virtue of its reliable resorption may be taken orally, and patients might thus be treated on an outpatient basis.

In the control of typhoid and paratyphoid fever in an endemic area like Jakarta, it can be useful to consider an analogy of the host-pathogen interaction with the environment, in the concept of a balance. The present situation in Jakarta could then be described as illustrated in model I, in which the pathogen and host factors in interaction with environment are in

balance but result in endemicity of typhoid and paratyphoid. This fragile balance could easily tip over to the disadvantage of the host, favoring further circulation of bacteria, e.g., when a new clone of bacteria that is more virulent becomes endemic, or when flooding or contamination of drinking water or food with pathogens increases exposure and causes an outbreak in the community. This situation is illustrated in the model II. To curtail transmission of disease, one must aim to achieve model III, where environmental changes favor containment of the pathogen and favor the human host.
Even though many environmental, major infrastructural modifications may sound ground shaking and complex to realize, already simple and practical interventions could be proposed that are quick to lessen at least some of the disease burden of enteric pathogens in Jakarta, e.g., through adequate treatment of cases to avoid emergence of multi-drug resistant strains, through control of the environment to allow a shift to the advantage of the host, e.g., by education of personal hygiene practice at school and through enforcing personal and food hygiene of the street food vendors (as they supply a nutrition source of many people in the society), and, if financially possible, by provision of adequate sanitation and safe water to households presently lacking these facilities. On the other hand, from the viewpoint of the human host, the identification of susceptible individuals at risk of disease or prone to become chronic carriers and targeted immunization of household contacts of typhoid and paratyphoid fever cases could further curtail transmission of disease.



I. Pathogen, host and environment in balance

II. Pathogen becomes more virulent or host becomes susceptible and the environment shifts to the disadvantage of the host

III. Shifting of the environment and control of pathogen to the advantage of the host