Typhoid fever : aspects of environment, host and pathogen interaction

Ali, S.

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Aspects of environment,

host and pathogen interaction

Soegianto Ali

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PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Dr. D.D. Breimer,
hoogleraar in de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 2 november 2006
klokke 16:15 uur

door

Soegianto Ali
geboren te Jakarta in 1967

Promotiecommissie

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ISBN-10 90-9021024-5
ISBN-13 978-90-9021024-7
Printed by Gildeprint, Enschede, the Netherlands
Cover photo - Marcel Malherbe, Copyright © Hollandse Hoogte
Graphic design - Jan Kleingeld, Leiden, the Netherlands

The study described in this thesis is part of a scientific cooperation
between the Netherlands and Indonesia,
and was supported by Atma Jaya Catholic University of Indonesia, Jakarta,
and by a grant of the Koninklijke Nederlandse Academie van Wetenschappen KNAW
(Royal Dutch Academy of Sciences project no. 99-MED-02)

Contents

This thesis is dedicated to my wife Novita and my son Toby

I General introduction

Typhoid fever is caused by infection of humans with the microorganism *Salmonella enterica* subspecies *enterica* serotype Typhi (*S. typhi* for short). It is a systemic disease characterized by a prolonged fever, malaise and weight loss. On physical examination, characteristic skin lesions, rose spots, usually accompany a hepatosplenomegaly. Without antibiotic treatment the fever may persist for several weeks, and the disease will be fatal in about 15 percent of those affected. The bacterium is transmitted by faecal-oral route, through contaminated water or food.
S. typhi is highly adapted to its human host; there is no reservoir but man. Therefore, every case of typhoid fever means an infection from a previous one. The immunopathogenesis is characterized by a sustained low-grade bacteremia with microbial invasion of and multiplication within the mononuclear phagocytes lining the sinoids of the liver, spleen, bone marrow, lymph nodes, and Peyer's patches. Bacterial multiplication at the latter sites, with necrosis and sloughing of the overlaying mucosal epithelium produces the characteristic ulcerations of Peyer's patches in the terminal ileum, a long recognized pathological entity that proved invaluable to distinguish typhoid fever from typhus.
Paratyphoid fever is clinically and pathologically a highly similar disease, but caused by *Salmonella enterica* subspecies *enterica* serotypes Paratyphi A, B or C (*S. paratyphi* for short). Enteric fever refers to both typhoid fever and paratyphoid fever (1).
P. Louis described typhoid fever in 1829 as distinct clinical entity, apart from typhus and other sustained fevers. It took another 60 years before the microbial etiological agent, *S. typhi*, was isolated by Gaffkey in Germany in 1884. By serendipity, T. Woodward discovered shortly after the second World War that chloramphenicol could be used to successfully treat typhoid fever patients and shortly after that the first clinical field studies were done in Malaysia (2).

Although practically eradicated from the developed Western countries, enteric fever remains a major global health problem due to its high incidence and significant morbidity and mortality in developing countries. For the year 2000, it was estimated that 21.650.974 patients contracted typhoid fever, and that 216.510 died due to the disease, whereas paratyphoid fever was responsible for about 25 percent of all enteric fever cases and was estimated to infect 5.412.744 individuals (3). In Indonesia, the annual costs of treatment of typhoid fever cases has been estimated at approximately US$ 60 million, with an additional US$ 65 million loss of income, and typhoid is cause of deaths of about 20.000 individuals (4). In Jakarta, clinicians and public health experts believe that typhoid fever still is one of the five most common febrile illnesses causing the highest mortality among hospitalized patients.

Pathogenesis of typhoid fever

Salmonella enterica subspecies *enterica* serotype typhi is a member of the family Enterobacteriaceae. The bacterium is serologically characterized by the lipopolysaccharide antigens O9 and O12, the flagellar protein antigen Hd, and a polysaccharide capsular antigen Vi. The Vi capsular antigen is largely restricted to *S. typhi*, although it is shared by some strains of *S. enterica hirschfeldii* (Paratyphi C) and *dublin*, and *Citrobacter freundii* (5). The bacterium is strictly confined to the human species and there is no other reservoir of *S. typhi* but man.

The infectious dose of *S. typhi* in volunteers varies between 1000 and 1 million organisms (6). In real life, the infectious dose may well be much lower, because of the limited number of volunteers that participated in experimental infection studies. Although the Vi-antigen may not be responsible for virulence, Vi-negative strains of *S. typhi* appear to be less infectious than Vi-positive strains. After ingestion by drinks or food, *S. typhi* must survive the gastric acid barrier and reach the small intestine. As proven for non-typhoidal *Salmonellae*, individuals with a reduced gastric acid barrier are likely more susceptible to disease, because they fail to reduce the inoculum. In the small bowel, the bacteria adhere to the mucosa and next orchestrate their ingestion by mucosal cells. The so-called M cells, specialized epithelial cells overlying Peyer's lymphoid patches, are probably the primary sites of internalization of *S. typhi*. After passing these cells, the bacterium is presented to the underlying lymphoid tissue. Invading microorganisms translocate to intestinal lymphoid follicles and are transported to draining mesenteric lymph nodes. From there on, some pass into the circulation ('primary bacteremia') and are cleared by the mononuclear phagocyte system (previously designated as reticuloendothelial cells) of liver and spleen (7).

Just like other intracellular bacterial pathogens, *Salmonellae* manage to survive, persist and multiply within the mononuclear phagocytes of the lymphoid follicles, liver and spleen (8). At a critical point– which is probably determined by the number of bacteria, their virulence, and the host response– bacteria are released or spill over from this sequestered intracellular habitat into the bloodstream and cause a low-grade bacteremia. Until the (second) bacteremia, the patient does not have manifestations of disease and is in the incubation period that depending on the inoculum usually takes between 7 and 14 days.

In the bacteremic phase, the organism becomes widely disseminated. The most common sites of dissemination are the liver, spleen, bone marrow, gallbladder, and lymphoid tissues including the Peyer's patches of the terminal ileum. Gallbladder invasion occurs either directly from the blood or by retrograde spread from bile. Microorganisms excreted in the bile may re-invade the intestinal lining or are excreted in the faeces. It has been estimated that in patients with typhoid fever the total bacterial count in the bone marrow amounts to approximately 9 bacteria per millilitre, and in the blood to 0.9 per millilitre (9). Because of this difference, bone marrow cultures may still become positive when blood cultures are negative, as may be the case in patients just started on empirical treatment.

Clinical manifestations of typhoid fever

The clinical manifestations and severity of disease in typhoid fever may vary widely, largely depending on the patient population, e.g., adults versus infants, studied. Typhoid fever is a disease of children and young adults, and most patients who present to hospitals with typhoid fever are in the age class of 5 to 25 years. However, community-based surveillance in high-endemic regions demonstrate that many cases of typhoid, in particular in children under five years of age, may have a non-specific less severe illness that is not recognized clinically as typhoid (10). In most developing countries, many patients with typhoid fever do not receive appropriate medical attention or are treated as outpatients (11, 12).
The disease typically presents with a step-like, daily increase in temperature (finally reaching up to 40-41°C) combined with headache, malaise and chills. The hallmark of typhoid fever is a prolonged fever that may persist up to 4 to 8 weeks in untreated cases. Even though the illness may be mild and brief, in rare cases an acute severe infection progresses into multiple organ failure, disseminated intravascular coagulation and central nervous system involvement ('typhoid', i.e., 'in the clouds') and may results in early death. In other instances, necrotising cholecystitis or intestinal bleeding and perforation of the necrotic Peyer's patches can occur in the third or fourth week of illness, and result in late death. In most cases, the onset of these late complications is dramatic and clinically obvious. Other gastrointestinal manifestations include constipation (especially in adults) rather than diarrhoea (in children) and often is accompanied by abdominal tenderness. After the first week, mild hepatosplenomegaly is detectable in the majority of patients. A bradycardia relative to height of the fever may be a clinical clue to typhoid but is found in only a minority of patients. Epistaxis may be noted in the early stages of illness. "Rose spots," appearing as small, pale red, blanching, slightly raised maculae, are occasionally seen on the chest and abdomen during the first week. They can evolve into non-blanching small haemorrhages and may be difficult to see in dark skinned patients (13).

Like many infectious diseases, typhoid fever is the manifestation of the outcome of a complex crosstalk between the human host, its environment and the microbe, with many acquired, random and genetic factors coming into play. Somewhat oversimplified, it may be said that the severity of a case of typhoid fever depends on genetic properties of the pathogen (e.g., expression of Vi-antigen, other virulence factors, multi-drug resistance), the bacterial inoculum that effectively reaches its site of entry into the body (influenced by many environmental, social and host-specific factors, such as population density, food and personal hygiene, but also gastric acid, competing microorganisms in the gut, etc), and specific resistance mechanisms of the host (influenced by environmental factors like nutrition, as well as its genetic make-up, age, immune status, etc). Host genetic factors are therefore one among many determinants of susceptibility and outcome of infectious disease (14).

Considering the importance of typhoid fever to public health in Indonesia, there is a need for a comprehensive study describing environmental, host genetic and bacterial-specific characteristics as interactive aspects resulting in the clinical entity of typhoid and paratyphoid fever. The present thesis makes a start with such an analysis by focusing on these contributing elements.

Environmental factors in typhoid fever

The basic route of transmission of typhoid and paratyphoid fever is well known. Worldwide experience has demonstrated that improvement of environmental sanitation, including adequate sewage disposal and provision of safe water, sharply reduces the incidence of typhoid fever (13). However, such large infrastructural works that took decades to realise in Western countries about a century ago, cannot be realised overnight in the developing countries of today. Therefore, in these countries it is still useful to identify risk factors for disease and the most critical routes of transmission of disease linked to their particular situation, to enable the design of rational, 'individualized' public health control strategies. Risk factors for typhoid fever have been identified in several epidemiologic studies indicating a role for either waterborne (15-17) or foodborne (18,19) transmission. The risk factors for paratyphoid fever have not been determined in similar detail. The comparison of routes of transmission of both diseases is becoming increasingly relevant, however, since recent reports indicate a relative increase in cases of paratyphoid fever (20,21). It is not clear whether this change is due to incomplete reporting or to a downward trend in the incidence of typhoid fever (4) and by consequence a relative or absolute increase in incidence of paratyphoid fever. This is an important issue, for instance because of recent interest in mass immunization as a control strategy in regions of endemicity. This needs to be reconsidered if the incidence of typhoid fever is decreasing and paratyphoid fever is on the rise, because current typhoid fever vaccines do not provide protection against paratyphoid fever (22). Although the possible transmission routes of enteric pathogens like *Salmonella* are known, the relative importance of the various factors, i.e., the weak link in the transmission chain in a particular situation (rural vs. urban, Asia vs. South America, etc) is uncertain but of great importance to help focus the most relevant and cost-effective local health interventions. An additional complicating factor is the fact that *Salmonella* bacteria can multiply in food and easily reach infective dose after an initial insignificant contamination (23). Therefore, determinants for transmission of enteric pathogens into commercial food and handling of food were examined in a cross-sectional study.

Host genetic factors as determinants in typhoid fever

As abovementioned, host genetic factors may influence susceptibility to and outcome of infectious diseases. Also, studying host and bacterial genetic factors provides insight into

relevant pathophysiological mechanisms in typhoid, by identifying critical pathways and mechanisms of bacterial invasiveness, host resistance and immunity, or tolerance. On the population level, studying genetic variation in relation to environmental factors may help us understand the perceived variation between individuals in susceptibility and clinical outcome (14).

Typhoid induces systemic and local humeral and cellular immune responses, but these confer incomplete protection against relapse and reinfection (24). The multitude of host mechanisms involved leaves open the possibility that (failure of) effector systems at multiple levels of host defence culminate in a differential susceptibility to typhoid and paratyphoid fever. Also, *S. typhi* is highly adapted to its human host and for its transmission relies not only on reconvalescent patients who temporarily excrete the bacterium, but also on subjects that become chronic, sometimes life-long faecal carriers. About 3 to 5 percent of typhoid patients, with a preference for females, become long-term asymptomatic carriers, and can excrete the bacterium at very high numbers without showing any signs of carriership. Many carriers even cannot recall a history of a typhoid fever attack and probably have had an undiagnosed mild infection (13). Obviously, typhoid carriers are of particular concern to the public health since they represent the reservoir for spread of typhoid in the situation that most typhoid patients are recognized, treated adequately and educated how by simple hygienic measures they can prevent passing on the disease as long as they excrete the bacterium. Chronic carriers of *S. typhi* have high levels of serum antibodies to Vi and flagellar antigens, which can be useful for diagnostic purposes (8). Investigating the cohabitation of host and pathogen in chronic carriers should provide a fascinating insight into bacterial survival and propagation strategies, as well as information that could be useful to develop new approaches for the treatment of typhoid and perhaps other persistent microbial infections. With respect to the analysis of host genetic factors, it can be said that functional polymorphisms in genes encoding pro- or anti-inflammatory cytokines and their association with infectious diseases had been studied extensively. In some cases, an association of a particular polymorphism and an infection seemed clear, whereas in other diseases, no significant influence of genetic variation was evident. In most studies, it is not so clear whether susceptibility to disease per se was studied, or an association of genetic variability and severity of disease manifestation.

The activation of infected macrophages by interferon–γ in synergy with TNF–α is a major effector mechanism of cell-mediated immunity to intracellular pathogens like *S. typhi* (25). TNF–α is synthesized by macrophages and T cells as a membrane protein, which is cleaved to produce its soluble 17 Kd form. Soluble TNF–α exerts a range of inflammatory and immunomodulatory activities that are of importance to host defence (26).

The gene for TNF–α is located within the MHC region on chromosome 6p21.3. This is a highly polymorphic region and the location of multiple genes involved in host defense. The TNF–α gene contains a large number of polymorphisms. Of these, single nucleotide polymorphisms (SNP) at position –238 and –308 are the most extensively studied. The role of TNF–α SNPs had been studied in viral infections, i.e., hepatitis B (27) and hepatitis C (28), in parasitic infection, i.e., malaria (29) and leishmaniasis (30), as well as bacterial infection, i.e., meningococcal disease (31), sepsis due to various microorganisms (32-34), and in Vietnam among patients hospitalized for typhoid fever (35). Interestingly, an ex-vivo whole blood study of the cytokine response to lipopolysaccharide (LPS) in patients with typhoid fever found no association between the *TNFA–308* promoter polymorphism and LPS induced TNF–α release, neither during active infection nor after treatment (36). Polymorphism of *TNFRSF1A +36*, a gene that encodes TNF receptor 1 has been associated with Crohn's disease (37).

In the scope of the present study, other genetic variation of interest involves polymorphisms in *IFNG SNP+874* and an allele characterized on the basis of CA repeat polymorphism in intron 5 of *IFNGR1* that have been associated with susceptibility to *Mycobacterium tuberculosis* infection, an intracellular pathogen like *S. typhi* (38-41).

The cytokine IL-1 has been implicated in many inflammatory diseases and the *IL1A SNP-889*, for instance, has been associated with juvenile rheumatoid arthritis (42). Polymorphism in *IL1β SNP+3953* appears to have functional consequences, as it was associated with quantitative differences in expression levels of IL-1β (43). The second allele of *IL1B SNP-511* was decreased in seronegative Epstein-Barr virus culture-positive patients (44). The *IL1R1 SNPsA124G* and *R456R* are both in the coding region and have not been studied extensively.

Interleukin–12, a heterodimer composed of a p40 and p35 subunit, is produced by subsets of dendritic cells and macrophages and acts on natural killer (NK)-cells and T cells. It initiates their proliferative response that leads to the production of IFN-γ. Thus, IL-12 constitutes a major link between innate and adaptive immunity. The overall importance of this cytokine in the pathogenesis of salmonellosis was demonstrated in studies of humans with severe and recurrent infections caused by salmonellae and non-tuberculous mycobacteria: these subjects were found to have genetic defects in the interleukin–12/interferon–γ mediated pathway of macrophage activation (45-47). A polymorphism in *IL12B SNP+1188* in the 3' untranslated region has been associated with levels of IL12B mRNA expression and IL-12p70 secretion (48).

Besides IL-12, the cytokine IL-18 plays a role in the activation of NK-cells and T-cells, likely by its co-stimulatory action on these cells to produce IFN–γ (49).

The IL-18 SNP in codon 35 has been identified as one of the genes that determines susceptibility to Crohn's disease (50).

Another candidate gene that may play a role in immunopathogenesis of typhoid fever is

CASP1, since genetic variation herein has been associated with *Salmonella enteritidis* infection in poultry (51). *CRP* SNP+1444 was found to influence the basal, as well as the stimulated CRP levels (52) and previously, CRP levels were associated with typhoid fever (53). Studying the association of these polymorphisms with susceptibility to typhoid fever in cases identified in a population-based surveillance in an endemic area such as Jakarta, together with well-defined random community controls should help elucidate the role of many of these SNPs as risk factors for susceptibility to or severity of clinical manifestations of typhoid fever.

Besides the variation in genes that have been linked to the host immune response, some other candidate genes should be considered. Since both *Salmonella* and *Mycobacteria* are intracellular pathogens, some immunopathogenic pathways may be quite similar (25,45,46,54). Of interest then, study on leprosy patients revealed that polymorphisms on *PARK2* and *PACRG* were associated with susceptibility to *Mycobacterium leprae* infection (55). Mutations in *PARK2*, the gene encoding Parkin, on chromosome 6, have been identified to cause autosomal recessive juvenile Parkinsonism (56,57). Parkin is an E3 ubiquitin ligase that is required for poly-ubiquitination of proteins before degradation by the proteasome (58). Parkin Co-Regulated Gene or *PACRG* is a reverse strand gene located upstream of the Parkin gene. The gene product, termed Glup, together with Parkin may deal with cytotoxic intermediates by breaking them down or turning them into harmless molecules in the proteasome (59). The ubiquitin-proteasome pathway is important in protein processing and degradation, and contributes to quality control of proteins in cells and antigen-processing for cross-presentation (60). An essential feature of the bacterial pathogen *Salmonella spp.* is its ability to enter cells that are normally non-phagocytic, such as those of the intestinal epithelium. The bacterium achieves entry by delivering effector proteins that cause physiologic changes in mucosal cells. These bacterial proteins must be degraded in exactly the right way and sequence to keep the cells intact and to provide a sustainable environment for *Salmonella* to multiply. The possible role of the ubiquitin-proteasome pathway in effector protein degradation has been cited by several studies on *Salmonella* and host cell interaction (61-63). Mutations in *PARK2/PACRG* might influence the ubiquitin-proteasome pathway; therefore it is interesting to extend the association found with leprosy to that with typhoid fever.

Another gene of potential interest in susceptibility to typhoid fever concerns the cystic fibrosis transmembrane conductance regulator (CFTR) protein. *Salmonella* infection starts with the invasion of bacteria into the mucosa of the small intestine (13). It has been hypothesized that the CFTR protein is used by *Salmonella* Typhi as a docking station, necessary as the first step in entering epithelial cells. CFTR is a chloride ion channel

expressed on many secretory epithelial cells. Many deleterious homozygous mutations of the CFTR result in an almost absence of membrane expression and are the cause of cystic fibrosis. Consistent herewith, cells expressing wild-type CFTR RNA internalize more *S. typhi* than isogenic cells expressing the most common CFTR mutation, a phenylalanine deleted at residue 508 (Δ508). Antibodies against CFTR and synthetic peptides mimicking a domain of CFTR inhibited the uptake of *S. typhi* (64). Finally, yet another study found that *S. typhi* was bound to the CFTR by interaction of its prePilS protein with a 15-mer peptide representing the first extra cellular domain of CFTR (65). In one study, *S. typhi* was even found to induce intestinal epithelial cells to increase membrane expression of CFTR, resulting in enhanced bacterial ingestion and sub-mucosal translocation. In conclusion, CFTR could well play as essential role in the first step in the infectious process leading to typhoid fever, i.e., adhesion to the gut mucosa (66).

Pathogen factors as determinant in typhoid fever

Controlling infectious diseases such as typhoid fever depends on the ability to rapidly detect, identify and characterize the etiological microbial agent. In turn, this relies on an adequate surveillance system to monitor prevalence, detect outbreaks, and assess effects of control programs, so that appropriate intervention strategies can be implemented (67). To be able to interpret the epidemiology of infectious diseases, e.g., to distinguish an outbreak from an overall increase in endemicity, or establish a common link between scattered cases, genetic identification of the various clones of bacteria has become an essential tool. Molecular typing has been used as a tool to identify different *Salmonella* strains. Distinction based on different plasmid profiles of *S. typhi* and *S. paratyphi* A is unsuitable, because only a small proportion of strains (10%) contain plasmids (68). Vi phage typing is technically demanding, and the analysis of envelope proteins detected only minor differences between strains (69). In recent years, macro restriction analysis using Pulsed Field Gel Electrophoresis (PFGE) methods has been used to analyze *Salmonella typhi* isolates in outbreaks (70-77).

Selective amplification of fragment length restriction (AFLP), a technique developed for DNA fingerprinting based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (78), has been used in the genotypic analysis of several species of bacteria which were highly related or identical using other typing methods (79-81). AFLP has the advantage of being more efficient and readily adapted for automation compared to PFGE (82). Recently, AFLP also has been used for genotypic characterization of *S. typhi* (83). A study comparing characterization of isolated *S. typhi* and *S. paratyphi* A using genomic typing by AFLP and phenotypic typing by biochemical and antibiotic sensitivity profiles and in combination with the data from our epidemiology study will give insight of how many genotypically-distinct *Salmonella* strains might circulate.

Besides the molecular typing methods, also phenotypic typing can be applied, for instance by looking at certain biochemical characteristics or resistance to multiple antibiotics. With respect to *S. typhi*, there is a lack of comparison between these phenotypic and genotypic methods.

Moreover, surveillance for antibiotic resistance is important also from the point of view of patient treatment. Especially so, as blood cultures supplemented by antibiotic sensitivity testing are rarely performed in Jakarta. For the treatment of patients, one has to rely on knowledge of the prevalence of antibiotic resistance of microorganisms in the population, but little data is available for Jakarta or Indonesia. Resistance to chloramphenicol in *S. typhi* was reported already in 1950, but it was not until 22 years later that the first large outbreaks of chloramphenicol-resistant typhoid fever occurred (84). Since 1992, multiple antibiotic resistance (MDR) among isolates of *S. typhi* has become an increasingly important and serious problem (4). In Asia, outbreaks of infections with these strains occurred in India (75,85), Pakistan (86,87), Bangladesh (88), Tajikistan (17) and in Vietnam (89). In Vietnam, MDR *S. typhi* were present in higher numbers in the blood of patients than the sensitive strains (9), in accordance with a previous notion that MDR strains of *S. typhi* are somehow more virulent (90). Little is known on antibiotic resistance of *S. typhi* and *S. paratyphi* in Indonesia. Trends in antibiotic resistance of *S. typhi* and *S. paratyphi* A in Europe are monitored by the Enter-net surveillance hub (91).

Outline of the thesis

In **Chapter 1** a general introduction to typhoid and paratyphoid fever is given.
In the introduction, the burden of disease by typhoid and paratyphoid fever, and clinical aspects are reviewed. Attention is given to the interaction between environment, host and pathogen. Environmental factors, such as personal hygiene and behaviour, contaminated food and water, and host genetic background as risk factors for contracting and defining clinical outcome of typhoid or paratyphoid fever are discussed, as are *Salmonella*-related factors.
In **Chapter 2** risk factors for typhoid and paratyphoid fever in Jakarta are discussed (12). The chapter deals with the influence of personal hygiene, water supply and quality, and eating habits as risk factors for typhoid and paratyphoid fever. Knowledge of the relative contribution of each of these risk factors will be essential to be able to design effective control strategies.
In **Chapter 3** risk factors for transmission of foodborne illness in restaurants and by street vendors in Jakarta are discussed (92). The chapter describes the identification of determinants of transmission of foodborne diseases such as typhoid and paratyphoid fever, in commercial food handling in restaurants, food stalls and pushcarts.
In **Chapter 4** the analysis of environmental determinants is replaced by an evaluation of genetic determinants of disease, by discussing polymorphisms in pro-inflammatory (cytokine) genes in relation to susceptibility to and severity of typhoid fever and paratyphoid fever (93).
In **Chapter 5** an interesting typhoid-susceptibility related candidate gene, i.e., *PARK2/PACRG*, and its polymorphisms in relation to susceptibility to typhoid and paratyphoid fever is discussed (94). Specifically, the chapter explores the association of *PARK2/PACRG* polymorphisms, genes that play a role in ubiquitin-proteasome pathway and were found to be associated with leprosy, with susceptibility to typhoid and paratyphoid fever.
In **Chapter 6** the hypothesis is tested that expression of the cystic fibrosis CFTR protein might be related to susceptibility to typhoid fever (95).
In **Chapter 7** a phenotypic analysis and molecular typing of *S. typhi* by AFLP is applied to assess which method is useful and contributes to the understanding of the epidemiology of typhoid fever and paratyphoid fever in Jakarta (96). To this end, the molecular method for strain typing, AFLP, is compared with conventional methods such as biochemical and antibiotic sensitivity profiles.
Chapter 8 concludes with a general discussion and a summary of the findings of this thesis.

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2

Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia

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Journal of the American Medical Association 2004; 291: 2607-2615

2

Abstract

Context: The proportion of paratyphoid fever cases to typhoid fever cases may change due to urbanization and increased dependency on food purchased from street vendors. For containment of paratyphoid a different strategy may be needed than for typhoid, because risk factors for disease may not coincide and current typhoid vaccines do not protect against paratyphoid fever.

Objective: To determine risk factors for typhoid and paratyphoid fever in an endemic area.

Design, Setting, and Participants: Community-based case-control study conducted from June 2001 to February 2003 in hospitals and outpatient health centers in Jatinegara district, Jakarta, Indonesia. Enrolled participants were 1019 consecutive patients with fever lasting 3 or more days, from which 69 blood culture–confirmed typhoid cases, 24 confirmed paratyphoid cases, and 289 control patients with fever but without *Salmonella* bacteremia were interviewed, plus 378 randomly selected community controls.

Main Outcome Measures: Blood culture–confirmed typhoid or paratyphoid fever; risk factors for both diseases.

Results: In 1019 fever patients we identified 88 (9%) *Salmonella typhi* and 26 (3%) *Salmonella paratyphi* A infections. Paratyphoid fever among cases was independently associated with consumption of food from street vendors (comparison with community controls: odds ratio [OR], 3.34; 95% confidence interval [CI], 1.41-7.91; with fever controls: OR, 5.17; 95% CI, 2.12-12.60) and flooding (comparison with community controls: OR, 4.52; 95% CI, 1.90-10.73; with fever controls: OR, 3.25; 95% CI, 1.31-8.02). By contrast, independent risk factors for typhoid fever using the community control group were mostly related to the household, ie, to recent typhoid fever in the household (OR, 2.38; 95% CI, 1.03-5.48); no use of soap for handwashing (OR, 1.91; 95% CI, 1.06-3.46); sharing food from the same plate (OR, 1.93; 95% CI, 1.10-3.37), and no toilet in the household (OR, 2.20; 95% CI, 1.06-4.55). Also, typhoid fever was associated with young age in years (OR, 0.96; 95% CI, 0.94-0.98). In comparison with fever controls, risk factors for typhoid fever were use of ice cubes (OR, 2.27; 95% CI, 1.31-3.93) and female sex (OR, 1.79; 95% CI, 1.04-3.06). Fecal contamination of drinking water was not associated with typhoid or paratyphoid fever. We did not detect fecal carriers among food handlers in the households.

Conclusions: In Jakarta, typhoid and paratyphoid fever are associated with distinct routes of transmission, with the risk factors for disease either mainly within the household (typhoid) or outside the household (paratyphoid).

Introduction

Typhoid fever, a food- and waterborne disease caused by *Salmonella enterica* serotype Typhi (*S. typhi*), is a serious public health problem in developing countries that claims 600 000 lives every year.[1] Paratyphoid fever, caused by *Salmonella paratyphi* A, B, or C, has a disease presentation similar to that of typhoid fever, but its incidence is reportedly about one tenth that of typhoid (ratio, 1:10-20).[2-3] In developing countries the identification of risk factors and relevant route of transmission for a disease such as typhoid fever is essential for the development of rational control strategies. Resources could consequently be allocated to where they count most, e.g., to the construction or expansion of water distribution networks or sewage systems, chlorination of drinking water, ensurance of food safety, hygiene education, mass vaccination campaigns, and/or the identification of carriers within or outside the households of patients.

Risk factors for typhoid fever have been identified in several epidemiologic studies suggesting either waterborne [4-8] or food borne transmission.[7,9-11] Whether these factors coincide with those for paratyphoid fever has not been determined. The assumption is that in paratyphoid fever, a higher dose of bacteria is required for infection than in typhoid fever; consequently, food is implicated as the major vehicle for transmission of paratyphoid fever, since *Salmonella* bacteria can multiply in food.[12] Comparison of the transmission of both diseases is becoming increasingly relevant, because recent reports have demonstrated an increasing occurrence of paratyphoid fever.[3,13] It is not clear whether this is due to incompleteness of epidemiologic data in endemic countries or to a downward trend in the incidence of typhoid fever [1,14] and a consequent relative or absolute increase in the incidence of paratyphoid fever. In consequence, however, public health measures may well be refocused. In particular, recent interest in mass immunization as a control strategy in regions of endemicity needs to be reconsidered if the incidence of typhoid fever is decreasing and para-typhoid fever is on the rise, because current typhoid fever vaccines (i.e., parenteral Vi and oral Ty21a vaccine) do not protect against paratyphoid fever.[2]

In this community-based case-control study in an endemic area in East Jakarta, Indonesia, we compared case patients having paratyphoid and typhoid fever with random community controls to identify hygienic practices, eating habits, and environmental and household characteristics that could elucidate prevailing transmission routes. For this purpose we also examined the microbiological quality of drinking water and cultured stools of intra-household food handlers to detect transient or chronic carriers. A second control group composed of patients with non-enteric fever was used for comparison and confirmation of the results. Patients with typhoid fever, paratyphoid fever, and non-enteric fever were identified in a prospective passive-surveillance study involving hospitals and outpatient health centers in the study area.

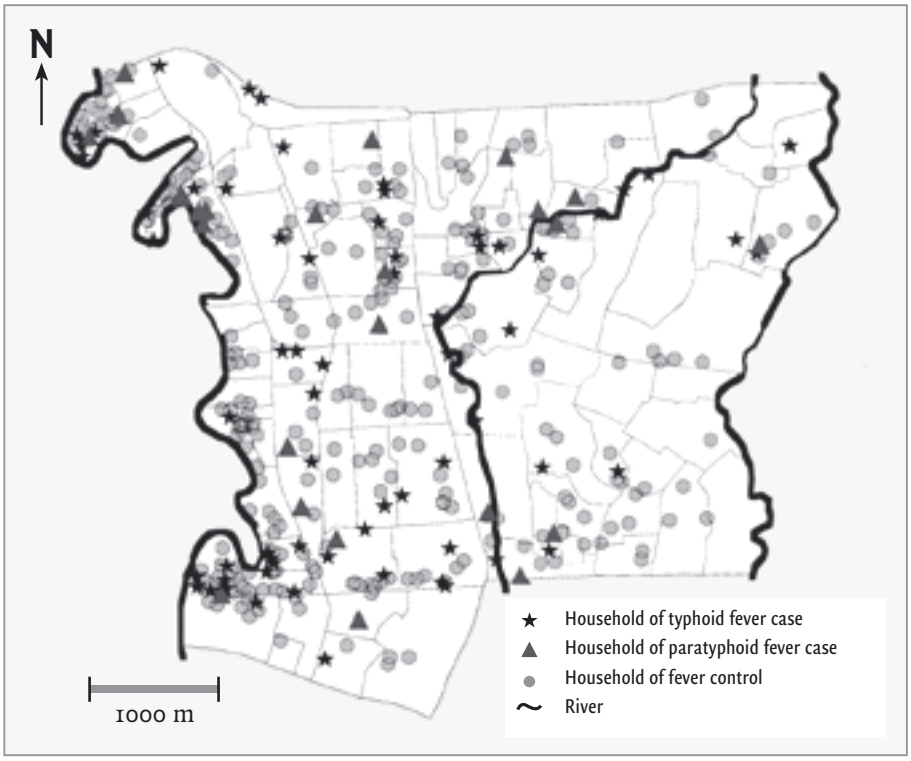
Methods

Study Area and Population: The Jatinegara district in East Jakarta, a 10.6 km² area with 262 699 registered inhabitants (as of March 2002), was selected as the study area **(Figure 1)** because of its varied socioeconomic conditions and good access to *puskesmas* (i.e., public community health centers providing medical care for low-income residents of Indonesia). The local climate has 2 distinctive seasons: a rainy season (December-April) and a dry season (May-November). Three rivers cross the area, making the adjacent subdistricts prone to flooding. There is no sewage system in the area. Vaccination campaigns have not been initiated in the area.

Study Design and Selection Criteria: The study was approved by the Indonesian National Institute of Health Research and Development (*Litbangkes*) and provincial authorities.

A passive surveillance system was established from June 11, 2001, to February 4, 2003. Health care facilities in the study area were approached for the surveillance study.

Figure 1. Study area (Jatinegara, Jakarta, Indonesia), showing households of cases with typhoid and paratyphoid fever and fever controls



Those participating included all 4 hospitals in the immediate vicinity, 8 of the 13 additional small private outpatient clinics in the area, and all 12 *puskesmas*. A fee of US $0.35 covers 3 days of antibiotic treatment, but cultures or Widal tests are not part of the usual diagnostic practice in *puskesmas*. Eligible patients were individuals living in the study area who consulted one of the participating health care facilities because of self-reported fever for 3 or more consecutive days. A single blood specimen for culture was collected from each eligible patient. Depending on the age of the patient, 5 to 10 mL of blood was collected into blood culture vials (aerobic) containing antibiotic-absorbing resins (Bactec; Becton Dickinson, Franklin Lakes, NJ) that were provided to the centers by the study group free of charge.

Cases were eligible patients with blood culture–confirmed *S. typhi* or *S. paratyphi* infection. All cases were subject to a household visit within a month after the febrile episode that prompted the blood culture.

Blood cultures of patients with non-enteric fever showed either no growth or bacteria other than *S. typhi* or *S. paratyphi* as cause of fever. Malaria could be excluded in the differential diagnosis of prolonged fever, because transmission does not occur in Jakarta. Every second consecutive patient with non-enteric fever was selected as a fever control and visited. Also, during the surveillance, community controls were randomly selected within a random household in every third *rukun tetangga* (i.e., the smallest administrative unit of 40-60 area households) of a total of 1140 *rukun tetanggas*. When a community control reported fever in the 30 days preceding the interview or refused participation, the house on alternating sides of the initially selected household was approached. The selection of both groups of controls was nonmatched for age, sex, or neighborhood (i.e., residence in 1 of the 8 subdistricts of Jatinegara) to limit selection bias and prevent overmatching. Four controls from both groups for every case of enteric fever were selected to increase statistical power.

Household Visits and Sample Collection: Cases and controls were interviewed by trained medical school graduates, using a standardized questionnaire that included the known risk factors from previous studies and questions from a questionnaire that was used in a similar risk factor study, which had been locally tested and validated.[6] Written informed consent was provided by all participants at the household visit. To prevent the overrepresentation of multiple-case households, only 1 patient (i.e., the first reported case or fever control) per household was interviewed. If cases or controls were younger than 13 years, the mother or guardian was interviewed. No time frame for hygiene behavior and food habits was mentioned, because it aimed at the description of usual practice. A *household* was defined as a dwelling whose inhabitants ate from the same pot. *Flooding* was defined as inundation of the house of a participant in the 12 months preceding the interview. *Intrahousehold food handlers* were defined as individuals preparing meals for cases or

controls 3 or more times a week. A single stool sample of 2 g was collected from all cases, controls, and their intrahousehold food handlers in a vial with Cary-Blair transport medium and samples were processed within 24 hours after collection. Water samples of 150 mL directly from the source of running drinking water were collected in the households of 62 typhoid and 20 paratyphoid cases, 341 community controls, and 233 fever controls using World Health Organization guidelines.[15]

Laboratory Methods: Blood culture vials from outpatient facilities were transported on the day of collection to Mitra Internasional, one of the participating private hospitals with a microbiology laboratory certified by the International Organization for Standardization. Blood cultures were incubated for up to 7 days. Samples demonstrating growth were plated on blood agar medium. *Salmonella typhi* or *S. paratyphi* A were identified by use of agglutination antisera (Polyvalent, D, Vi, H, and Paratyphi A; Murex Biotech Ltd, Dartford, England) and biochemical tests (Microbact; Medvet Diagnostics, Adelaide, Australia). Susceptibility against chloramphenicol, ampicillin, cotrimoxazole, and ciprofloxacin was tested by disk diffusion on Mueller-Hinton agar. Stool samples were cultured for *Salmonella* bacteria using selenite enrichment broth (Oxoid Ltd, Hampshire, England). Suspected colonies as identified by visual inspection were plated on xylose-lysine-desoxycholate agar and *Salmonella-Shigella* agar, and on triple sugar iron agar, SIM (sulphide and indole production and motility) medium, and Simmons citrate (Oxoid). Bacterial identification was identical to that for bacteria from blood cultures.

Samples from the sources of drinking water were transported on ice and processed within 6 hours after collection at the Nusantara Water Centre.[15] In samples from piped water the bactericidal effect of chlorine during transport was neutralized by 0.1 mL of 10% sodium thiosulphate. Water samples were examined for total and fecal coliforms by use of most probable number method.[15] Fecal contamination was defined as a most probable number index for fecal coliforms of 1/100 mL or greater.

Statistical Methods: Data from the questionnaires were entered twice using EpiInfo 6.04b software (US Centers for Disease Control and Prevention, Atlanta, Ga), validated, and imported into SPSS version 11.5 (SPSS Inc, Chicago, Ill) for statistical analysis. After the first 3 months of surveillance, an interim analysis was performed and the needed sample size was calculated; a minimum sample size of 80 enteric fever cases (assuming 4 times as many fever controls) was required to detect significant associations ($P<.05$) between key exposure variables and outcome, with a power of 0.80. Normally and nonnormally distributed numerical variables were analyzed using t tests and Mann-Whitney U tests, respectively. Measures for association were expressed as odds ratios (ORs) for disease with their 95% confidence intervals (CIs) for categorical variables. To control for confounding, a multivariate analysis was performed using logistic regression with a forward likelihood ratio test with the significantly associated variables from the bivariate analysis

and potential confounders (e.g., age, sex, income, and neighborhood residence).[16] Sex and income were also included in the bivariate analysis; age and neighborhood residence were not. Effect modification by interaction of age, sex, or income was tested, but these terms were not significantly associated and did not change the ORs of associated variables. The attributable risk of each independently associated variable from the multivariate analysis was calculated.[17]

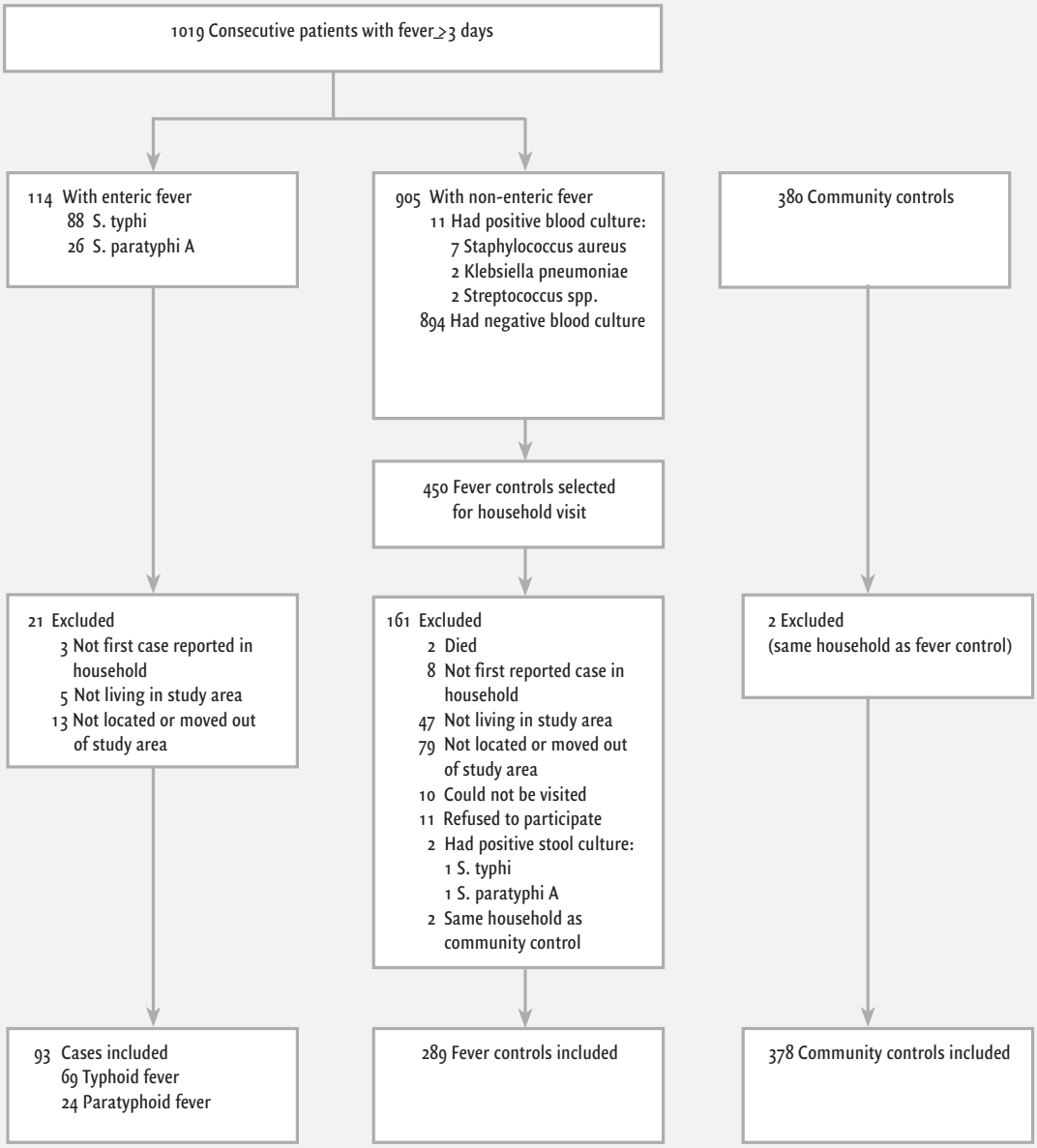
Results

Surveillance Study: During the study period 1019 consecutive patients with fever lasting 3 or more days were included. We identified 88 *S. typhi* and 26 *S. paratyphi* A infections. In 905 patients with non-enteric fever, 11 had bacteremia of another cause (*Staphylococcus aureus* [n = 7], *Klebsiella pneumoniae* [n = 2], and *Streptococcus* spp [n = 2]), whereas the remaining 894 patients were culture-negative **(Figure 2)**. Most of the patients were treated in the *puskesmas* (n = 717 [70%]), and fewer patients in hospitals (n = 113 [11%]) and outpatient clinics (n = 189 [19%]). The relative number of patients with typhoid or paratyphoid fever among febrile patients was similar for all health care centers (P = .81). Typhoid and paratyphoid fever accounted for 114 (11%) of the febrile episodes identified. Twenty-three percent (26/114) of enteric fevers were paratyphoid fever. Three (3%) of the 88 *S. typhi* strains were resistant to chloramphenicol, ampicillin, and cotrimoxazole; all *S. paratyphi* A strains were susceptible to these antibiotics.

Patients with typhoid and paratyphoid fever reported a median of 4 days (interquartile range [IQR], 3-7) of fever before blood cultures were taken. This period was similar to that in patients with non-enteric fever (median, 4 days; IQR, 3-54). The age of all patients enrolled in the surveillance study ranged from 1 to 76 years (3-59 years for patients with enteric fever and 1-76 years for those with non-enteric fever). The number of enteric fever cases enrolled in the dry season was higher than that in the rainy season (ratio, 7:3) and this ratio was similar (P>.05) in patients with non-enteric fever (ratio, 6:4). Referring physicians reported prior use of antibiotics in 26 patients (23%) with typhoid or paratyphoid fever and in 200 patients (22%) with nonenteric fever (P = .86).

Household Visits: In total, 69 typhoid fever cases, 24 paratyphoid fever cases, 289 fever controls, and 378 community controls were available for analysis (**Figure 2**). Not all of the cases and fever controls could be interviewed. Two fever controls died. Three cases (3%) and 8 fever controls (2%) were secondary patients from households in which only the first patient was interviewed to prevent overrepresentation of these households. Five cases (4%) and 47 fever controls (10%) were not living in the study area. Some addresses could not be found or patients had migrated out of the area (13 [11%] and 79 [18%] for cases and fever controls, respectively). Due to manpower constraints, 10 fever controls

Figure 2. Study inclusion of typhoid and paratyphoid fever cases, fever controls and community controls in Jatinegara, Jakarta, Indonesia, June 2001 – February 2003



(2%) could not be visited; 11 fever controls (2%) but none of the remaining cases refused cooperation. Two fever controls had positive stool culture results (for *S. typhi* [n = 1] and *S. paratyphi* A [n = 1]) at the household visit and were therefore excluded from the analysis. Enteric fever cases and fever controls were visited a median of 24 (IQR, 21-29) days after the blood culture. Fever controls reported to be diagnosed and treated for the following diagnoses: suspected typhoid fever (n = 126 [44%]), dengue fever (n = 11 [4%]), respiratory tract infections (n = 10 [3%]), tuberculosis (n = 3 [1%]), influenza (n = 3 [1%]), gastroenteritis (n = 2), urinary tract infection (n = 1), and encephalitis (n = 1); 132 patients (46%) were not informed of the working diagnosis.

During the study period, 380 random households in the study area community were visited; 289 (76%) of the community controls agreed to participate at the first approach and the remaining 91 (24%) were the neighbors from the initially selected households. From 2 households of community controls a patient with non-enteric fever was included later in the course of the study period. These 2 households were excluded from the analysis.

Demographic Data From the Visited Cases and Controls: The median age of the typhoid cases was 16 (range, 3-57) years; of paratyphoid cases, 22 (range, 4-59) years; of community controls, 27 (range, 1-80) years; and of fever controls, 20 (range, 1-75) years (**Table 1**). Typhoid and paratyphoid fever cases and fever controls were significantly younger than the community controls ($P<.01$). The age of patients with typhoid fever did not differ significantly from that of those with paratyphoid fever ($P = .12$). Fever controls were significantly more often of male sex than were community controls ($P = .003$ by χ^2 test) and typhoid cases ($P = .03$). No significant differences in the sex ratio were found when typhoid or paratyphoid cases were compared with community controls. Compared with the number of community controls per subdistrict, who had been included proportionally to the size of the population, in 1 subdistrict proportionally more typhoid cases than community controls were enrolled ($P = .07$), whereas in another subdistrict more patients with paratyphoid fever were enrolled ($P = .05$). Within the group of patients with enteric fever itself, no significant overrepresentation of any subdistrict was found in the comparison of patients with typhoid and paratyphoid fever ($P = .37$).

Risk Factors for Typhoid and Paratyphoid Fever: Risk factors for typhoid and paratyphoid fever in comparison with community and fever controls are shown in **Table 1**. Compared with paratyphoid cases the typhoid cases were more often female, lived in more crowded conditions, were more frequently from a lower income category, more frequently reported recent typhoid fever among household contacts in the preceding 12 months, used ice cubes more often, shared food more often, and observed poor handwashing hygiene. Flooding and eating food purchased from street vendors were more frequently reported by patients with paratyphoid fever than by those with typhoid fever. Among the 2 control groups, fever controls were more often male, from a lower income group, observed

Table 1. Risk factors for typhoid and paratyphoid fever in Jakarta

	Cases		Controls	
Risk factor	**Typhoid fever (n=69)**	**Paratyphoid fever (n=24)**	**Community (n=378)**	**Fever (n=289)**
Age, median (range), y	16 (3-57)	22 (4-59)	27 (1-80)	20 (1-75)
Female sex	40 (58%)	9 (38%)	211 (56%)	126 (44%)
Low family income [a]	40 (58%)	9 (38%)	182 (48%)	174 (60%)
Household size, median (range) [b]	6 (3-200)	5 (2-8)	6 (1-50)	6 (1-20)
Crowding [c]	34 (49%)	8 (33%)	137 (36%)	101 (35%)
Recent typhoid fever in the household	11 (16%)	3 (13%)	23 (6%)	27 (9%)
No use of soap for hand washing	49 (71%)	15 (63%)	214 (57%)	183 (63%)
No toilet in household	15 (22%)	5 (21%)	33 (9%)	38 (13%)
Eating food from street vendors	22 (32%)	13 (54%)	85 (23%)	59 (20%)
Consumption of iced drinks	17 (25%)	5 (21%)	51 (14%)	62 (22%)
Consumption of ice cubes	45 (65%)	14 (58%)	176 (47%)	131 (45%)
Sharing food from same plate	31 (45%)	7 (29%)	102 (27%)	101 (35%)
Eating with hands	33 (48%)	11 (46%)	121 (42%)	164 (43%)
Drinking water: piped water	7 (10%)	2 (8%)	77 (20%)	42 (15%)
Faecal contamination of drinking water source [d]	30 (48%)	11 (55%)	192 (56%)	125 (54%)
Flooding	26 (38%)	14 (58%)	79 (21%)	99 (34%)

a: Defined as below the median monthly income of the community controls (900,000 Rupiah [US $105]).

b: Includes 2 outliers: an orphanage with 200 individuals and a dormitory with 50 individuals in the typhoid cases and community controls, respectively.

c: Defined as more than the median number of household members of community controls (median, 6)

d: Water samples obtained from 62 typhoid and 20 paratyphoid cases, 341 community and 233 fever controls.

Table 2. Bivariate analysis of risk factors for typhoid and paratyphoid fever in comparison with community controls and fever controls

Risk factor	**Odds ratio (95% Confidence interval)**			
	Typhoid fever		**Paratyphoid fever**	
	Community controls	**Fever controls**	**Community controls**	**Fever controls**
Female sex	1.09 (0.65-1.84)	1.78 (1.05-3.04)	0.48 (0.20-1.11)	0.78 (0.33-1.83)
Low family income	1.49 (0.88-2.50)	0.91 (0.54-1.55)	0.65 (0.28-1.51)	0.40 (0.17-0.94)
Crowding	1.71 (1.02-2.86)	1.81 (1.06-3.07)	0.88 (0.37-2.11)	0.93 (0.39-2.25)
Recent typhoid in the household	2.93 (1.36-6.32)	1.84 (0.86-3.92)	2.21 (0.61-7.94)	1.39 (0.39-4.95)
No use of soap for hand washing	1.88 (1.07-3.28)	1.42 (0.80-2.52)	1.28 (0.55-2.99)	0.97 (0.41-2.28)
No toilet in household	2.90 (1.48-5.70)	1.84 (0.94-3.57)	2.75 (0.97-7.85)	1.74 (0.61-4.93)
Eating food from street vendors	1.61 (0.92-2.83)	1.83 (1.02-3.26)	4.07 (1.76-9.42)	4.61 (1.96-10.81)
Consumption of iced drinks	2.10 (1.13-3.90)	1.20 (0.65-2.22)	1.69 (0.60-4.72)	0.96 (0.35-2.68)
Consumption of ice cubes	2.15 (1.26-3.68)	2.26 (1.31-3.91)	1.61 (0.67-3.71)	1.69 (0.73-3.93)
Sharing food from same plate	2.21 (1.31-3.74)	1.52 (0.89-2.59)	1.11 (0.45-2.77)	0.77 (0.31-1.91)
Drinking water: piped water	0.44 (0.19-1.01)	0.66 (0.29-1.55)	0.36 (0.08-1.54)	0.54 (0.12-2.36)
Faecal contamination of drinking water source	0.73 (0.42-1.25)	0.81 (0.46-1.42)	0.95 (0.38-2.35)	1.06 (0.42-2.64)
Flooding	2.29 (1.33-3.95)	1.16 (0.67-2.00)	5.30 (2.27-12.38)	2.69 (1.15-6.27)

poorer handwashing hygiene, had fewer toilets and connections to the water mains in their houses, shared food more frequently, were more likely to consume iced drinks, and were more likely to report flooding than were community controls (**Table 1**).
In addition, for all interviewed participants, low income was significantly associated with purchasing food from street vendors (OR, 1.58; 95% CI, 1.03-2.41). When ice cubes were used, these were purchased from ice vendors by equal proportions in the groups: 41 (69%) patients with typhoid or paratyphoid fever, 107 (61%) community controls, and 93 (71%) fever controls (P = .12).

Bivariate Analysis

Risk Factors for Typhoid Fever: Bivariate analysis of risk factors comparing typhoid cases with community controls showed the following significantly associated risk factors for typhoid fever: crowding (>6 household members) and recent typhoid fever of household contacts (**Table 2**). The association of recent typhoid fever of household contacts and typhoid fever also remained significant in a subgroup of households with more than 6 household members: from the 34 typhoid cases, 8 (24%) reported recent typhoid fever in a household contact, whereas from 137 community controls, 9 (7%) did (OR, 4.38; 95% CI, 1.54-12.40). In the comparison with community controls, other significantly associated risk factors for typhoid fever were no use of soap for handwashing, no toilet in the household, and flooding. With respect to eating habits, typhoid was not significantly associated with eating food from street vendors, but a significant association was found with consuming iced drinks, use of ice cubes, and sharing food from the same plate. Sharing of food occurred mostly with household contacts: 84% (26/31) of typhoid cases and 84% (85/101) of community controls and in lower frequencies in all groups at work or school.
Female sex was associated when typhoid cases were compared with fever controls, which was likely due to the overrepresentation of males in the fever control group (**Table 2**).
In the fever-control comparison crowding was associated with typhoid fever, as was eating foods from street vendors and use of ice cubes. None of the hygiene-related risk factors (i.e., no use of soap for handwashing, no toilet in the household) was significantly associated with typhoid in comparison with fever controls.

Risk Factors for Paratyphoid Fever: In comparison with community controls and fever controls, paratyphoid fever among cases was significantly associated with eating foods from street vendors and flooding. Fever controls had a lower family income than did patients with paratyphoid fever.
Water Examination: During the study period, 656 samples from the sources of running drinking water of cases and controls were collected; 358 (55%) contained fecal coliforms (median, 30; IQR, 6-250 per 100 mL). Fecal contamination of drinking water was not

significantly associated with either typhoid or paratyphoid fever in comparison with both control groups (**Table 2**). Also, bacterial numbers in water samples were not significantly different for typhoid or paratyphoid fever cases vs those for fever controls (P = .54 and P = .90, respectively, by Mann-Whitney U test) or community controls (P = .43 and P = .95, respectively). All respondents reported that they boiled drinking water before consumption and that they kept water boiling for several minutes.

Food Handlers: A food handler was not present in all households of cases or controls because some cases and controls always ate outside of the household or cooked their own food. No *S. typhi* or *S. paratyphi* A were isolated in the single stool samples that could be obtained from 96% of the 78 food handlers of (para)typhoid cases, 246 of the fever controls, and 298 of the community controls, respectively.

Multivariate Analysis

Residence of participants in 1 of the 8 subdistricts was not evaluated in the bivariate analysis, but was included in the multivariate analysis as a potential confounder. In this analysis, neighborhood residence was not independently associated with either typhoid fever or paratyphoid fever. The significant risk factors for typhoid and paratyphoid fever from the bivariate analysis that were evaluated in the multivariate analysis are shown in **Table 3.**

Risk Factors for Typhoid Fever: Using the community control group, typhoid fever continued to be independently associated with hygienic practices (no use of soap for handwashing, sharing of food, and no toilet in the household) and recent intrahousehold typhoid fever in the preceding 12 months. These are presented in order of decreasing magnitude of attributable risk (**Table 3**). Typhoid cases were significantly younger than community controls, suggesting that either exposure to *S. typhi* or susceptibility to symptomatic infection when exposed is greater among young people.

Using the fever controls for comparison, we identified ice cubes and female sex (related to the high percentage of male participants in the fever control group) as independent risk factors for typhoid fever. Hygiene-related factors were not independently associated.

Risk Factors for Paratyphoid Fever: In the multivariate analysis, paratyphoid fever continued to be independently associated with eating foods from street vendors when paratyphoid cases were compared with both control groups (**Table 3**). Flooding also remained a significant risk factor for paratyphoid fever. The individual contribution of eating habits and flooding as calculated by the attributable risk alternated in importance for both control groups. Low income was inversely associated with paratyphoid fever in the comparison with fever controls.

Table 3. Multivariate analysis of independent risk factors for typhoid and paratyphoid fever in comparison with community controls and fever controls

	Typhoid fever (n=69)		Paratyphoid fever (n=24)	
Risk factor	**OR (95% CI)**	**Attributable Risk, %**	**OR (95% CI)**	**Attributable risk, %**
Comparison with community controls (n=378)				
No use of soap for handwashing	1.91 (1.06-3.46)	34	NA	
Sharing food from same plate	1.93 (1.10-3.37)	22	NA	
No toilet in household	2.20 (1.06-4.55)	12	NA	
Recent typhoid in household	2.38 (1.03-5.48)	9	NA	
Young age	0.96 (0.94-0.98)		0.99 (0.96-1.02)	
Flooding	1.65 (0.88-3.08)		4.52 (1.90-10.73)	45
Eating food from street vendors	NA		3.34 (1.41-7.91)	38
Use of iced drinks	1.12 (0.55-2.26)		NA	
Consumption of ice cubes	1.34 (0.73-2.44)		NA	
Crowding	1.54 (0.88-2.72)		NA	
Comparison with fever controls (n=289)				
Consumption of ice cubes	2.27 (1.31-3.93)	36	NA	
Female sex	1.79 (1.04-3.06)	26	1.10 (0.43-2.84)	
Low income	0.85 (0.49-1.49)		0.28 (0.11-0.71)	49
Eating food from street vendors	1.62 (0.88-2.98)		5.17 (2.12-12.60)	48
Flooding	NA		3.25 (1.31-8.02)	42
Crowding	1.60 (0.92-2.76)		NA	

Abbreviations: CI, confidence interval; OR, odds ratio.
NA: not significantly associated in the bivariate analysis and not included in the multivariate analysis.

Comment

The main finding of this study is that in Jatinegara, Jakarta, typhoid and paratyphoid fever largely follow distinct routes of transmission. Typhoid is spread predominantly within the household, whereas paratyphoid is mainly transmitted outside the home. No fecal carriers among food handlers in the households were detected and there was no association between the level of contamination of drinking water and either typhoid or paratyphoid fever. Apparently, *S. typhi* is introduced into households by convalescent cases transiently excreting the bacterium. Consistent with this, independent risk factors for the intrahousehold spread of typhoid were poor handwashing hygiene and sharing of food from the same plate. On the other hand, risk factors for transmission of paratyphoid were outside the household (i.e., flooding, consumption of foods from street vendors). Furthermore, in this community-based passive surveillance study, paratyphoid comprised 23% of all enteric fever cases, an apparent rise in relative incidence of paratyphoid compared with earlier studies.

To reach the conclusion concerning the distinct route of transmission of paratyphoid and typhoid fever, we compared characteristics of cases with those of community controls and fever controls. Some potential pitfalls that may affect complete recruitment of patients in the area, and individual classification of cases and fever controls, need to be considered. Not all eligible fever patients might have been included, although we performed blood cultures free of charge to preclude economic barriers for inclusion. Self-treatment with over-the-counter antibiotics and an atypical presentation of enteric fever (e.g., as observed in young children) may have influenced inclusion. [18] Even so, the proportional representation of typhoid fever of 8.6% of illnesses with fever for 3 or more days is comparable with rates in other active and passive surveillance studies for typhoid fever using the same inclusion criteria (4.6%-8.5%). [19-23] Furthermore, the sensitivity of the microbiological methods never reaches 100%. [24] However, because most patients with fever were included in the first week of illness, the sensitivity of blood culture comes close to that of quantitation in bone marrow and is superior to the Widal test. [25,26] Also, the interference of antibiotics, which can yield false-negative results, was limited due to this short period before inclusion and to the antibiotic-neutralizing resins in the blood culture vials. Accordingly, equal proportions of typhoid and paratyphoid fever cases and non-enteric fever controls had previously taken antibiotics. To further minimize misclassification of fever controls, stool cultures were performed 3 to 4 weeks after blood culture (i.e., at a time when bacteria may still be excreted in feces of patients with typhoid or paratyphoid fever). The 2 febrile patients with negative blood culture results at inclusion, whose stool cultures yielded *S. typhi* and *S. paratyphi* A, were accordingly excluded from the analysis. Another potential limitation of this study concerns the screening for *Salmonella* carriers by a single stool culture that might not suffice because of intermittent excretion of the bacteria in stools. [12]

The use of a representative community control group allowed us to determine the prevalence of risk factors in the whole population at risk. Our study demonstrates that risk estimates from case-control studies could be affected by the selection of the control-group used for comparison. For instance, when typhoid fever cases were compared with community controls, most of the independent risk factors for typhoid fever were intrahousehold factors (i.e., no use of soap for handwashing, sharing of food, and recent typhoid fever in a household member), whereas those factors were not associated in the comparison with fever controls. This suggests that hygiene practices of both cases and fever controls were of a standard below that of community controls. In addition, partially overlapping routes of transmission of typhoid fever and other febrile illnesses could be interdependent and result in the demonstrated similar intrahousehold risk profile of typhoid fever cases and fever controls with similar socioeconomic characteristics.

Food obtained from street vendors was a likely vehicle for extrahousehold transmission

of paratyphoid fever because it contributed significantly to transmission in contrast to hygiene-related risk factors. This is consistent with the notion that multiplication of paratyphoid bacteria in food is required to reach a number sufficient to cause disease. Street vendors have only limited facilities for cooled storage of foods and for washing of hands, foods, and dishes. The low hygienic standards could therefore contribute not only to the transmission of paratyphoid fever but of other foodborne diseases such as typhoid, as well. [7,11,27-29] Due to the Asian economic crisis starting in 1997, the expanding urban population became even more dependent on inexpensive food obtained from street vendors, which may explain the relatively high proportion of paratyphoid fever in enteric fever in Jakarta. Low-income groups more frequently ate food obtained from street vendors than did individuals with high income, but all income groups who purchase food from street vendors may be at risk.

In contrast to the largely extra-household transmission of paratyphoid fever, typhoid fever was more of an intrahousehold affair introduced by recent typhoid cases in the households and facilitated by poor hand-washing hygiene and sharing of food from the same plate, consistent with an earlier report. [10] The association of poor handwashing hygiene and typhoid fever was shown before in Indonesia and India. [6,9,11] A recent review stressed the importance of the use of soap for the reduction of the incidence of diarrheal diseases. [30] In our study we also identified a significant association between not using soap for handwashing and all febrile illnesses (OR, 1.40; 95% CI, 1.05-1.88). The combination of poor handwashing hygiene, eating with hands, and sharing food from the same plate can understandably facilitate transmission of typhoid, but apparently the infective dose to allow transmission of paratyphoid is only infrequently met. Because we observed no intrahousehold outbreaks and detected no fecal carriers among the food handlers in the households of cases, intrahousehold person-to-person spread through convalescent patients observing poor hygiene seems a more likely scenario than transmission by chronic carriers among food handlers in households.

Apart from the above-mentioned risk factors, some additional observations should be considered. First, the total number of interviewed patients with typhoid and paratyphoid fever in our study was limited, which may have influenced the statistical power of the analysis, especially in small subgroups, and the demonstrated associations of specific risk factors. Second, food purchased from street vendors could be implicated as a vehicle for transmission of typhoid as well, as shown in the bivariate analysis. Also, the consumption of ice cubes obtained from street vendors might expose clients to *Salmonella* bacteria because these bacteria can survive in ice. [31] Another extrahousehold location of acquisition of typhoid fever could be public toilets, which generally lack handwashing facilities. Third, there was an association between flooding and paratyphoid fever. Two hypotheses may explain this association: flooding could introduce bacteria from

contaminated surface water into sources of drinking water. However, since most cases of typhoid and paratyphoid fever occurred during the dry season, flood-related waterborne transmission seemed not to play a major role. Alternatively, flooding may be an income-associated geographic marker that coincides with the distribution of carriers among food vendors in the area. This could also explain the clustering of paratyphoid fever cases in some regions, but since community controls were nonmatched for subdistrict neighborhood residence, this assumption could not be verified. Finally, although a considerable proportion of the sources of drinking water contained fecal coliforms that were used as indicator organisms, contamination itself was not associated with enteric fever. Dilution of *S. typhi* or *S. paratyphi* in water might generate too low a dose to infect partially immune residents. More likely, however, the entrenched habit of boiling drinking water from the water mains or groundwater pumps explains the lack of an association between water contamination and enteric fever and should certainly be continued to prevent possible outbreaks of disease, in combination with proper storage of boiled water to prevent domestic contamination.

In conclusion, the present findings suggest that public health policies for control of typhoid and paratyphoid fever in Jakarta should focus on hygiene education as well as monitoring of the street-food trade, although such strategies would have to be tested in intervention trials to prove their value. First, instruction on proper handwashing hygiene using soap could reduce the overall incidence of infectious diseases in Jakarta and especially preclude transmission of typhoid fever among contacts of cases. Second, prevention of bacterial contamination of street food and ice cubes could contribute to containment of enteric fever, paratyphoid in particular. Follow-up of enteric fever cases, especially among food vendors, should be prioritized to reduce the role of transient or chronic carriers in the foodborne transmission.

If vaccination were to be considered as a means of controlling typhoid, an individualized approach rather than mass vaccination (i.e., targeted vaccination of young household contacts of cases) may be a cost-effective approach when public health resources are scarce.[32] But, because of the increasing incidence of paratyphoid fever in Jakarta, as well as readily available antibiotic treatment and the potentially effective intervention of education to increase appropriate handwashing, mass immunization programs for typhoid fever in Jakarta may not be appropriate at this time.

Acknowledgment

We thank J.P. Vandenbroucke, MD, PhD, from the Leiden University Medical Centre (LUMC) Department of Clinical Epidemiology and Nico Nagelkerke, PhD, from the LUMC Department of Medical Statistics for critically reviewing the document and Wil Dolmans, MD, PhD, for his help in the preparatory stage of this project. We thank the physicians, nurses, and technicians of the participating health centers in Jakarta for their cooperation: Mitra Internasional hospital and microbiology laboratory, Budhi Asih, St Carolus and Persahabatan hospital, all *puskesmas* in Jatinegara, the local private practitioners, as well as the microbiology department of Atma Jaya University Hospital and Nusantara Water Centre, West Jakarta. This study could not have been done without our research assistants: Billy Hunsinger, Ferry Kandaw, Rinny Listyani, Meily, Vea Noveria, Carmelita Ridwan, Min Ali Sugiharto, Lidwina Sutikno, Mariana Tasman, and Lily Yaputra.

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3

Risk factors for transmission of food borne illness in restaurants and street vendors in Jakarta, Indonesia

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Epidemiology and Infection 2004, 132: 863-872

3 Abstract

In a previous risk factor study in Jakarta we identified purchasing street food as an independent risk factor for paratyphoid fever. Eating from restaurants, however, was not associated with disease.

To explain these findings we compared 128 street food vendors with 74 food handlers from restaurants in a cross-sectional study in the same study area. Poor hand-washing hygiene and direct hand contact with foods, male sex and low educational level were independent characteristics of street vendors in a logistic regression analysis. Faecal contamination of drinking water (in 65% of samples), dishwater (in 91%) and ice cubes (in 100%) was frequent. Directly transmittable pathogens including *S. typhi* (n=1) and non-typhoidal *Salmonella* spp. (n=6) were isolated in faeces samples in 13 (7%) vendors; the groups did not differ, however, in contamination rates of drinking water and *Salmonella* isolation rates in stools.

Poor hygiene of street vendors as compared to restaurant vendors, in combination with faecal carriage of enteric pathogens including *S. typhi*, may help explain the association found between purchasing street food and food borne illness, in particular *Salmonella* infections.

Public health interventions to reduce transmission of food borne illness should focus on general hygienic measures in street food trade, i.e., hand-washing with soap, adequate food handling hygiene, and frequent renewal of dishwater.

Introduction

In a previous case-control study in Jakarta, Indonesia, we identified purchasing foods from street vendors as an independent risk factor for (para)typhoid fever, whereas no such association was found with eating in restaurants. [1] Similarly, in other studies in Indonesia street food was associated with typhoid fever. [2,3] Several factors may explain this association of street food and (para)typhoid fever, a systemic febrile illness caused by *Salmonella typhi* and *S. paratyphi* A,B or C that only affects humans. For instance, personal hygiene and knowledge of hygienic food preparation [4-6], faecal contamination of basic ingredients or water used for food preparation [7] and/or isolation rates of enteric pathogens [8], may differ between street food vendors and vendors in restaurants. Although the possible transmission routes of enteric pathogens like *Salmonella* are well-known, the relative importance of the various factors, i.e., the weak link in the transmission chain, is uncertain but of great importance to help focus the most relevant health intervention.

We therefore examined determinants for transmission of enteric pathogens in commercial food handling in a cross-sectional study in Jakarta. Because of our previous findings in the same area we compared street vendors with vendors from restaurants. In both groups of food handlers we determined faecal isolation rates of enteric pathogens including *Salmonella* spp., assessed the hygiene practices and knowledge about safe food preparation and examined water reservoirs and ice cubes used for consumption. Our findings should be helpful to health authorities for the development of effective methods for the containment of food borne diseases in commercial food handling especially in food stalls and pushcarts.

Material and methods

Study population: From 17 February until 21 May 2003 all food vendors working in the Bidara Cina sub-district in East-Jakarta were approached by graduated medical school students. During the study period the study area was visited daily, during daytime and evenings, until all present food vendors were interviewed. This area of 126 hectares houses 43 829 inhabitants (December 2002) and has been subject to a typhoid fever risk factor study as described elsewhere. [1] Ethical clearance was obtained from the Indonesian National Institute of Health Research and Development (*Litbangkes*) and the local provincial authorities. A written informed consent was obtained from all food vendors.

A study subject was defined as an individual working as a vendor of foods or drinks in the study area who was physically involved in the preparation or handling of foods. All types of units were eligible for inclusion: restaurants, food stalls, and pushcarts. Some

restaurants and *warung* (i.e., small-scale restaurants often connected to the household of the owner) are subject to six-monthly visits by local health authorities for inspection and education on food hygiene, but food hawkers are not visited. Food stalls are stationary roadside facilities with or without seats. Pushcarts are mobile units that lack seating facilities.

Questionnaires: A standardized questionnaire was used to obtain data on demographic and socio-economic characteristics of the food vendors, recent disease history, hygiene practice, and water sources in the units. Measure of hygiene that were assessed were: defecation during working hours, hand washing before food preparation and after defecation, the use of soap for hand washing, direct hand contact with food items, available water sources for hand washing and dishwashing, the use of soap for dishwashing and the frequency of renewal of dishwater, and the presence of flies on food items. Diarrhoea was defined as three or more loose stools per day. During and following the interview (i.e., a period of in total 30 minutes) the interviewers observed the hand washing hygiene and food handling of the vendors to compare the given answers with the actual practice. Any reported use of soap was verified by screening for the presence of soap in the unit. Knowledge about safe food preparation was tested by a scoring system. Eight diseases were mentioned: diarrhoea, typhoid fever, jaundice, worm infections, pneumonia, skin infections, AIDS, and tuberculosis. Vendors were asked whether these illnesses could be transmitted by food. Also knowledge about vehicles for disease transmission in food processing was tested: i.e., flies, dirty hands, polluted water, cutting boards, traffic fumes, and ill food handlers. For every correct answer one point was given, no point if the answer was not known, and one point subtracted for an incorrect answer.

Sample collection: At every location 150 mL samples were collected from the water source or container with drinking water and dishwater. If piped water was sampled, the bactericidal effect of chlorine during transport was neutralized by addition of 0.1 mL 10% sodium-thiosulphate. Ice cubes (150 mL) were collected from cool boxes into sterile bottles. Two stool samples were collected: two gram of faeces into a vial with Cary Blair transport medium for bacteriological examination and ten gram of fresh stool for parasitological examination.

Water examination: The samples were transported on ice, processed within six hours after collection and examined for total and faecal coliform counts by use of Most Probable Number method. [9] Serially diluted water samples were incubated in Endolactose broth and Brilliant Green to detect specific colour changes and gas formation. Presence of faecal coliforms (>1 MPN Index / 100 mL) was defined as faecal contamination. [9] The upper detection limit was 1600/100 mL.

Stool cultures: Stool samples were cultured in the central reference lab using Selenite enrichment broth (Oxoid Ltd, Hampshire, England). Colonies were plated on xylose-

lysine-desoxycholate, *Salmonella Shigella* agar, and on Triple Sugar Iron agar, SIM Medium (sulphide and indole production and motility) and Simmons Citrate (Oxoid Ltd, Hampshire, England). *Salmonella* bacteria were identified using agglutination anti-sera (Polyvalent, O-9, Vi, h, paratyphi A; Murex Biotech Ltd., Dartford, England) and biochemical tests (Microbact: Medvet Diagnostics, Adelaide, Australia).

Parasitologic stool examination: The second stool sample was processed within 24 hours after collection and microscopically examined after lugol staining, Kato Katz technique, and Harada Mori method for the detection of hookworms.

Feedback: Food vendors were informed about their water quality, instructed on safe food preparation methods, and if necessary treated (worm infections: mebendazole, *Giardia lamblia*: metronidazole). When *Salmonella* was isolated in stool cultures, vendors were subject to follow-up and treatment was administered in case of repeated positive stool cultures.

Statistical methods: Data was entered twice in EpiInfo 6.04 (CDC, Atlanta, USA), validated and imported in SPSS (SPSS Inc., Chicago, IL, USA) for analysis. T-tests were used for evaluation of normally distributed numerical variables and Mann Whitney U-tests for not-normally distributed numerical variables. Proportions within the group of street food vendors and within the group of vendors from restaurants or *warung* were compared using Chi square tests (χ^2). Measures for association were expressed as odds ratios (OR) with their respective confidence limits (95%-CI) for categorical exposures. To control for confounding a multivariate analysis was performed on the significantly associated risk factors from the bivariate analysis in a logistic regression model by forward likelihood ratio test. For the comparison of hygiene parameters between the two groups we depended on the self-reported methods of hand-washing hygiene after defecation, but not all food vendors reported to defecate during working hours (e.g., due to non-availability of facilities, limited working hours per day, or to business activity). Hygiene parameters were consequently evaluated by multivariate analysis for all food vendors, and additionally in the sub-group of subjects who told to defecate during working hours to confirm overall trends. Significance levels were p-values < 0.05.

Results

Study population: In total 238 food vendors were found to be working in the study area. From these 202 food vendors (85%) were interviewed. Thirty-six food vendors refused participation: 6 worked in restaurants, 13 worked in *warung*, and 17 worked in roadside stalls or pushcarts. Stool specimens could be collected from 175 of the 202 vendors; 27 (13%) refused a sample. We also collected 139 drink water samples from the 149 vendors who offered drinking water to customers, and 172 dishwater samples. The age of food

Table 1. Characteristics of food vendors

Variables	Selling unit			
	Restaurant	*Warung*	Food stall	Pushcart
n	11	63	110	18
Sex:				
- Male	10 (91%)	15 (24%)	76 (69%)	18 (100%)
- Female	1 (9%)	48 (76%)	34 (31%)	0
Age: median years (IQR)	30 (24-37)	40 (35-47)	39 (30-44)	34 (30-46)
Finished education:				
- Primary school or less	4 (36%)	33 (52%)	70 (64%)	14 (78%)
- Secondary school	7 (64%)	30 (48%)	40 (36%)	4 (22%)
Time working as food vendor:				
- Median (IQR) years	6 (0-18)	5 (2-8)	5 (1-13)	9 (5-20)
Number of customers/day:				
- ≤ 50 customers	9 (82%)	48 (76%)	70 (64%)	5 (28%)
- > 50 customers	2 (18%)	15 (24%)	40 (36%)	13 (72%)
Ownership of the unit:				
- Self owned by respondent	2 (18%)	46 (73%)	93 (85%)	13 (72%)
- Family, rented or employee	9 (82%)	17 (27%)	18 (15%)	5 (28%)
Daily sales [a]**:**				
- ≤ 100 000 Rp	1 (10%)	33 (53%)	65 (59%)	12 (67%)
- > 100 000 Rp	9 (90%)	29 (47%)	45 (41%)	6 (33%)

a: Missing data: one food vendor from a restaurant and one from a *warung*, Exchange rate: 9 400 Rupiah = US $ 1 (June 2004).
IQR, interquartile range

Table 2. Food supply

Variables	Selling unit			
	Restaurant	*Warung*	Food stall	Pushcart
n	11	63	110	18
Number of sold items	2-87	1-35	1-10	1
Sold foods and drinks:				
- Rice dishes	7 (64%)	46 (73%)	42 (38%)	-
- Noodle dishes	5 (46%)	13 (21%)	14 (13%)	5 (28%)
- Meat dishes	10 (91%)	41 (65%)	52 (47%)	1 (6%)
- Seafood and fish	4 (36%)	35 (56%)	24 (22%)	1 (6%)
- Boiled and fresh vegetables	5 (46%)	48 (76%)	27 (25%)	2 (11%)
- Fried snacks	-	6 (10%)	17 (16%)	2 (11%)
- Fruit juices	7 (64%)	15 (24%)	14 (13%)	-
- Es *cendol* or *es cincau* [a]	3 (27%)	1 (2%)	6 (6%)	4 (22%)

a: Iced flavoured coconut milk with insoluble flour particles or leave extracts.

vendors ranged from 18-68 years, no significant difference in age between vendors from the four units was found (p = 0.11, ANOVA). Vendors in *warung* were significantly more often female (p < 0.001, χ^2) (**Table 1**).

Education level of the group of vendors from stalls and pushcarts was lower than that of vendors in restaurants and *warung* (p = 0.03, χ^2) (**Table 1**). For 95% of the respondents food vending was a fulltime economic activity during six or seven days a week. Mobile vendors proportionally served most customers per day: 72% served more than 50 customers a day. The small-scale entrepreneurs in food stalls and pushcarts tend to specialize in food items which limits their supply to a few or single items (**Table 2**).

Hygiene in the grouped units: Seventy (55%) of the vendors from food stalls and pushcarts did not wash their hands before food preparation as compared with 21 (28%) of the vendors in restaurants/*warung* (p < 0.001) (**Table 3**). Non-use of soap for hand-washing before food preparation was reported in 79% vs. 51%, respectively (p = 0.002). Although all vendors reported to wash their hands after defecation during working hours, non-use of soap occurred significantly more frequent in stalls and carts than in restaurants/*warung* (37% vs. 10%, p< 0.001). Direct hand contact with ready-to-eat foods occurred more often in food stalls and pushcarts (63% vs. 36%, p < 0.001). The limited facilities for hand- and for dishwashing were demonstrated for 86% of the pushcarts and stalls and 58% of the *warung* and restaurants, because the same water reservoir was used for both purposes (p = 0.01). Vendors reported to renew the dishwater in buckets 0-20 times during working hours with the lowest mean frequency in the food stalls and pushcarts (3.1 vs. 6.2, p < 0.001). In restaurants/*warung*, flies on ready-to-eat foods were observed more often (p = 0.01) and ice cubes were used more often (p < 0.001). Refrigerators for storage of ready-to-eat foods were lacking in 99% of the *warung*, food stalls and pushcarts and 54% of the restaurants.

Knowledge of safe food preparation and recent illness: The score for the knowledge of safe food preparation (maximum score: 14) was not significantly different between the two groups of units (mean score: 5.0 and 5.5 for stalls/pushcarts and restaurants/*warung*, respectively: p = 0.15, t-test). Vendors most frequently indicated diarrhoea (89% of the vendors) and least frequently AIDS (6%) as food borne illness. Ninety-one percent of the vendors from food stalls and pushcarts and 93% from restaurants and *warung* were aware that diarrhoeal diseases could be transmitted by hands (p = 0.52, χ^2). In the 30 days prior to the interview 24% of the vendors reported to have suffered from fever, and 23% of the vendors told that they had experienced at least one diarrhoeal episode in the preceding three months. The isolation rate of enteric pathogens and occurrence of diarrhoea in the preceding three months was not correlated (p = 0.35, χ^2). The reported occurrence of diarrhoea did not differ between the two groups (p = 0.19): OR 0.64 (95%-CI 0.33-1.25) (**Table 3**).

Table 3. Comparison of hygiene parameters between two groups of food vendors: bivariate analysis

Variable [a]	Food stalls and pushcarts	Restaurants and *warung*	OR (95% CI)	p
- **n** (202)	128	74		
Hand-washing hygiene:				
- No use of soap for hand washing after defecation (n=74 vs 63) [b]	27 (37%)	6 (10%)	5.46 (2.08-14.33)	< 0.001
- Not washing hands before food preparation (n=128 vs 74)	70 (55%)	21 (28%)	3.05 (1.65-5.63)	< 0.001
- No use of soap if washing hands before food preparation (n=58 vs 53)	46 (79%)	27 (51%)	3.69 (1.61-8.49)	0.002
- Direct hand contact with ready-to-eat food (n=128 vs 74)	80 (63%)	27 (36%)	2.90 (1.60-5.25)	< 0.001
Dishwater:				
- Dishwater is used for washing hands (n=36 vs 31) [c]	31 (86%)	18 (58%)	4.48 (1.37-14.63)	0.01
- Mean number of times dishwater is renewed per day (range)	3.1 (0-15)	6.2 (1-20)		< 0.001
Other factors:				
- Use of ice cubes (n=128 vs 74)	62 (48%)	63 (85%)	0.16 (0.08-0.34)	< 0.001
- Flies on food items (n=127 vs 73)	7 (6%)	12 (16%)	0.30 (0.11-0.79)	0.01
- Diarrhoea last 3 months (n=128 vs 74)	26 (20%)	21 (28%)	0.64 (0.33-1.25)	0.19

a: Number of vendors from stalls/pushcarts versus restaurants/warung available for analysis

b: n = 137: only those vendors who reported to defecate during working hours.

c: n = 67: only those vendors who washed utensils/dishes and/or hands before food preparation in buckets.

Table 4. Comparison of water examination results between two groups of food vendors: bivariate analysis

Variable [a]	Food stalls, pushcarts	Restaurants, *warung*	OR (95% CI)	p
Water examination:				
- Faecal contamination of sampled drinking water (n=67 vs 72)	40 (60%)	50 (69%)	0.65 (0.32-1.31)	0.23
- Median faecal coliform count in drinking water [b] (n=40 vs 50)	34 (13-105)	46 (19-1075)		0.12
- Faecal contamination of sampled dishwater (n=102 vs 70)	95 (93%)	62 (89%)	1.75 (0.60-5.07)	0.30
- Median faecal coliform count in dishwater [b] (n= 95 vs 62)	425 (33-1600)	39 (20-900)		0.006

a: Number of vendors from stalls/pushcarts versus restaurants/*warung* available for analysis.

b: Median (IQR) MPN index /100 mL, comparison of numbers by Mann Whitney U-test.

Table 5: Results of the stool examination (n=175)

Enteric pathogen	Food stalls and pushcarts (n=110)	Restaurants and *warung* (n=65)	Total
Non-typhoidal *Salmonellae*	4 (4%)	2 (3%)	6 (3%)
Salmonella typhi	1 (1%)	0	1 (0.6%)
Hookworms	32 (29%)	14 (22%)	46 (26%)
Trichuris trichiura	26 (24%)	13 (20%)	39 (22%)
Ascaris lumbricoides	3 (3%)	5 (8%)	8 (5%)
Giardia lamblia	2 (2%)	1 (2%)	4 (2%)
Entamoeba histolytica/dispar	2 (2%)	0	2 (1%)

Pathogens were isolated in 86 individuals.

Examination of drinking water: Drinking water sources were bottled water (2), piped water (49), and groundwater extracted by pumps (98). Fifty-three food handlers did not serve drinking water. All respondents reportedly boiled drinking water before storage and serving. The majority of vendors (129, 88%) kept the boiled water in closed plastic jars, jerry-cans or kettles, while 18 vendors (12%) kept it in open containers such as buckets or pans. In the latter case utensils had to be immersed to collect the water from the reservoirs. Of the 139 examined samples 90 (65%) contained faecal coliforms with median 39 (IQR 17-450)/100 mL in the contaminated samples. The location ($p = 0.23$, χ^2), the storage method (i.e., closed or open container) ($p = 0.82$), or the source (pump or piped water) ($p = 0.39$) did not significantly influence the contamination rate. No significant differences were found in the number of faecal coliforms in the contaminated samples for the two groups of units ($p = 0.12$, Mann Whitney U-test) (**Table 4**). Also, the bacterial numbers in the tap or groundwater samples from either closed or open containers did not differ significantly ($p = 0.64$, Kruskal Wallis test).

Examination of dishwater: In 172 units (i.e., 102 street vendors and 70 restaurants/*warung*) dishwater was present at the location of vending and this was consequently examined; 157 (91%) of the 172 dishwater samples were contaminated with a median faecal coliform count of 140 (IQR 23-1600)/100 mL. The faecal coliform counts in dishwater from stalls and pushcarts were higher than that from the restaurants and *warung* ($p = 0.01$, Mann Whitney U-test) (**Table 4**). The median faecal coliform count in 46 buckets used both for washing hands and dishes was higher than in the 17 buckets only used for dishwashing: 323 (IQR 28-1600) vs. 20 (IQR 15-1600)/100 mL ($p = 0.06$, Mann Whitney U-test). The presence of detergent significantly decreased the number of faecal coliforms in dishwater: median 40 (IQR 17-1600) vs. 900 (IQR 34-1600) /100 mL where soap was absent ($p = 0.005$, Mann Whitney U-test).

Examination of ice cubes: Ice cubes were used in drinks by 125 (62%) of the vendors. We collected 23 ice samples from 3 pushcarts, 14 food stalls, 4 *warung* (two samples at one location) and 1 restaurant. All ice cubes were contaminated, with a median faecal coliform count 500 (IQR 170-1600)/100 mL. Most of the ice cubes had been purchased from ice vendors (70%), but no significant differences in faecal coliform numbers between purchased or self-made ice cubes were observed ($p = 0.15$, Mann Whitney U-test). Fifteen food vendors (68%) collected ice cubes with their hands and seven used tools in cool boxes, but faecal coliform counts did not differ significantly by method of handling ($p = 0.25$, Mann Whitney U-test).

Stool examination: In 86 vendors (49%) pathogens were detected. Directly transmittable pathogens (i.e., *Salmonella* spp., *Giardia*, and *Entamoeba*) were isolated in 13 (7%) (**Table 5**). *S. typhi* was isolated in the stool from a 25 year-old male mobile vendor selling iced flavoured drinks. Two repeated stool cultures in three week-intervals were negative. He

reported not to have suffered from prolonged fever in the preceding six months or from previous typhoid fever. Both *Salmonella* spp. and hookworms were detected in the stools from two food vendors. Faecal carriage of non-typhoidal *Salmonellae* was equally frequent in both groups (p = 0.33): OR 1.19 (0.18-9.65).

Parasitology: Single parasite infestations were detected in the stools of 63 vendors (36%), and dual infestations in 18 vendors (10%) (**Table 5**). The most frequent combination was hookworm infection with *Trichiuris trichura* (n = 12) or with *Ascaris* (n = 3). Two other combinations were *Ascaris* or *Giardia* with hookworms and *Trichiuris* with *Giardia*. Infestation rates of street food vendors (49%) and restaurant/*warung* employees (42%) were non-significantly different (p = 0.63): OR 1.36 (0.73-2.52).

Differences in hygiene parameters between restaurants/*warung* and stalls/carts: All study findings were summarized to compare hygiene parameters of the two groups by bivariate analysis (**Table 3, 4**). Significantly different features in food stalls and pushcarts were poor hand-washing hygiene including less use of soap, direct hand contact with food items, and poor standards of dishwashing with higher median faecal coliform counts in dishwater. In restaurants and *warung* ice cubes were used more often because of the available cooling facilities and/or more frequent supply of drinks, and flies were observed more often on ready-to-eat foods. In a multivariate analysis including only the subjects who reported defecation during working hours (n = 137), independently associated features of food vendors from stalls and carts were not washing hands before food preparation (OR 7.51 [2.44-23.05]), direct hand contact with foods (OR 2.76 [1.04-7.33]), and male sex (OR 7.81 [2.79-21.83]). Also the numerical variable 'frequency of renewal of dishwater' was independently associated with food stalls and pushcarts (OR 0.77 [0.65-0.91]) which means that the lowest frequencies of renewal occurred significantly more often in the latter group. In a multivariate analysis for all vendors (i.e., without the variable of hand-washing hygiene after defecation and without the dishwater examination results, which reduced the number of vendors available for analysis) poor hand-washing before food preparation (OR 4.20 [1.97-8.93]), direct hand contact with foods (OR 2.54 [1.22-5.29]), and male sex (OR 5.45 [2.59-11.48]) remained independently associated, but then also less use of ice cubes (OR 0.25 [0.11-0.57]) and lower educational level (OR 2.35 [1.13-4.88]) were independently associated with food stalls and pushcarts (**Table 6**).

Table 6. Multivariate comparison of vendors from food stalls/pushcarts and vendors from restaurants/warung using logistic regression analysis

Variable	Odds ratio (95% CI)
No hand-washing before food preparation	4.20 (1.97-8.93)
Direct hand contact with foods	2.54 (1.22-5.29)
Use of ice cubes	0.25 (0.11-0.57)
Male sex	5.45 (2.59-11.48)
Low educational level	2.35 (1.13-4.88)

Discussion

This cross-sectional study in Jakarta compared street food vendors with vendors from restaurants to identify specific risk factors for the transmission of food borne illness, in particular (para)typhoid fever, in pushcarts and food stalls that could explain the association of street food and (para)typhoid fever observed in a previous study. The main findings are that one in every twenty-five food vendors excreted *Salmonella* spp. including one *S. typhi* in their faeces, but that isolation rates did not differ between the two groups. Similarly, reported diarrhoeal episodes occurred equally frequent in both groups and drinking water of poor quality was found in all units. Consequently, as possible pathogens are equally prevalent in both groups, other determinants of transmission, such as hygiene, should determine the association of (para)typhoid fever and street food. We demonstrated that infrequent hand-washing, non-use of soap, direct hand contact with foods and inadequate dishwashing hygiene in food stalls and pushcarts – all characteristics that could likely result in bacterial contamination of street food – may help explain the abovementioned association. In addition, the street food vendors had a lower educational level than the other vendors, yet were equally aware of transmission factors. However, that knowledge was not applied to food-handling practice. One reason is that most street vendors are small-scale entrepreneurs with limited (washing) facilities and limited financial resources who tend to compromise food safety for financial issues. [4]

These conclusions depend on the validity of our study design and in this respect some issues should be raised. First, we included all present food vendors in the study area by active search during daytime and evenings until all food vendors were approached. This method of inclusion and the variety of included units in terms of the vended food items provide a reliable representation of food vending units and the Indonesian cuisine. Since the offered food items are prepared in characteristic ways to guarantee an universal taste of specific dishes all over Indonesia, and the preparation occurs in similar conditions (i.e., the same limitations as found in the food stalls and pushcarts), we assume that our findings are representative for food preparation procedures in Indonesia, especially in urban districts of lower socio-economic standards. Second, the prevalence of faecal excretion of *Salmonella* bacteria of four percent is likely an underestimation, because we cultured a single stool sample from every vendor. Multiple stool cultures are advocated to establish carrier rates more definitively, because of the intermittent excretion of pathogenic bacteria in faeces. [10] Indeed, an earlier cross-sectional study in Jakarta found a prevalence of *Salmonella* spp. carriers of 8.4%. [11] The identification of 1 typhoid carrier in 175 individuals (0.6%) from our study is in line with that observed in other regions of endemicity, e.g., in Chile (0.69%). [12] However, the essential issue is not the exact rate of faecal carriage per se but the finding that prevalence of faecal carriage was equal in both groups.

Third, we were unable to examine the direct health risk for consumers of street food, since bacterial contamination of the foods and drinks or basic ingredients was not examined. However, a previous study in Jakarta had demonstrated that beverages and meals are frequently contaminated with faecal coliforms, *Salmonella-Shigella* spp., and *Vibrio cholerae*. [13] As a consequence, we focused on the role of food handlers in the transmission of food borne illness.

Last, the more frequent use of ice cubes and observation of flies on foods in restaurants and *warung* could certainly contribute to transmission of food borne diseases by this group as well. Enteric pathogens can survive freezing [14] and flies have been implicated as vehicles for transmission of food borne diseases. [15-17] The contamination level of ice cubes was not influenced by unhygienic handling in the units, suggesting that contamination may as well originate from the production or transport of the ice cubes by the ice distributors. Although these two risk factors for food borne illness were more prominent in the restaurants and *warung*, the poor hand-washing hygiene and direct contact with foods in food stalls and pushcarts most likely outweigh the two other transmission routes of food borne illness because of a greater probability of a high inoculation size.

From literature it is evident that proper hand washing is one of the most effective measures to control the spread of pathogens in food handling. [18] Greater priority to hand washing with soap should be given, considering the high isolation rates of enteric pathogens and also the poor sanitary conditions in Jakarta. The latter could be concluded from the high prevalence of trichiuriasis and hookworm infections, which is an indirect indicator of unhygienic human waste disposal. Also, in Jakarta bacterial gastro-intestinal diseases such as (para)typhoid fever, shigellosis and Campylobacter infections are endemic. [19] These data imply frequent faecal-oral transmission, probably by inadequate hand washing hygiene. Bacteria can multiply rapidly, particularly when food items are stored in stalls and pushcarts that lack cooling facilities. Therefore, initial contamination of food with low numbers of bacteria as a consequence of improperly washed hands can result in sufficient numbers to cause disease in customers. Food can also be contaminated on soiled dishes or kitchen surfaces, because Gram-negative bacteria can survive on hands, dishes, washing-up sponges, and kitchen surfaces and be transmitted in sufficient numbers to foods. [20-23] The immersion of soiled hands in dishwater, the infrequent use of detergent, and the infrequent refilling of buckets were three factors that generated favourable conditions for survival of pathogens in dishwater and on dishes. Our study also demonstrated that the use of detergent was effective in reducing the bacterial numbers in dishwater.

Next to food as a vehicle for transmission of *Salmonella* infections drinking water might also play a role in Jakarta. More than half of the water samples were faecally contaminated which implies that drinking water sources and human excreta disposal are not fully sepa-

rated. However, contamination rates and levels in the two groups of food vendors did not differ. We are uncertain whether all vendors boiled their drinking water, but boiling water before consumption is not the ultimate safeguard against waterborne diseases, if storage methods and handling are insufficient to prevent contamination. [7,24] However, no recommendations on safe drinking water sources or storage methods could be made on the basis of our data.

Our report should not be interpreted as a plea to stop the street food trade. Street-vended foods are an essential part of the daily diet for low-income groups in Indonesia and its variety allows the uptake of most essential nutrients. Food vending is also an essential economic activity for many low-educated residents. Rather, practical modifications should be introduced to reduce the risk of bacterial contamination of foods and spread of food borne diseases in Jakarta, while nutritional and economic benefits are preserved. [25] First, the presence of carriers among food vendors gives cause for close monitoring of newly diagnosed cases of typhoid and paratyphoid fever among food handlers. Public health authorities should incorporate food stalls and pushcarts in their inspection and education programmes to monitor hygienic food preparation and hand-washing hygiene. In this respect, the distribution of soap, detergent or hypochlorite can be considered as an effective intervention method for the reduction of food borne illness. [7,26] Second, street food vendors should be stimulated to use public pumps or taps from local health centres for the frequent renewal of dishwater. Third, the production, transport and handling of ice cubes merit the attention of public health authorities. Finally, the protection of foods from flies in restaurants and *warung* should be promoted.

Acknowledgements

Funding was obtained through the Royal Netherlands Academy of Sciences and Arts (KNAW). We thank the technicians of the Mitra Internasional Hospital Microbiology Lab, Jakarta, and the technicians of the department of Parasitology of Universitas Indonesia for the examination of the stools samples. We also thank the technicians of the Nusantara Water Centre in West-Jakarta for the water examination. This study could not have been done without our research-assistants.

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4

Polymorphisms in pro-inflammatory genes and susceptibility to typhoid and paratyphoid fever

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Submitted for publication

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Abstract

Host genetic factors are thought to contribute to susceptibility and outcome in infectious diseases. A polymorphism in a pro-inflammatory gene, tumor necrosis factor alpha (*TNFA–308*), was recently found to be associated with susceptibility to typhoid fever. Polymorphisms in other pro-inflammatory genes may also contribute to susceptibility to typhoid fever. We tested this hypothesis in a case-control study in an endemic area in Indonesia. Samples of patients with blood culture-confirmed typhoid fever (n=90) and paratyphoid fever (n=26), and fever controls (n=337), were compared with those of community controls (n=322). In these groups we analyzed polymorphisms in *TNFA* by PCR and RFLP, polymorphisms of *IFNG*, *IL1A*, *IL1B*, *IL1R1*, *TNFRSF1A*, *CASP1* and *CRP* by Sequenom MassArray, and polymorphisms in *IL12B* and *IFNGR1* by fragment length analysis. The *IL1R1* polymorphisms were nearly absent in the Indonesian population. The *TNFA–308* polymorphism was not associated with typhoid fever (OR 0.35 [95%-CI: 0.1-1.0]) in this population. The polymorphisms at *TNFA–238* or in *IFNG*, *IL1A*, *IL1B*, *IL12B*, *TNFRSF1A*, *IFNGR1*, *CASP1* and *CRP* were also not associated with typhoid or paratyphoid fever. We conclude that polymorphisms in pro-inflammatory genes appear not to contribute to susceptibility to typhoid fever and, in view of earlier findings, suggest that the *TNFA–308* polymorphism might be related to severity of established disease rather than to susceptibility per se.

Introduction

Typhoid fever, caused by *Salmonella typhi* and transmitted by fecal-oral route through contaminated food, water and drinks, is a health concern in many developing countries such as Indonesia [1]. Paratyphoid fever, caused by *Salmonella paratyphi A, B* or *C*, has a disease presentation highly similar to that of typhoid fever, but – at least in Jakarta – seems to follow a distinct route of transmission: whereas typhoid fever is spread predominantly within the household, paratyphoid fever is mainly transmitted outside the patient's home [2].
The identification of risk factors and most relevant route of transmission of enteric fever (encompassing both typhoid and paratyphoid fever) are essential for the development of control strategies and allocation of public health resources.
Salmonellae are able to survive and multiply within mononuclear phagocytes of lymphoid follicles, liver and spleen [3]. In reaction to the presence of this facultative intracellular pathogen, the host mounts an immune response. The development of the systemic and local immune response is a complex balancing act, in the course of which host immunological mediators as well as bacterial factors may contribute to tissue damage such as the necrosis of Peyer's patches and thereby complications in severe typhoid fever, i.e., bleeding and bowel perforation [4].
Unlike for some other infectious diseases caused by an intracellular pathogen such as tuberculosis and leprosy, no historical data is available suggesting typhoid fever would have a genetic component. Recently however, an association between single nucleotide polymorphism (SNP) *TNFA –308* and typhoid fever has been reported in Vietnam. Together with HLA-DRB1*0301/6/8 and HLA-DQB1*0201-3, the *TNFA–308*A* allele was thought to be associated with susceptibility to typhoid fever [5]. No association of a second common polymorphism, i.e., *TNFA–238*, and susceptibility to typhoid fever was found [5]. Similar to *TNFA-308*, polymorphisms in and around other genes encoding pro-inflammatory cytokines and their receptors, may also contribute to an individual's susceptibility to enteric fever. Besides Tumor Necrosis Factor-α (TNF-α), also Interferon-γ (IFN-γ), Interleukin-1α (IL-1α), Interleukin-1β (IL-1β), Interleukin-12p40 (IL-12p40), the receptors Tumor Necrosis Factor Receptor 1 (TNFR1), Interferon-γ receptor 1 (IFN-γR1), and IL-1 receptor (IL-1R1), and the IL-1β-activating Caspase-1 (CASP1) and the inflammatory biomarker C-reactive protein (CRP) are involved in many of the cellular and inflammatory processes underlying the immune reaction. Indeed, the *IFNG* SNP +874 influences IFN-γ production [6] and has been found in various studies to be associated with susceptibility to tuberculosis [6-8]. An allele of the CA repeat polymorphism in intron 5 of *IFNGR1* is associated with susceptibility to tuberculosis [9]. In the IL-1 complex *IL1A* SNP -889 in the promoter is associated with juvenile rheumatoid arthritis [10]. *IL1B* SNP -511 in the promoter is associated with amongst others EBV seronegativity [11] and homozygosity of this marker is associated with mortality

from meningococcal disease [12]. *IL1B* SNP +3953 in exon 5 influences IL-1β expression levels [13] and is associated with several inflammatory disorders. The *IL1R1* SNPs A124G and R456R are both in the coding region and have not been studied extensively yet. Associations between polymorphisms in *CASP1* (also known as *ICE*) and disease have not been studied in humans so far, a polymorphism in *Casp1* has however been found to influence salmonella disease resistance in poultry [14]. The human *CASP1* SNP in codon 235 has been extensively validated and has a high percentage of heterozygosity (25%, source: SNP database). The *IL12B* insertion/deletion polymorphism in the promoter has been reported to influence both *IL12B* mRNA expression levels [15] and, as a result, nitric oxide (NO) production [16], this polymorphism is in addition associated with a.o. mortality from cerebral malaria [16] and outcome of hepatitis C virus infection [17]. *TNFRSF1A* SNP +36 is associated with Crohn's disease [18] and familial rheumatoid arthritis [19]. *CRP* SNP +1444 influences CRP production [20] while serum CRP levels were found to be a marker for infection with *Salmonella typhi* [21].

In the present study, to determine whether polymorphisms of *TNFA* and other pro-inflammatory genes are associated with an individual's susceptibility to typhoid fever and paratyphoid fever we assessed the prevalence of the polymorphic alleles in patients enrolled in a community based surveillance. To this end we analyzed the above described polymorphisms in the genes *TNFA*, *IFNG*, *IL1A*, *IL1B*, *IL12B*, *TNFRSF1A*, *IFNGR1*, *IL1R1*, *CASP1* and *CRP* and studied the association of these polymorphisms in a typhoid fever case-control study.

Materials and Methods

Study design and participants

From June 2001 to February 2003 patients with bloodculture-confirmed *Salmonella typhi* (n=69) or *Salmonella paratyphi A* (n=24) were identified in a prospective, community-based, passive surveillance study in the Jatinegara district of Jakarta, Indonesia [2]. Participants were 1019 consecutive individuals living in the study area who presented with fever lasting 3 days or more to one of 24 healthcare facilities in the district. Blood cultures were collected into Bactec bottles (aerobic) containing antibiotic absorbing resins (Becton Dickinson, USA) that were provided to the centres by the study group free of charge. Every second consecutive non-enteric fever patient was selected as a fever control. Also, during the surveillance community controls were randomly selected within a random household in every third *rukun tetangga* (RT) of a total of 1140 RTs in the study area, RT being the smallest administrative unit of 40-60 households. When a community control reported fever in the 30 days preceding the interview or refused participation, the house on alternating sides of the initially selected household was approached. The selection of both groups

of controls was non-matched for age, sex or neighbourhood to limit selection-bias and prevent overmatching. Four controls from both groups for every case of enteric fever were selected in order to increase statistical power. Full details of the enrollment of patients have been described in detail elsewhere [2]. Furthermore, between March and October 2003, 4 participating centers and the Medistra Hospital adjacent to the study area contributed another 23 cases of enteric fever (i.e., 21 typhoid fever and 2 paratyphoid fever cases). All cases and fever controls were visited at home within one month after the febrile episode that led to the blood culture. Community controls were visited randomly throughout the study period. This study was approved by the Indonesian National Institute of Health Research and Development (*Litbangkes*) and provincial authorities. From all participants or their guardians a written informed consent was obtained.

Household visits and sample collection

Typhoid and paratyphoid cases, fever controls and randomly selected community controls were interviewed by trained medical school graduates using a validated, standardized questionnaire as described [2,22]. Blood was collected using an EDTA-containing vacutainer system (Becton Dickinson, USA). Genomic DNA from white blood cells was isolated essentially as described by Sambrook [23]. The isolated DNA was dissolved in 10 mM Tris, 0.1 mM EDTA, pH 7.5.

TNFA Single Nucleotide Polymorphism (SNP) genotyping

Determination of *TNFA–238* and *TNFA–308* SNPs was done using PCR amplification followed by restriction enzyme digestion as described [24]. Samples were run on EL300 Spreadex gels in a SEA 2000 electrophoresis apparatus (Elchrom Scientific AG, Cham, Switzerland). Interpretation of gels demonstrating the *TNFA–238* and *TNFA–308* SNPs alleles was done twice in a blinded fashion and independently by two laboratory workers.

Sequenom typing of single nucleotide polymorphisms in pro-inflammatory genes

SNPs in the genes *IFNG*, *IL1A*, *IL1B*, *TNFRSF1A*, *IL1R1*, *CASP1* and *CRP* were selected from literature and databases on the web (**Table 2**). Multiplex assays were designed using Assay designer software (Sequenom). Genotyping was performed using the MassArray platform according to manufacturers protocols (Sequenom). In brief, after PCR on 2.5 ng of DNA a primer extension reaction was performed to introduce mass-differences between alleles and, after removing salts by adding a resin, ~15 nl of the product was spotted onto a target chip with 384 patches containing matrix. Mass differences were detected using a Bruker Autoflex MALDI-TOF mass spectrometer and genotypes were assigned real-time using Typer 3.1 software (Sequenom). As quality control, 10% of samples were genotyped in duplo and no inconsistencies were observed. Primer sequences are available upon request.

Analysis of IFNGR1 CA repeat and IL12B ins/del polymorphisms

PCRs for *IFNGR1* CA and *IL12B* ins/del polymorphisms were performed using 100 ng of genomic DNA, 200 µM of each dNTP, 10 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1 % Triton X-100, 1.5 mM $MgCl_2$, 0.5 U of Taq DNA polymerase (Promega) in a total volume of 25 µl. Forward primers were 5'-labeled with FAM and TET respectively, primer sequences are available on request. PCR conditions used: 5 min 95˚C, followed by 30 cycles of 95˚C 30 s, 55˚C 30 s, 72˚C 30 s and 5 min 72˚C. PCR products were diluted 1:20 in H_2O, 1 µl of diluted product was added to 8.8 µl HiDi Formamide, 0.2 µl 400 HD-ROX size standard (Applied Biosystems) and heated to 95˚C for 5 min. Products were run on an ABI Prism 3700 DNA Analyzer (Applied Biosystems), results were analysed using GeneScan Analysis and Genotyper software (Applied Biosystems). Several homozygous alleles were sequenced to verify allele lengths.

Statistical methods

Data from the questionnaires were entered twice using EpiInfo 6.04b software (US Centers for Disease Control and Prevention, Atlanta, Ga), validated and together with data from *TNFA* SNP typing, Sequenom MassArray typing and fragment length analysis imported into SPSS version 11.5 (SPSS Inc, Chicago, Ill) for statistical analysis. The Hardy-Weinberg equilibrium of each polymorphism was checked in the total population and in each group of respondents using the program HWE [25], *IFNGR1* CA repeat alleles with < 5% frequency were grouped for this computation. For the comparisons of the proportion, either the Pearson's Chi-Square test or Fisher's exact test was used. Association analyses of the CA repeat polymorphism were carried out using the program CONTING v2.62 [25].

Results

Cases of typhoid fever and paratyphoid fever

In all, 90 bacteremic typhoid fever patients and 26 bacteremic paratyphoid fever patients were enrolled in this study. The demographic characteristics of the cases are given in **Table 1**; only about 13 percent of the typhoid fever and paratyphoid fever cases were hospitalized, all other patients were treated in an outpatient setting.

Fever controls and randomly selected community controls

We included 337 fever controls. Of 378 randomly selected community controls, 56 (15%) refused to give blood. Sixty-two (19%) of the 322 community controls had a self reported history of typhoid fever; in none of these cases, however, was that history at the time

confirmed by a positive blood culture.
The gender distribution of the typhoid and paratyphoid cases was about even: 57:59 for the female and male, respectively; in the randomly selected community controls it amounted to 179:143. The difference of gender distribution between cases and community controls is not significant (p=0.37). The median age of cases, 20 years [Inter Quartile Range: 12-26.8], was similar to that of fever controls, both being significantly lower than that of the random community controls, i.e., 31.5 years [IQR: 18-49]. Of note, the age of typhoid fever cases did not differ significantly from that of paratyphoid fever cases (p=0.10). The population of Jatinegara is a mixture of Indonesians from different islands of the archipelago and in individual cases it is not possible to designate a subject to one group, or exclude admixture with certainty. However, based on the sublocation in the area and the subjects' names the ethnic makeup of our three study populations did not differ; no stratification with respect to possible admixture was made. In the non-enteric fever control group, patients could be infected with various bacterial and viral pathogens, each having distinct disease mechanisms. Therefore, the underlying genetic susceptibilities for this group could also be diverse. Although the inclusion of a fever control group would not provide a consistent reference group, we decided to present the findings in this group as well to further illustrate allelic frequencies in the population. Some additional characteristics of the respondent groups in the study can be found in **Table 1**.

Hardy-Weinberg equilibrium calculation

The single nucleotide polymorphisms (SNPs) analyzed in this study are summarized in **Table 2**. We started with calculating the Hardy-Weinberg equilibrium for all polymorphisms. The prevalence of the *TNFA–238* and *TNFA–308* genotypes in the total population group, case group and fever control group were in equilibrium. However, they are not

Table 1. Demographic data of typhoid fever and paratyphoid fever cases, community controls, and fever controls

	Cases (n=116)	Community controls (n=322)	Fever controls (n=337)
Age, median [IQR]	20 [12-26.8]	31.5 [18-49]	21 [14-30.5]
Gender, F/M	57/59	179/143	156/181
Low Family income (%) [a]	58 (51)	148 (46)	197 (59)
Household size, median (range) [b]	6 (1-200)	6 (1-50)	6 (1-20)
Hospitalized (%)[c]	15 (12.9)	N.A.	39 (11.7)
Days of inactivity (range)	10.9 (0-36)	N.A.	7.1 (0-50)

a Defined as below the median monthly income of the community controls (900.000 Rupiah [US$ 97])
b Includes 2 outliers: an orphanage with 200 individuals and a dormitory with 50 individuals in the typhoid fever cases and community controls, respectively.
c Respondents who were initially admitted to the hospital when the blood culture was taken or who were admitted to the hospital in later stage during the disease episode.

in equilibrium for the community control group (p<0.02 and p<0.01 for *TNA–238* and *TNFA–308*, respectively). This is due to the presence of 1 and 3 individuals with the AA genotype in the respective polymorphisms where the A allele proportion is very low. As the expected numbers of AA homozygotes are 0.1 and 0.8, we believe this is not a major deviation. The SNP *IFNG +874* was in equilibrium for the total population group, case group and community control group but not for the fever control group (p<0.02).

The two SNPs in *IL1R1* were nearly absent in our population (2 C alleles in 710 genotyped individuals and 2 G alleles in 728 genotyped individuals for *rs3917320* and *rs3917287*, respectively) and were therefore not studied further. All other polymorphisms studied were in Hardy-Weinberg equilibrium in the total population as well as in the separate groups of cases, fever controls and community controls.

Single Nucleotide Polymorphisms TNFA–238 and TNFA–308

The allele and genotype distributions for *TNFA–238* and *–308* in cases, randomly selected community controls and fever controls are given in **Table 3**. When comparing the allele distribution of *TNFA–238* between typhoid and paratyphoid fever patients and community controls, there was no significant difference (p=0.83). Similar findings were made when the typhoid fever cases only were compared with the community controls (p=0.75). Exclusion of the community controls with an unconfirmed history of typhoid fever did not change significantly the distribution of *TNFA–238* alleles in this group (p=0.55), nor did it affect the

Table 2 Distribution of alleles according to SNP database

Common SNP name	Official SNP designation	Frequency (%) of major allele in various populations [a] (Asian population)	Frequency (%) of major allele in Indonesian cohort
TNFA –238	rs361525	G 92-96 (n.a.)	G 98
TNFA –308	rs1800629	G 80-93 (n.a.)	G 95
IFNG +874	rs2430561	T n.a. [b]	T 68
IL1A –889	rs1800587	C 61-88 (88 in Chinese)	G 92
IL1B +3953	rs1143634	C 71-98 (98 in Chinese)	C 97
IL1B –511	rs16944	G 53-82 (53 in Japanese)	G 55
TNFRSF1A +36	rs767455	A 50-94 (94 in Asians [c])	A 89
IL1R1 A124G	rs2228139	C 89-100 (100 in Asians [d])	C 100
IL1R1 R456R	rs3917320	A 91-100 (100 in Chinese)	A 100
CASP1 codon 235	rs580253	C 73-100 (100 in Asians [d])	C 97
CRP +1444	rs1130864	C 70-93 (n.a.)	C 90

a as obtained from the SNP database at www.ncbi.nlm.nih.gov
b present at 0.32-0.50 in various populations according to literature
c population not specified
d both Chinese and Japanese
n.a. not available

outcome of the comparisons with typhoid and paratyphoid fever patients taken together, or typhoid fever patients only (i.e., p= 0.51 and p=0.34, respectively).

Furthermore, the distribution of the TNFA–238 alleles was not significantly different in the fever control group as compared to enteric fever cases or typhoid fever patients only (p=0.54 and p= 0.37, respectively). Similar results were obtained upon comparison of the TNFA–308 genotype between the total number of enteric fever patients or typhoid fever case group only, and the community controls group (p=0.16 and p=0.11, respectively). Again, essentially identical results were obtained after exclusion of community controls with an unconfirmed history of typhoid fever (p= 0.29 and p=0.20 for typhoid and paratyphoid fever cases taken together, and typhoid fever only, respectively). Furthermore, the distribution of the TNFA–308 genotypes in the typhoid and paratyphoid cases group and fever controls group was not statistically significant (p=0.26 and p=0.13 for enteric fever case group and typhoid fever only, respectively).

We calculated odds ratios (OR) upon comparison of the alleles or genotypes of *TNFA–238* and *–308* in typhoid fever and paratyphoid fever cases with community controls and fever controls. For the *TNFA–238* polymorphism, we could not find an association of a particular allele or genotype and susceptibility or resistance against community acquired typhoid and paratyphoid fever or typhoid fever. For the *TNFA–308* polymorphism, in contrast to a previous study that associated allele A with the disease [5], the allele G appeared to be significantly associated with occurrence of typhoid and paratyphoid fever or typhoid fever specifically (OR = 4.42 [95% CI: 1.11-38.41] and OR = 2.87 [95% CI: 1.00-11.28], respectively). However, this association does no longer reach a level of significance when the comparison is made against the randomly selected community controls that did not have an -unconfirmed- history of typhoid or paratyphoid fever.

SNPs in IFNG, IL1A, IL1B, TNFRSF1A, CASP1 and CRP

The majority of the SNPs in this study were analyzed using a Sequenom MassArray system, which fully automatically determines the genotypes. There were no significant differences when we compared the genotypes or alleles of the SNPs in *IFNG*, *IL1A*, *IL1B*, *TNFRSF1A*, *CASP1* or *CRP* between typhoid and paratyphoid fever cases and community controls. Similar findings were made when the typhoid fever cases only were compared with the community controls. Exclusion of the community controls with an unconfirmed history of typhoid fever did not change significantly the distribution of the alleles in these groups. One of the SNPs, *IFNG +874* was not in Hardy-Weinberg equilibrium within the fever control group which may indicate the presence of an association between the cause of the fever in this group and certain genotypes. This was not further studied since we did not determine the causative

Table 3 Allele and genotype distributions of SNPs in pro-inflammatory genes

		Cases	Community controls	Fever controls
SNP	Alleles/ genotypes			
TNFA –238	A	0.02	0.02	0.02
	G	0.98	0.98	0.98
	AA	0 (0 %)	1 (0.3 %)	0 (0 %)
	AG	4 (3.6 %)	11 (3.4 %)	11 (3.3 %)
	GG	107 (96.4 %)	308 (96.3 %)	320 (96.7 %)
TNFA –308	A	0.02	0.05	0.03
	G	0.98	0.95	0.97
	AA	0 (0 %)	3 (0.9 %)	0 (0 %)
	AG	4 (3.6 %)	26 (8.1 %)	23 (6.9 %)
	GG	107 (96.4 %)	291 (90.9 %)	308 (93.1 %)
IFNG +874	A	0.31	0.32	0.38
	T	0.69	0.68	0.63
	AA	11 (10.5 %)	26 (8.6 %)	54 (17.3 %)
	AT	44 (41.9 %)	144 (47.4 %)	126 (40.4 %)
	TT	50 (47.6 %)	134 (44.1 %)	132 (42.3 %)
IL1A -889	A	0.11	0.08	0.09
	G	0.89	0.92	0.91
	AA	0 (0 %)	1 (0.3 %)	3 (0.9 %)
	AG	23 (21.9 %)	44 (14.4 %)	52 (16.2 %)
	GG	82 (78.1 %)	260 (85.2 %)	266 (82.9%)
IL1B +3953	A	0.04	0.03	0.03
	G	0.96	0.97	0.97
	AA	0 (0 %)	0 (0 %)	0 (0 %)
	AG	9 (8.6 %)	17 (5.6 %)	18 (5.6 %)
	GG	96 (91.4 %)	288 (94.4 %)	303 (94.4 %)
IL1B -511	A	0.40	0.45	0.45
	G	0.60	0.55	0.55
	AA	13 (12.5 %)	62 (20.3 %)	56 (17.7 %)
	AG	57 (54.8 %)	147 (48.4 %)	172 (54.4 %)
	GG	34 (32.7 %)	95 (31.3 %)	88 (27.9 %)
TNFRSF1A +36	C	0.09	0.11	0.10
	T	0.91	0.89	0.90
	CC	2 (1.9 %)	4 (1.4 %)	0 (0 %)
	CT	14 (13.3 %)	54 (18.3 %)	61 (19.2 %)
	TT	89 (84.8 %)	237 (80.3 %)	256 (80.8 %)
CASP1 CODON 235	A	0.02	0.03	0.03
	G	0.98	0.97	0.97
	AA	0 (0 %)	0 (0 %)	1 (0.3 %)
	AG	4 (3.8 %)	19 (6.2 %)	19 (5.9 %)
	GG	100 (96.2 %)	287 (93.8 %)	302 (93.8 %)
CRP +1444	T	0.09	0.10	0.11
	C	0.91	0.90	0.89
	TT	0 (0 %)	5 (1.7 %)	6 (1.9 %)
	CT	20 (18.7 %)	49 (16.7 %)	57 (17.8 %)
	CC	87 (81.3 %)	239 (81.6 %)	257 (80.3 %)

bacterial strains in this group other than exclusion of *S. (para)typhi*.
The distribution of alleles and genotypes of these SNPs are shown in **Table 3**.

Variable length polymorphisms in IFNGR1 and IL12B

There were no significant differences when we compared the genotypes or alleles of the CA repeats in *IFNGR1* and the ins/del polymorphism in *IL12B* between enteric fever cases and community controls. Also no significant differences were found between the fever controls and the enteric fever cases. The distribution of the alleles is shown in **Table 4**.

Discussion

The main finding of the present study is that polymorphisms in a series of pro-inflammatory genes were not associated with typhoid fever and thus appear not to contribute to susceptibility to acquire typhoid fever unlike the previously described polymorphisms in *CFTR* and *PARK2/PACRG* in the same typhoid fever cohort [26,27]. In view of earlier findings the *TNFA–308* polymorphism, however, might be related to severity of established disease rather than to susceptibility per se.

To analyze association of polymorphisms with susceptibility to typhoid fever and paratyphoid fever, we compared the prevalence of the polymorphisms in typhoid and paratyphoid cases with those of fever controls and community controls. Some of the potential pitfalls that may affect complete enrollment of patients, most of whom were recruited in outpatient facilities in the area, and the classification of cases and fever controls have been described in detail elsewhere [2]. In short, provisions were taken to minimize misclassification of patients, including stool cultures 3 to 4 weeks after the blood culture, when *S. typhi* and *S. paratyphi A* may still be excreted in the feces of patients [28]. For every patient with typhoid fever or paratyphoid fever, we included 4 controls of two groups recruited from the same study area: fever controls who like typhoid fever patients presented with 3 days of fever but of whom blood cultures either showed no growth or growth of bacteria other than Salmonellae, or randomly selected community controls. Although the fever controls likely suffered from a divergent spectrum of diseases other than enteric fever, and as such do not constitute a consistent reference group as the random community controls do, we decided to include the findings in this group to further illustrate the allelic frequencies found in the Indonesian population. Nineteen percent of the randomly selected community controls reported a possible episode of typhoid fever in the past. Likely, this percentage is an overestimation of the real number of cases since most fever patients are empirically treated in outpatient clinics without confirmatory diagnosis, but importantly,

the distribution of polymorphisms in the community control group was not significantly different when these community controls were left out of the analysis. The age of the typhoid cases and the random community controls did differ, whereas the incidence of typhoid is higher in the age group below twenty. In this respect, however, the distribution of polymorphisms in the community control group did not differ for different age cohorts, e.g., those under or above their 20s. Although the sample size may have influenced the outcome to some extent we have more than 80% power to detect significant differences with an OR of 3 in e.g. *TNFA-308* allele distribution, this should have provided sufficient power to replicate the findings in Vietnam where an OR of >5 was found [5].

The role of pro-inflammatory proteins like TNF-α, IFN-γ, IL-1α, IL-1β, IL-12, TNFR1, IFN-γR1, IL-1R1, CASP1 and CRP in induction of expression of a variety of genes and the synthesis of several proteins that induce acute and chronic inflammatory changes are well established [19;19;29]. Although polymorphisms in some of these genes were found to

Table 4 Allele distribution of IFNGR1 CA repeat and IL12B ins/del polymorphisms

		Cases	Community controls	Fever controls
POLYMORPHISM	ALLELES OR GENOTYPES			
IFNGR1 CA	CA_{11}	1 (0.4 %)	0 (0 %)	0 (0 %)
	CA_{12}	82 (35.7 %)	183 (28.5 %)	211 (31.4 %)
	CA_{13}	0 (0 %)	1 (0.2 %)	0 (0 %)
	CA_{16}	5 (2.2 %)	18 (2.8 %)	21 (3.1 %)
	CA_{17}	4 (1.7 %)	16 (2.5 %)	11 (1.6 %)
	CA_{18}	32 (13.9 %)	109 (17.0 %)	96 (14.3 %)
	CA_{19}	27 (11.7 %)	66 (10.3 %)	81 (12.1 %)
	CA_{20}	7 (3.0 %)	32 (5.0 %)	27 (4.0 %)
	CA_{21}	1 (0.4 %)	11 (1.7 %)	16 (2.4 %)
	CA_{22}	22 (9.6 %)	65 (10.1 %)	57 (8.5 %)
	CA_{23}	31 (13.5 %)	78 (12.1 %)	82 (12.2 %)
	CA_{24}	16 (7.0 %)	61 (9.5 %)	63 (9.4 %)
	CA_{25}	2 (0.9 %)	2 (0.3 %)	7 (1.0 %)
	total	**230**	**642**	**672**
IL12B ins/del [a]	short allele	127 (56.2 %)	336 (53.2 %)	382 (57.4 %)
	long allele	99 (43.8 %)	296 (46.8 %)	284 (42.6 %)
	short/short	38 (33.6 %)	81 (25.6 %)	108 (32.4 %)
	short/long	51 (45.1 %)	174 (55.1 %)	166 (49.8 %)
	long/long	24 (21.2 %)	61 (19.3 %)	59 (17.7 %)

a The short allele contains GC that in the long allele is replaced by CTCTAA, resulting in a 4 nt difference in length.

be associated with susceptibility to infectious disease like tuberculosis [7,9], we failed to associate these polymorphisms with manifest infection by the intracellular pathogens *Salmonella typhi* and *Salmonella paratyphi* A.
Unlike in Vietnam, 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 significantly from those in fever controls or community controls. This discrepancy might be explained by the way the patients were selected for the respective studies, i.e., use of typhoid fever patients admitted to hospital in Vietnam [5] as compared with consecutive (para-) typhoid fever patients enrolled in the community-based surveillance study in Jakarta. In our study, only a minority of the patients (~13 %) was hospitalized. Patients admitted to hospital usually have more severe disease than patients treated in an outpatient setting. Together, the findings suggest that *TNFA* promoter polymorphisms, the *TNFA–308* in particular, may have a role not in susceptibility to acquire (para-)typhoid fever but in determining the course and severity of the established disease requiring hospitalization of patients. Such an explanation is consistent with earlier findings that the *TNFA–308*A* allele was associated with severity and mortality of meningococcal infection [30], and bacterial sepsis [31], cerebral malaria [32], mucocutaneous leishmaniasis [33], as well as some viral diseases [34] and trauma [30-33,35,36]. Although the presence of *TNFA–308*A*, may result in somewhat higher constitutive and inducible levels of gene transcription than *TNFA–308*G* [37,38] in clinical practice it cannot be determined readily whether greater TNF-α production causes a more severe inflammatory response or, conversely, whether more severe inflammation elicits greater TNF-α synthesis [39].

Due to the lack of a suitable animal model for *S. typhi* infection the pathogenesis of typhoid fever has not been studied extensively. Gene expression profiling of human model intestinal cells infected with *Salmonella typhi* has shown that *S. typhi*, in contrast to a well-defined pro-inflammatory pathogen like *S. typhimurium*, does not elicit a pro-inflammatory response [40]. In human colonic tissue explants infected with *S. typhi* it was found that the Vi antigen expressed by *S. typhi* is able to reduce TLR5 and TLR4 mediated responses which may account for the lack of inflammatory infiltrates in the human intestinal mucosa [41]. Both these *in vitro* experiments and our genetic data suggest that the role of pro-inflammatory cytokines may be limited in determining the outcome of *S. typhi* infection. We recently found an association of susceptibility to typhoid fever (OR 2.6) with polymorphisms in *CFTR*, which encodes for a protein expressed on the intestinal epithelium that is utilized by *S. typhi* to enter the epithelial cells before entering the bloodstream [26]. A crucial step in developing typhoid fever is the ability of the bacteria to penetrate the gut epithelium and this may mean that the genes that will be found to influence the susceptibility to typhoid fever will be encoding proteins that are expressed in the gut epithelium and are involved in

S. typhi entry or passage. Once the salmonellae have passed the epithelial layer and entered the bloodstream the pro-inflammatory genes may merely be able to influence disease severity and speed of recovery.
Two SNPs (in *IL1R1*) of which the minor alleles were reported with a low frequency in the SNP database were virtually absent in the Indonesian population. Most of the other SNPs in the pro-inflammatory genes we studied proved to have a low frequency of the minor allele: *TNFA-238*, *TNFA-308*, *IL1A -889*, *IL1B +3953*, *TNFRSF1A +36* and *CASP1 codon 235* (**Table 2**). No Indonesian panel has thus far been used for establishing allele frequencies for the SNP database. Before embarking on this study we therefore did not know whether allele frequencies would be similar to other Asian populations or would diverge greatly. To have the necessary power to detect an association with polymorphisms of which the minor allele frequency is < 0.05 (as was the case for *TNFA –238*, *IL1B+3953* and *CASP1 c235* in this population) a study with a larger sample size is required. Ideally, one would want to analyze SNPs of which the allele frequencies have been validated in the population that one is about to study, these may however not be the polymorphisms one would prefer to analyze based on functional data (expression) and association studies from the literature.

In conclusion, we did not find an association of susceptibility to typhoid and paratyphoid fever and *TNFA* promoter polymorphisms as was previously described for the *TNFA–308*A* allele and suggest that this polymorphism might be related to severity of established disease rather than to susceptibility per se. We also did not find an association between a series of other pro-inflammatory genes and conclude that polymorphisms in pro-inflammatory genes appear not to contribute to susceptibility to typhoid fever.

Acknowledgements

Financial support was provided by the Royal Netherlands Academy of Arts and Sciences (KNAW) and by the Centre for Medical Systems Biology (CMSB), a centre of excellence approved by the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (NWO). We thank the physicians, nurses and technicians of the participating health centers in Jakarta for their cooperation: Mitra International hospital and microbiology lab, Budhi Asih, St. Carolus, Medistra and Persahabatan hospital, all puskesmas' in Jatinegara and local private practitioners.
We thank our research assistants in Jakarta: Billy Hunsinger, Ferry Kandaw, Rinny Listyani, Meily, Vea Noveria, Carmelita Ridwan, Min Ali Sugiharto, Lidwina Sutikno, Mariana Tasman and Lily Yaputra. We also thank Leo G. Visser and Henry A.G.H. van Asten for their contribution to the project.

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PARK 2/PACRG polymorphisms and susceptibility to typhoid and paratyphoid fever

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Clinical and Experimental Immunology 2006; 144: 425–431

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Clinical and Experimental Immunology ORIGINAL ARTICLE

PARK2/PACRG polymorphisms and susceptibility to typhoid and paratyphoid fever

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Accepted for publication 8 March 2006

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Summary

Host genetic factors may contribute to susceptibility to and outcome in infectious diseases. Recently polymorphisms in *PARK2/PACRG*, a gene cluster linked to ubiquitination and proteasome-mediated protein degradation, were found to be associated with manifest infection by *M. leprae*. Here, we address whether these polymorphisms are associated with susceptibility to infection with *Salmonella typhi* and *S. paratyphi A*, intracellular pathogens that upon infection of humans share with mycobacteria aspects of the hosts' immune response. The polymorphisms of *PARK_e01(−697)*, *PARK2_e01(−2599)*, *rs1333955* and *rs1040079* were analysed by polymerase chain reaction and restriction fragment length polymorphism in a case-control study of typhoid and paratyphoid fever patients in an endemic area in Jakarta, Indonesia. For this study, samples were obtained from patients with blood culture-confirmed typhoid fever ($n = 90$), paratyphoid fever ($n = 26$) and fever controls ($n = 337$) in a passive, community-based surveillance and compared to those of randomly selected community controls ($n = 322$) from the same city area. The *PARK2_e01(−2599)* allele T was significantly associated with typhoid and paratyphoid fever (OR: 1·51, 95%CI: 1·02–2·23) but the other polymorphisms, *PARK2_e01(−697)*, *rs1333955* and *rs1040079*, were not associated. Although within the *PARK2/PACRG* gene cluster the *PARK2_e01(−2599)* allele T was most strongly associated with leprosy (OR= 3–5), the association with typhoid is much less strong. Our findings suggest that this polymorphism in *PARK2/PACRG* plays a small but significant role in susceptibility to the intracellular pathogens *S. typhi* and *S. paratyphi*.

Keywords: *PARK2*, *PACRG*, *S. typhi*, *S. paratyphi*, typhoid fever, gene polymorphism

Introduction

Typhoid fever constitutes a serious public health problem in the world, especially in the developing countries, claiming over 200 000 lives in 2000 [1]. Typhoid fever is a systemic infection caused by *Salmonella enterica* serotype typhi (*S. typhi*). Paratyphoid fever, caused by *Salmonella paratyphi A, B* or *C*, has a disease presentation highly similar to that of typhoid fever, but, at least in Jakarta, seems to follow a distinct route of transmission: whereas typhoid fever is spread predominantly within the household, paratyphoid fever is mainly transmitted outside the patient's home [2]. The identification of such risk factors and the most relevant route of transmission of the disease are essential for the development of control strategies and the allocation of public health resources.

Several case-control studies have documented risk factors for typhoid fever at the community level, such as inadequate hygiene, lack of microbiologically safe drinking water or the consumption of street food [2]. Within this environmental context, an individual's genetic makeup may predispose subjects to acquisition of typhoid fever or development of severe disease [3]. For instance, an association between the single nucleotide polymorphism (SNP) *TNFA* −308 and typhoid fever has been reported in Vietnam. Together with HLA-DRB1*0301/6/8 and HLA-DQB1*0201–3, the *TNFA*−308*A allele was thought to be associated with susceptibility to typhoid fever [4,5].

In families with members displaying increased susceptibility to infection with the intracellular pathogens salmonella and mycobacteria, defects in IL-12/IFNγ type-1 cytokine mediated activation of macrophages have been found [6], suggesting that there is considerable overlap in immune responses against these unrelated intracellular bacterial pathogens. Recently, variants in the shared *PARK2* and *PACRG* regulatory region have been found to act as common risk factor for manifest infection by *M. lepraе*: a strong association (i.e. OR of 3–5) was demonstrated between the PARK2_e01(−2599) polymorphism and leprosy [7]. Mutations in the *PARK2* gene encoding Parkin, have been identified as the cause of autosomal recessive juvenile Parkinsonism [8]. Parkin is a E3 ubiquitin ligase that is required for polyubiquitination of proteins before degradation by the proteasome [9]. Parkin Co-Regulated Gene (*PACRG*) is a reverse strand gene located upstream of the *PARK2* gene. The gene product, termed Glup, forms a large molecular chaperone complex containing heat shock proteins (Hsp) and chaperonin components. Glup binds Parkin via Hsp70, and this multicomponent aggregate may deal with bacterial proteins by breaking them down or turning them into harmless molecules [10]. *PARK2* and *PACRG* share a common promoter regulating their expression [11]. Of interest here, variants in this shared regulatory region have been found to act as a common risk factor for the acquisition of leprosy and there was some evidence that the T allele of this polymorphism acts in a dominant fashion [7].

Given the overlap in immune response to Salmonellae and mycobacteria, and its proven role in leprosy, we hypothesized that *PARK2/PACRG* polymorphisms may be associated with clinical typhoid and paratyphoid fever. In addition, *in vitro* studies suggested a possible role for *PARK2/PACRG* regulated genes in *Salmonella* pathogenesis, since they link this pathway to intracellular bacterial evasion mechanisms [12–14]. Parkin, the protein encoded by *PARK2* has ubiquitin ligase (E3) activity [15]. The ubiquitin-proteasome pathway is important in protein processing and degradation, and contributes to quality control of proteins within cells and antigen processing for cross-presentation [16]. Invasion of host cells by *Salmonellae* requires the reversible activation of Cdc42 and Rac1 by bacterial encoded SopE and SptP, which must exert their function at different times during uptake. Although both proteins are delivered into the host cell cytoplasm at approximately equivalent amounts, SopE is rapidly degraded through a proteosome-mediated pathway, while SptP exhibits much slower degradation kinetics. Stabilization of SopE by proteasome inhibition prevents cellular recovery after bacterial infection and therefore continuation of a permissive environment for the bacteria to replicate or evade host defences [12,13]. This mechanism is important in *Salmonella* interaction with its host cells, and we hypothesized that modification of its activity might result in an association between *PARK2/PACRG* polymorphism and typhoid and paratyphoid fever.

Given the above hypothesis on the possible role of ubiquitination and degradation of bacterial proteins in the cellular pathogenesis of these diseases [12–14,16], we investigated the role of *PARK2* and *PACRG* polymorphisms as host-dependent risk factors for acquisition of *Salmonella typhi* and *S. paratyphi* infection.

Materials and methods

Study design

From June 2001 to February 2003 patients with blood culture-confirmed *Salmonella typhi* ($n = 69$) or *Salmonella paratyphi A* ($n = 24$) were identified in a prospective, community-based, case-controlled passive surveillance study in the Jatinegara district of Jakarta, Indonesia [2]. In this surveillance study, we enrolled 1019 consecutive individuals living in the study area who presented with fever lasting ≥ 3 days to one of 24 healthcare facilities in the district. The study was designed to address both environmental [2,17,18] and genetic determinants of susceptibility to enteric fever and was aimed at investigating new associations as well as identifying specific candidate genes that might reveal meaningful immunological insights [19]. Full details of the enrolment of patients have been described elsewhere [2].

Blood cultures were collected into Bactec bottles (aerobic) containing antibiotic absorbing resins (Becton Dickinson, Sparks, MD, USA) that were provided to the centres by the study group free of charge. Every second consecutive fever patient with a negative blood culture, or having a pathogen other than *Salmonella* cultured, was selected as a fever control. Also, during the surveillance community controls were randomly selected within a random household in every third *rukun tetangga* (RT) from a total of 1140 RTs in Jatinegara, RT being the smallest administrative unit of 40–60 households in the area. When a community control reported fever in the 30 days preceding the interview or refused participation, the house on alternating sides of the initially selected household was approached. The selection of both groups of controls was nonmatched for age, sex or neighbourhood to limit selection-bias and prevent overmatching. Four controls from both groups for every case of blood culture-confirmed enteric fever were selected in order to increase the statistical power. Furthermore, between March and October 2003, 4 participating centres and the Medistra Hospital adjacent to the study area contributed another 23 cases of enteric fever (i.e. 21 typhoid fever and 2 paratyphoid fever cases).

All cases and fever controls were visited at home within one month after the febrile episode that led to the blood culture. Community controls were visited randomly throughout the study period. This study was approved by the Indonesian National Institute of Health Research and Development (*Lithangkes*) and provincial authorities. Written informed consent was obtained from all participants or their guardians.

Household visits and sample collection

Cases, fever controls and randomly selected community controls were interviewed by trained medical school graduates using a validated, standardized questionnaire as described previously [2,20]. Three ml of blood was collected using an EDTA-containing vacutainer system (Becton Dickinson). Blood samples were stored in a cool box until processing in the Biomedical Laboratory, Faculty of Medicine, Catholic University of Atma Jaya. The plasma was separated and erythrocytes were lysed using lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA, pH = 7·4). White blood cells were washed twice with phosphate buffered saline (PBS) and stored in a freezer until transport to the Laboratory of Infectious Diseases, Leiden University Medical Centre. Genomic DNA was isolated from the cells essentially as described by Sambrook and Russell [21]. Before assay, DNA was diluted to a suitable concentration and stored in microplates at −20 °C.

Single nucleotide polymorphism (SNP) analysis

SNP analysis was performed using polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion. PCRs were performed using 100 ng of genomic DNA, 200 µM of each dNTP, 10 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 9·0), 1% Triton X-100, 1·5 mM $MgCl_2$, 0·5 U of Taq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 25 µl. PCR cycling conditions were as follows: 94 °C for 5 min once, 30 cycles of 94 °C for 45 s, annealing temperature for 45 s, 72 °C for 45 s and 72 °C for 7 min once. Primers used for PCR are based on [9] with major modifications for *PARK2_e01 (−697)* and *rs1040079*.

Digestion reactions using Bsu36 I, *HPy*CH4 IV, *Bsl* I (New England Biolabs, Ipswich, MA, USA) and *Bfm* I (MBI Fermentas, Vilnius, Lithuania) were performed according to the manufacturers protocols.

We chose to study four SNPs within the shared 5′ regulatory region of *PARK2* and *PACRG*, *PARK2_e01(−2599)*, *PARK2_e01(−697)*, *rs1333955* and *rs1040079* that were independently associated with an increased risk of leprosy [7].

The sequence of the primers, annealing temperatures and restriction enzymes used, the length of the products and the type of alleles are given in Table 1.

Statistical methods

Data from the questionnaires and polymorphisms were entered twice using EpiInfo 6·04b software (US Centers for Disease Control and Prevention, Atlanta, GA, USA), validated and imported into SPSS version 11·5 (SPSS Inc, Chicago, IL, USA) for statistical analysis. The Hardy–Weinberg equilibrium of each SNP was checked in the total population and in each group of respondents. For the comparisons of the proportion, either the Pearson's χ^2 test or Fisher's exact test was used.

Results

Study population

Of 1019 consecutive individuals living in the study area who presented with fever lasting ≥ 3 days, 116 individuals were enrolled with enteric fever and 337 as fever controls. In addition, 322 randomly selected community controls were included as detailed previously [2] and in Fig. 1. Of the 116 cases of enteric fever, 90 were caused by *Salmonella typhi* and 26 by *Salmonella paratyphi A*. Sixty-two community controls reported a possible history of enteric fever; in none of them had this past diagnosis been confirmed by (blood) culture.

Demographic background of cases, fever controls and randomly selected community controls have been described elsewhere [2]. In short, the gender distribution of the typhoid and paratyphoid cases was about even (56 female from 116 cases); in the community controls this increased to 177 female from 322 cases. The median age of cases was 20 years (Interquartile range (IQR): 12–26·5) which was similar to that of fever controls, whereas both were significantly lower than that of the random community controls, i.e. 32 years (IQR: 18–49). Of note, the median age of the

Table 1. Tools to investigate *PARK2/PACRG* polymorphisms: primers, annealing temperature, and product length of SNPs.

SNPs	Direction	Primer sequence*	Annealing temperature	Restriction enzyme	Product length	Allele
PARK2_e01(−697)	Forward	ACAGCCGCTCCCCGGTGCAC	62 °C	Bsu36 I	Uncut: 292 bp	Allele C
	Reverse	ATGGGCAGAGTACATCACTTG			Cut: 139 and 153 bp	Allele T
PARK2_e01(−2599)	Forward	TTTAGCAGTATAGACTTCTCAGC	60 °C	HpyCH4 IV	Uncut: 102 bp	Allele T
	Reverse	GAGCATGAGGTTGCAATTAAGA			Cut: 45 and 57 bp	Allele C
rs1333955	Forward	TTGGATTTTCAGGATTTTATAGC	58 °C	Bsl I	Uncut: 153 bp	Allele T
	Reverse	CTGGCCAGCCAGGTTTCTG			Cut: 66 and 87 bp	Allele C
rs1040079	Forward	CCATGAGTATAGGAGGAACTGT	52 °C 5×	Bfm I	Uncut: 103 bp	Allele G
	Reverse	GGACTAAAGGGCATGGTGAG	62 °C 25×		Cut: 23 and 80 bp	Allele A

*Primer sequences are based on [9] with major modifications for *PARK2-01(−697)* and *rs1040079*.

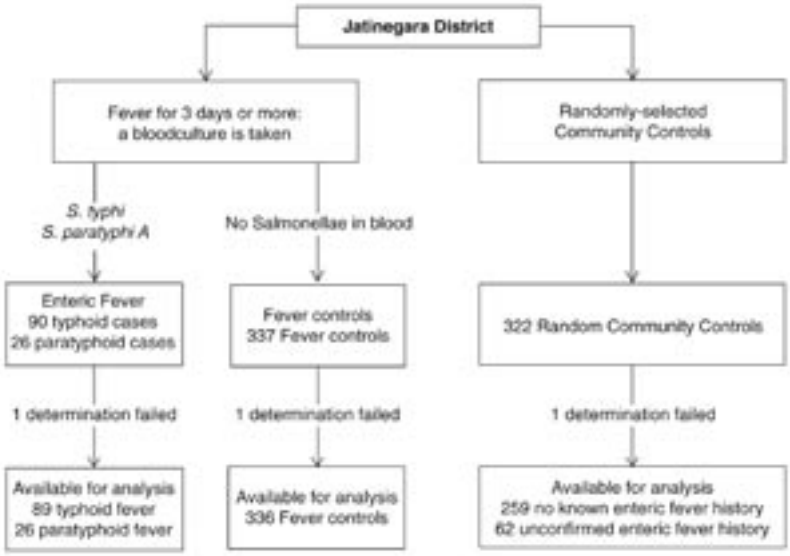

Fig. 1. Flow chart detailing the inclusion of typhoid and paratyphoid fever patients, and fever controls and the randomly selected community controls.

community controls that did not have a history of typhoid or paratyphoid fever was 32·5 years (IQR: 18–50), which was identical to that in community controls with a self-reported history of enteric fever. The age of typhoid fever cases did not differ significantly from that of paratyphoid fever cases. The population of Jatinegara is a mixture of, mainly, Indonesians from different islands of the archipelago and in individual cases it is not possible to designate a subject to one group or to exclude admixture with certainty. However, based on the sublocation in the area and the subjects' names the ethnic makeup of the three study populations did not differ; no stratification with respect to possible admixture was made. In the nonenteric fever control group, patients could be infected with various bacterial and viral pathogens, each having distinct disease mechanisms. Therefore, the underlying genetic susceptibilities for this group could also be diverse. Although the inclusion of a fever control group would not provide a consistent reference group, we decided to present the findings in this group to further illustrate allelic frequencies in the population.

In 1 (1%) of 90 typhoid fever cases, 1 (0·3%) of 337 fever controls and 1 (0·3%) of 322 community controls, we could not determine the SNP alleles due to technical difficulties.

Hardy–Weinberg equilibrium calculation

The genotypes of *PARK2_e01(−697)*, *PARK2_e01(−2599)*, *rs1333955* and *rs1040079* were found to be in Hardy–Weinberg equilibrium in all cases, fever controls, community controls and in the total group of respondents ($P > 0{\cdot}7$ in every group and in total for every SNP).

Genotyping PARK2/PACRG SNPs

The genotypic frequencies in cases, fever controls and randomly selected community controls are given in Table 2. In cases, fever controls and community controls alike, TT was found to be the most common genotype for *PARK2_e01(−697)* and *PARK2_e01(−2599)* (62% and 57%, respectively). CC was the most common genotype for *rs1333955* (50%), whereas AA was the most common genotype for *rs1040079* (63%). The most common allele for *PARK2_e01(−697)* and *PARK2_e01(−2599)* is the T-allele (proportion of 79% and 75%, respectively). For *rs1333955*, C is the most common allele with a proportion of 70%. A is the most common allele for *rs1040079* with a proportion of 79%.

In a previous study demonstrating a strong association between the PARK2_e01(−2599) polymorphism and leprosy, there was some evidence that the T allele of this polymorphism acts in a dominant fashion. Because only 3 typhoid fever cases and 1 paratyphoid fever case were CC homozygotes, the present study has limited power to test such a hypothesis. Futhermore, the T allele appears to have a higher frequency in the two control populations in this study (75%) than in the Vietnamese and Brazilian population (67 and 61%, respectively) [9], possibly reflecting differences in ethnic background.

Table 2. Genotypic frequencies in typhoid and paratyphoid cases, fever controls and randomly selected community controls.

	Typhoid cases (n = 89)		Paratyphoid cases (n = 26)		Community controls (n = 321)		Fever controls (n = 336)	
Locus/genotype	n	%	n	%	n	%	n	%
PARK2_e01(−697)								
CC	6	7	2	8	14	5	16	5
TC	29	33	10	38	110	34	104	31
TT	54	60	14	54	197	61	216	64
PARK2_e01(−2599)								
CC	3	3	1	4	24	7	20	6
TC	29	33	7	27	121	38	124	37
TT	57	64	18	69	176	55	192	57
rs1333955								
CC	49	55	16	61	154	48	167	50
TC	34	38	9	35	139	43	137	41
TT	6	7	1	4	28	9	32	9
rs1040079								
AA	58	65	15	57	203	63	207	62
AG	27	30	9	35	106	33	115	34
GG	4	5	2	8	12	4	14	4

PARK2/PACRG alleles and risk of developing enteric fever

When comparing the frequencies of these alleles amongst the case group and randomly selected community controls, we found that the frequency of allele T of *PARK2_e01(−2599)* was significantly higher in enteric fever cases ($P = 0{\cdot}03$). This difference was also significant when we excluded the community controls with an (unconfirmed) history of enteric fever ($P = 0{\cdot}02$). We did not observe a significant difference in frequency of allele T of *PARK2_e01(−2599)* when we compared fever controls to community controls nor upon comparison of enteric fever cases and fever controls. The allele distribution of the other polymorphisms studied, *PARK2_e01(−697)*, *rs1333955* and *rs1040079* was not significantly different in individuals with enteric fever when compared to those with fever due to other causes or randomly selected community controls.

Odds ratios (OR) were calculated on comparison of the alleles of *PARK2_e01(−697)*, *PARK2_e01(−2599)*, *rs1333955* and *rs1040079* in typhoid fever and paratyphoid fever with randomly selected community controls. Allele T *PARK2_e01(−2599)* was significantly but weakly associated with enteric fever (OR: 1·51, 95%CI: 1·02–2·23). This association became somewhat stronger when we compared the enteric fever cases to community controls without a history of enteric fever (OR: 1·58, 95%CI: 1·06–2·36). For *PARK2_e01(−697)*, *rs1333955* and *rs1040079* polymorphisms, we did not observe an association of a particular allele or genotype with susceptibility to or resistance against typhoid fever and paratyphoid fever grouped as enteric fever, or typhoid fever alone.

Discussion

The main finding of this study is that the common allele T of the *PARK2_e01(−2599)* polymorphism is significantly but weakly associated with typhoid and paratyphoid fever patients as compared to randomly selected community controls. The same polymorphism, i.e. *PARK2_e01(−2599)* within *PARK2* and *PACRG*, was, of all the polymorphisms in this gene region, most strongly associated with clinical leprosy in a Vietnamese population as well as a Brazilian population [7]. Alleles within this gene region that were less strongly but significantly associated with leprosy, i.e. *PARK2_e01(−697)*, *rs1333955* and *rs1040079*, were not found to be associated with typhoid and paratyphoid fever. The findings of the present study therefore, support a role for the *PARK2* and *PACRG* genes in susceptibility to *S. typhi* and *S. paratyphi* as they do, more strongly, in susceptibility to *M. leprae*, and they suggest that the implicated mechanism linked to ubiquitination and proteasome-mediated protein degradation could be a common pathway in the intracellular fate of these intracellular pathogens [6,22].

To study the association of *PARK2/PACRG* polymorphisms and susceptibility to typhoid fever and paratyphoid fever, we compared the prevalence of the polymorphisms in blood culture-confirmed cases of typhoid fever and paratyphoid fever to those of randomly selected community controls from the same study area. Provisions taken to minimize misclassification of cases and controls have been described in detail elsewhere [2]. To get a robust estimate of the prevalence of *PARK2/PACRG* polymorphisms in the population, we included 4 controls for every typhoid or paratyphoid fever patient. Furthermore, fever controls that, similarly to the

cases, presented with ≥ 3 days of fever, but from whom blood cultures showed either no growth or growth of bacteria other than *Salmonellae*, were recruited during the whole study period. Although the fever controls probably suffered from a divergent spectrum of diseases other than enteric fever, and therefore do not constitute a consistent reference group as the random community controls do, we decided to include the findings in this group to further illustrate the allelic frequencies found in the Indonesian population. Nineteen percent of the randomly selected community controls reported a possible episode of typhoid fever in the past. Probably, this percentage is an overestimation of the real number of cases since most fever patients are empirically treated in outpatient clinics without confirmatory diagnosis, but importantly, the distribution of polymorphisms in the community control group was not significantly different when these community controls were left out of the analysis. The age of the typhoid cases and the randomly selected community controls did differ, as the incidence of typhoid is higher in the age group < 20 years. In this respect, however, the distribution of polymorphisms in the community control group did not differ for different age cohorts, e.g. those < or > 20 years old. Of note, local HIV prevalence is low (e.g. 9 HIV positives were detected among 572 TB patients in a parallel study in Jakarta) and is unlikely to be an important confounder.

We hypothesized that *PARK2/PACRG* polymorphisms might be associated with typhoid and paratyphoid fever, given the overlap in immune responses to salmonella and mycobacteria [6], the finding that *PARK2/PACRG* polymorphisms are strongly associated with clinical leprosy [7] and *in vitro* studies that suggest a possible role for *PARK2/PACRG* regulated genes in *Salmonella* pathogenesis and link this pathway to intracellular bacterial evasion mechanisms and antigen processing for cross-presentation [12–14,16]. The study was powered to discern a similar strong association as described for leprosy, i.e. an OR of 3–5. To have the necessary power to confirm that the T allele of the *PARK2_e01 (–2599)* polymorphism acts in a dominant fashion, a study with a much larger sample size would be required, because only three typhoid and one paratyphoid case were CC homozygotes. In addition, the T allele appears to have a higher frequency in the Indonesian population (i.e. about 75%) than that previously found in the Vietnamese and Brazilian populations (about 65%) [7]. Given the weak but significant association between a *PARK2* polymorphism and typhoid and paratyphoid fever compared to randomly selected community controls, future studies of larger typhoid cohorts in different populations should elucidate to what extent these processes may play a role in the complex host defence mechanisms against *Salmonella*.

Acknowledgements

Financial support was provided by the Royal Netherlands Academy of Arts and Sciences (KNAW). We thank the physicians, nurses and technicians of the participating health centres in Jakarta for their cooperation: Mitra International Hospital and Microbiology Laboratory, Budhi Asih, St. Carolus, Medistra and Persahabatan Hospitals, all puskesmas' in Jatinegara and local private practitioners. We thank Adriëtte W. de Visser who isolated DNA from the blood samples, and our research assistants in Jakarta: Billy Hunsinger, Ferry Kandaw, Rinny Listyani, Meily, Vea Noveria, Carmelita Ridwan, Min Ali Sugiharto, Lidwina Sutikno, Mariana Tasman and Lily Yaputra. We also thank Leo G. Visser, and Henry A.G.H. van Asten, for their contribution to the project.

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6

Susceptibility to typhoid fever is associated with a polymorphism in the cystic fibrosis transmembrane conductance regulator (CFTR)

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Human Genetics 2005; 118: 138-140

Hum Genet (2005)
DOI 10.1007/s00439-005-0005-0

SHORT REPORT

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Susceptibility to typhoid fever is associated with a polymorphism in the cystic fibrosis transmembrane conductance regulator (CFTR)

Received: 14 January 2005 / Accepted: 23 May 2005

Abstract The cystic fibrosis transmembrane conductance regulator (CFTR) is the affected protein in cystic fibrosis (CF). The high rate of CF carriers has led to speculation that there must be, similar to the sickle cell haemoglobin advantage in malaria, a selective advantage for heterozygotes. Such a selective advantage may be conferred through reduced attachment of *Salmonella typhi* to intestinal mucosa, thus providing resistance to typhoid fever. We tested this hypothesis by genotyping patients and controls in a typhoid endemic area in Indonesia for two highly polymorphic markers in CFTR and the most common CF mutation. We found an association between genotypes in CFTR and susceptibility to typhoid fever (OR = 2.6). These analyses suggest that the role CFTR plays in vitro in *S. typhi* infection is also important for infection in the human population.

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel and is the affected protein in cystic fibrosis (CF). The CFTR mutation F508del is present in 30–75% of CF chromosomes and heterozygously in 2–5% of the caucasian population (numbers based on percentages in various populations). A few other mutations in CFTR are present at high frequency in specific subpopulations (e.g. W1282X in Ashkenazi Jews (Kornreich et al. 2004) and 1677delTA around the Black Sea (Angelicheva et al. 2005)). The high rate of CF carriers has led to speculation that there must be, similar to the sickle cell haemoglobin (HbS) advantage in malaria, a selective advantage for CF heterozygotes. In the past, suggestions have been made that this selective advantage could be due to increased resistance to tuberculosis, influenza, malaria, bubonic plague, syphilis or cholera. However, the evidence in support for selection by these infectious diseases has mostly been circumstantial (Jorde and Lathrop 1988). A CFTR null allele (S489X) in mice was found to confer resistance to cholera toxin (Gabriel et al. 1994), however, this particular mutation is very rare in humans (found in one patient) and may not be comparable to F508del, W1282X and 1677del TA in survival in a population. More importantly, cholera pandemics have mostly originated in India where CF incidence is much lower than in Europe, rendering resistance to cholera an unlikely explanation for the heterozygote advantage.

In vitro experiments suggest that *Salmonella typhi* uses CFTR to attach to the intestinal mucosal cells, and that this interaction may be crucial to initiate typhoid fever. Experiments in human epithelial cells and in mice suggest that *S. typhi* internalization is reduced by the F508del mutation (Pier et al. 1998). Also, blocking CFTR with antibodies or competition with synthetic peptides reduced *S. typhi* uptake in vitro (Pier et al. 1998). Internalization (and thus elimination) of *P. aeruginosa* is also hampered in F508del expressing cells (Pier et al. 1996).

Thus, the F508del mutation and possibly other variations in CFTR may provide a selective advantage in

Electronic Supplementary Material is available for this article at http://dx.doi.org/10.1007/s00439-005-0005-0

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case of exposure to salmonellae causing (para)typhoid fever, yet provide a selective disadvantage to infections with, for instance, *P. aeruginosa*, that require CFTR for elimination.

We hypothesized that F508del will be present at a lower rate in typhoid fever patients than controls. To test this hypothesis we examined the presence of F508del in a typhoid endemic country, Indonesia (Vollaard et al. 2004). No data has been published on the prevalence of CF or the CFTR mutation spectrum in Indonesia and CF is thought to be extremely rare in Indonesia (Dr. Taralan Tambunan, personal communication). In order to determine whether F508del is present in the Indonesian population for selection by *S. typhi* (and possibly *S. paratyphi*), we analyzed the presence of this mutation in 775 individuals enrolled in a case-control study into the epidemiology of (para)-typhoid fever in Jakarta, Indonesia. Cases ($n = 116$) were patients with blood-culture confirmed *S. typhi* ($n = 90$) or *S. paratyphi* ($n = 26$) infection. Fever controls ($n = 337$) were patients with other bacteria as the cause of fever or no growth of bacteria. Selection of patients and random community controls ($n = 322$) has been described elsewhere (Vollaard et al. 2004). All protocols are available on request.

Consistent with the apparently very low incidence of CF in Indonesia the common CFTR mutation F508del was not present in any of the subjects. The estimated prevalence of F508del heterozygotes is therefore <0.13%. Mutations in CFTR have thus far been analysed in few Asian populations. CF is extremely rare in Japan, and in the few Japanese CF patients who were analysed, the F508del mutation was absent (Yamashiro et al. 2004). Although the incidence of CF in India is unknown, CF appears to be somewhat more common and the F508del mutation was found in 19% of CF chromosomes (Kabra et al. 2003). The low prevalence of F508del in Indonesia may be a consequence of a very limited introduction of this mutation into the Indonesian population. However, since the overall CF incidence is also extremely low, another explanation may be that in Indonesia and other countries with a low CF incidence a different, locally endemic infectious disease provides a selection against CFTR mutations. This would be a pathogen that, like *P. aeruginosa*, depends on CFTR for elimination.

Based on the above information, the infrequent CFTR mutations are unlikely to contribute to susceptibility or resistance to typhoid fever in the Indonesian population. However, more subtle genetic variations can also reveal associations between a gene and susceptibility to disease when a functional link exists. Therefore, we continued to analyze two highly polymorphic microsatellites, IVS17bCA and IVS8CA, in intronic regions of the CFTR gene.

In the IVS17bCA analysis we observed 10 alleles. The predominant allele (94%) has 13 CA repeats. This IVS17bCA polymorphism is not polymorphic enough to differentiate between groups, accordingly no associations could be observed between any IVS17bCA allele or genotype and susceptibility to (para)typhoid fever (data available upon request).

In the IVS8CA analysis we also observed ten alleles (Table 1). The two most common ones were 181 and 183 bp and because the other alleles were so infrequent we grouped them as 'other'. The alleles are in Hardy Weinberg Equilibrium, in the combined groups (2-sided P-value = 0.50) as well as in the separate groups. IVS8-CA displayed a heterozygosity of 51% (in community controls).

When testing for homogeneity using the χ^2 test for the IVS8CA alleles 181 or 183 alleles versus 'other', we observed a highly significant ($P = 0.005$, $P = 0.015$ after correction for multiple testing) difference between the cases and community controls but not between community controls and fever controls ($P = 0.275$). The odds ratio (OR) for developing (para) typhoid fever when having 'other' alleles than the two common ones is 2.5 (95% CI: 1.4–4.8). Testing for homogeneity for the two genotypes 181181 or 181183 versus 'other' genotypes (Table 2) shows a significant difference ($P = 0.003$, $P < 0.01$ after correction for multiple testing) between cases and community controls for carriers of 'other' genotypes. This difference was more pronounced ($P < 0.0001$) when comparing typhoid cases only (infected with *S. typhi*, not with *S. paratyphi*) to community

Table 1 Allele distribution of CFTR IVS8 CA repeat

Allele (bp)	CA_n	Cases	Typhoid cases only	Community controls	Fever controls
175	13	1 (0.4)	1 (0.6)	0 (0)	0 (0)
177	14	9 (3.9)	8 (4.5)	11 (1.7)	12 (1.8)
179	15	0 (0)	0 (0)	2 (0.3)	1 (0.1)
181	16	118 (51.3)	91 (51.1)	375 (58.4)	403 (60.1)
183	17	93 (40.4)	72 (40.4)	245 (38.2)	235 (35.1)
185	18	2 (0.9)	1 (0.6)	4 (0.6)	8 (1.2)
191	21	1 (0.4)	1 (0.6)	0 (0)	2 (0.3)
193	22	3 (1.3)	3 (1.7)	0 (0)	4 (0.6)
195	23	1 (0.4)	0 (0)	3 (0.5)	5 (0.7)
197	24	2 (0.9)	1 (0.6)	2 (0.3)	0 (0)
total		230	178	642	670

Of both the cases and community controls one sample failed in the PCR. Between brackets the percentages are indicated

Table 2 Genotype distribution of CFTR IVS8 CA repeat

Genotype	Cases	Typhoid cases only		Community controls		Fever controls
181/181 or 181/183	73 (63)	57 (64)		251 (78)		262 (78)
Other genotypes	42 (37)	32 (36)		70 (22)		73 (22)
Total	115	89		321		335
	←	$P = 0.003^a$		→		
	←			$P = 0.003$		→
				←	$P = 0.996$	→
		←	$P < 0.0001^b$	→		

Between brackets the percentages are indicated
[a]OR = 2.1, 95% CI: 1.3–3.3 [b]OR = 2.6, 95% CI: 1.6–4.3

controls (OR = 2.6). The results show that the genotypes 181/181 and 181/183 have a protective effect towards susceptibility to typhoid fever.

While we found the IVS8CA alleles 181 (CA_{16}) and 183 (CA_{17}) in combination with IVS17bCA allele 136 (CA_{13}) to be present in 90% of the Indonesians tested, these combinations are present in only 64% of normal Caucasians tested (Morral et al. 1996). This could be an indication of selection by different pathogens in the two populations, or may be due to causal events and genetic history

Salmonella typhi produces type IVB pili which are required for bacterial self-association. These pili also bind to CFTR (Tsui et al. 2003) and may therefore be an important factor in entering the epithelial cells. Note, the *pilS* operon encoding these pili is also present in *P.aeruginosa*, although it is not known whether these pili are required for binding to CFTR and subsequent internalization.

In conclusion, although we were unable to attest or refute whether a F508del heterozygote advantage is due to selection through (para)typhoid fever, we did find a significant association between a CFTR polymorphism and (para)typhoid fever. Although it is also possible that the association we found is due to a linked gene, these genetic analyses suggest that, in accordance with CFTR's role in *S. typhi* infection in laboratory experiments (Pier et al. 1996), (Pier et al. 1998), CFTR is a factor in the susceptibility to *S. typhi* infection in the human population.

Acknowledgements We thank all patients, their families and population controls that participated in this study. We thank the physicians, nurses and technicians of the participating health centers in Jakarta for their cooperation. Financial support was provided by the Royal Netherlands Academy of Arts and Sciences (KNAW).

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Epidemiological analysis of typhoid fever and paratyphoid fever in Jakarta: phenotypic analysis versus strain typing by selective restriction fragment amplification analysis AFLP of *Salmonella typhi* and *Salmonella paratyphi* isolates.

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Submitted for publication

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Abstract

Typhoid and paratyphoid fever are a major health issue in developing countries. In a risk factor analysis study in Jakarta, we found that typhoid and paratyphoid fever follow distinct routes of transmission. To elucidate routes of transmission in more detail and enable clustering of cases, risk factor analysis should be complemented with microbiological methods that distinguish between circulating strains of *Salmonella typhi* and *Salmonella paratyphi*. Herein, we compared locally available biochemical and antibiotic susceptibility profiling with high resolution strain typing by whole genomic fingerprinting using selective restriction fragment amplification by AFLP^{TM}. Overall, the strains of *Salmonella typhi* (n=82) and *Salmonella paratyphi* (n=21) isolated in the surveillance study in Jatinegara, Jakarta, were remarkably homogeneous. With few exceptions, the strains of both species could be allocated to two major groups only, by either antibiogram typing and biochemical characterization or by state-of-the-art AFLP. The findings indicate that both *Salmonella typhi* and *Salmonella paratyphi* are highly homogeneous species; even high resolution typing by AFLP does not provide insight into how many strains circulate within the population and does not permit to assess strain transmission at the individual local level in Jatinegara, Jakarta.

Introduction

Typhoid and paratyphoid fever are a major health issue in many developing countries. *Salmonella enterica* serovar Typhi (*Salmonella typhi*), the etiologic agent of typhoid fever, globally accounts for over 20 million cases and more than 200.000 deaths each year (1). *Salmonella enterica* serovar Paratyphi A, B or C (*Salmonella paratyphi* A, B or C) that is the cause of paratyphoid fever, has a disease presentation similar to that of typhoid fever albeit milder, and globally accounts for more than 5 million cases per year (1).

In a passive surveillance study in Jakarta, we found that risk factors for typhoid fever are mainly within the household and those for paratyphoid fever are mostly outside the household of the cases (20). Distinct risk factors may implicate different modes of transmission and, consequently, suggest divergent public health interventions. To further elucidate the route of transmission of a pathogen and enable epidemiological clustering of individual cases, analysis of risk factors should be complemented with microbiological methods that distinguish between circulating strains of *Salmonella typhi* and *Salmonella paratyphi*.

Antibiotic susceptibility testing of bacteria has become a standard procedure in hospitals in Indonesia and also in many other developing countries. Antibiotic susceptibility profiles

and biotypes obtained by use of commercial identification systems are frequently used as a presumptive epidemiological marker. However, biochemical and antibiotic susceptibility profiles do not allow for high resolution strain typing, and can only be of any use if the microorganisms differ substantially from one another. To address this issue, several molecular methods have been used for typing Salmonella strains. Of these, plasmid profiling of *Salmonella* (para) typhi appeared unsuitable, because only a small proportion of strains (10%) contained plasmids (4). Vi phage typing has also been applied, but it is technically demanding, while analysis of cell envelope proteins revealed only minor differences between strains (5). In recent years, macro restriction analysis using Pulsed Field Gel Electrophoresis (PFGE) has been used successfully to analyse *Salmonella typhi* isolates for outbreak as well as general epidemiological reasons (6-13). Using multilocus enzyme electrophoresis, a method widely applied in bacterial population genetics in the 1980s and 1990s, *Salmonella typhi* was found to be a highly homogeneous microorganism (2). Another method, AFLP™, a DNA fingerprinting technique based on the selective PCR amplification of restriction fragments of genomic DNA (14), has been used in genotypic analysis of several species of bacteria (15-17). AFLP has the advantage of being more readily adapted for automation as compared to PFGE so that large numbers of strains can be studied (18). Recently, AFLP has also been used for genotypic characterization of *Salmonella typhi* (19). The aim of the present study was to set up and apply AFLP to complement our previous study on risk factors of typhoid and paratyphoid and elucidate in more detail the transmission route of *Salmonella typhi* and *Salmonella paratyphi* in the Jatinegara district, Jakarta. In addition, the concordance between biochemical and antibiotic sensitivity profiling and AFLP was evaluated.

Material and Methods

Bacterial isolates

Eighty-two isolates of *Salmonella typhi* and twenty-one of *Salmonella paratyphi* A were obtained by blood culture of patients with 3 or more days of fever in an epidemiology study in Jatinegara, Jakarta (20). Two additional isolates from the Laboratory of Clinical Microbiology, Leiden University Medical Center (LUMC), one isolate from a local hospital (Rijnland Hospital, Leiderdorp), Netherlands, five isolates from different parts of the world by courtesy of National Institute of Public Health and Environment in Netherlands (RIVM), and 5 isolates from a previous study in Sulawesi, Indonesia (21) by courtesy of Royal Tropical Institute, in Netherlands (KIT). The number of isolates and their origin are given in Table 1 and Table 2.

Table 1 Number and sources of isolated

Source	Serovars	N	Received from
Jatinegara, Indonesia	Salmonella typhi	82	Study area
	Salmonella paratyphi A	21	
	Salmonella spp.	2	
	Hafnia alvei	1	
	Citrobacter freundii	1	
Sulawesi, Indonesia	Salmonella typhi	2	KIT
	Salmonella spp.	3	
Ghana	Salmonella typhi	2	LUMC
India	Salmonella typhi	1	RIVM
	Salmonella spp.	1	
Pakistan	Salmonella typhi	1	RIVM
Morocco	Salmonella typhi	1	RIVM
	Salmonella spp.	1	
Unknown	Salmonella typhi	2	LUMC/Rijnland ZH

Table 2 Characteristics of patients infected by Salmonella in Jatinegara, Jakarta, Indonesia

Salmonella groups from AFLP	Age of patients Mean (SD)	Days of Fever Mean (SD)	Days of inactivity Mean (SD)	Type of treatment Ambulatory	Hospitalized
Salmonella paratyphi A group 1	21.4 (13.1)	7.5 (7.6)	8.8 (8.8)	13	0
Salmonella paratyphi A group 2	28.8 (15.7)	6.3 (4.2)	14.3 (8.4)	5	1
Salmonella typhi group 1	20.7 (11.6)	6.8 (5.8)	10.6 (9.3)	61	10
Salmonella typhi group 2	19.9 (8.9)	7.8 (4.0)	12.8 (11.8)	10	1
Salmonella spp.	50	0	0	1	0
Other bacteria	34 (12.1)	0	0	2	0

Table 3. Antibiotic sensitivity of Salmonella typhi, S. paratyphi and nontypoidal Salmonella spp by disc diffusion (median and range of mm inhibition zones)

N*	API-code	Salmonella	Amoxicillin	Amox-clavulan.	Cotrimoxazole	Chloramphenicol	Ciprofloxacin
21	4004540	typhi	39.5 (38-42)	40 (38-42)	45 (36-50)	36 (33-40)	42.5 (39-48)
91	4404540	typhi	39 (32-42)	39 (36-40)	45 (40-50)	36 (32-40)	42 (36-46)
15	0104552	paratyphi	36.5 (32-38)	36.5 (33-37)	44 (36-48)	34 (32-38)	40 (29-42)
26	6704552	non (para)typhi	35 (32-37)	34.5 (32-37)	43 (41-50)	32 (30-37)	39.5 (37-42)
16	6704752	non (para)typhi	34 (33-36)	34 (33-36)	44.5 (44-46)	32 (31-36)	40(34-42)

N*: numbers differ from those in the text, because from some cases, more than one colony of S. typhi or S. paratyphi was stored at -700C. Most of the time storage decisions were based on subjective arguments of minor macroscopic differences.

Bacteria identification and antibiotics sensitivity test

All isolates were recultured on MacConkey agar no. 3 (Oxoid, Hampshire, UK). For the purpose of the present study, identification of isolates was confirmed using API-E 20 strips (Biomérieux, Marcy l'Etoile, France) and agglutination test using polyvalent, anti O-9, anti dH and anti-Vi Salmonella agglutinating sera (Abbott-murex, Dartford, UK). For antibiotic susceptibility, isolates were cultured on Iso-Sensitest agar (Oxoid, Hampshire, UK) and assayed for sensitivity to amoxicillin, amoxicillin-clavulanic acid, ceftazidime, meropenem, gentamicin, chloramphenicol, cotrimoxazole, and ciprofloxacin by disc diffusion testing, using Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark). Strains were grouped according to their antibiotic susceptibility profile as derived from the zone diameter of inhibition zones. Squared Euclidian distances were used as similarity measure and UPGMA as the clustering algorithm.

DNA isolation and AFLP method

For DNA isolation, all isolates were cultured onto Iso-Sensitest agar (Oxoid, Hampshire, UK). Bacterial genomic DNA was isolated using a QIAamp DNA mini kit (QIAGEN Benelux, Venlo, Netherlands). The AFLP fingerprinting was performed essentially as described previously (22). Optimization was done using eight sets of primers: Cy5-labelled *Eco*RI primer and *Mse*I+G primer (set 1, 0-G), Cy5 labelled *Eco*RI primer and *Mse*I+C primer (set 2,0-C), Cy5-labelled *Eco*RI+C primer and *Mse*I primer (set 3,C-0), Cy5-labelled *Eco*RI+C primer and *Mse*I+G primer (set 4,C-G), Cy5-labelled *Eco*RI+A primer and *Mse*I+A primer (set 5,A-A), Cy5-labelled *Eco*RI+C primer and *Mse*I+G primer (set 6,C-G), Cy5-labelled *Eco*RI+AT primer and *Mse*I primer (set 7, AT-0) and finally, Cy5-labelled *Eco*RI+C primer and a *Mse*I+A primer (set 8,C-A) (A, C, G and T represent selective nucleotides). The ALFexpress II DNA analysis system (Amersham Biosciences, Uppsala, Sweden) was used for fragment separation and data recording. The profile of *Acinetobacter baumanii* (strain LUH 1091) from the collection of the Leiden University Medical Center was used as external reference for normalization of the gel. Fragments of 50-500 bp were subjected to cluster analysis by using BioNumerics software package, release 2.5 (Applied Maths, St-Martens-Latem, Belgium). The Pearson product-moment coefficient (r) was calculated and used as the measure of similarity and the unweighted pair group method using arithmetic averages (UPGMA) was used for grouping.

Results

Biochemical and antibiotic sensitivity testing of isolates

By the use of API20, a total of four biochemical profiles could be distinguished that identified 82 isolates as *Salmonella typhi*, whereas four other profiles identified 21 isolates as *Salmonella paratyphi* A. Five additional control isolates with three different profiles were

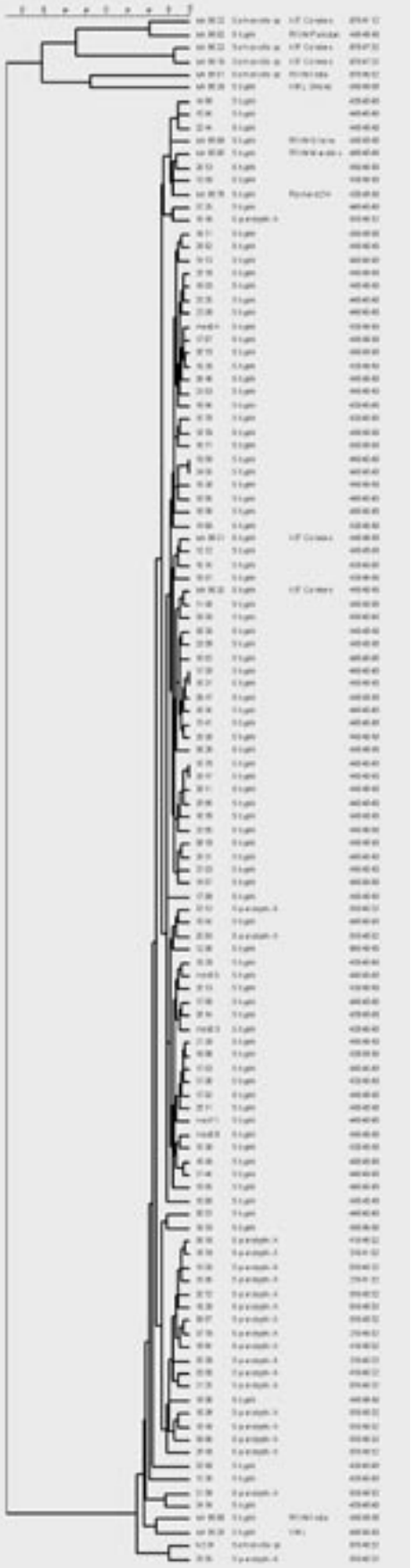


Figure 2. Banding patterns of several isolates using different sets of primers. Primer set OG and primer set AA which were previously found to be the most discriminatory primer sets did not allow differentiation in our system. Primer sets ATO and CA were the most discriminatory primer sets in our study.

Figure 1. The dendrogram from hierarchical clustering based on phenotypic characteristics, i.e., API number and antibiotics susceptibility profiles. All Salmonella typhi isolates and Salmonella paratyphi A isolates are clustering at a very high level.

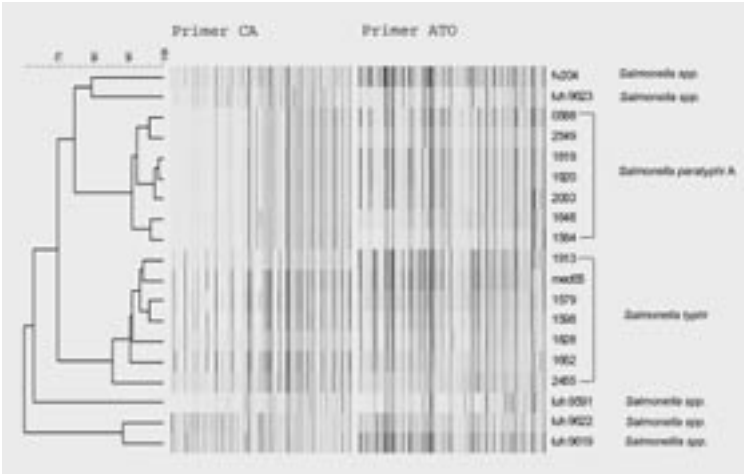
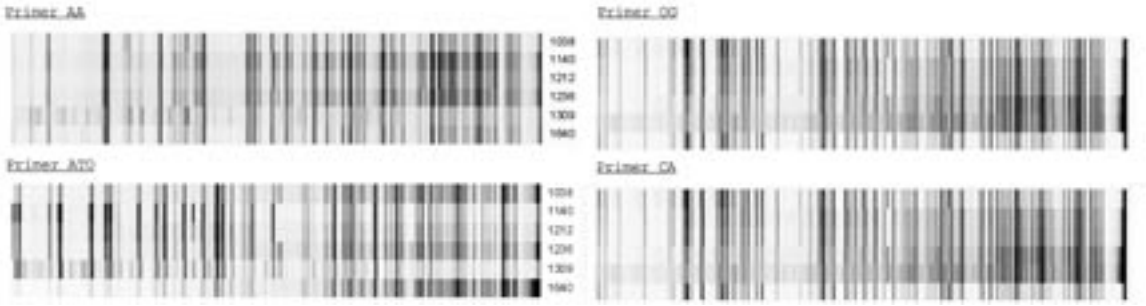


Figure 3. Composite banding patterns resulting from primer sets CA and ATO from different serovars of Salmonella in our study.



correctly identified as nontyphoidal *Salmonella species*.
The *Salmonella typhi* and *Salmonella paratyphi* A isolates from our study area in Jatinegara, Jakarta, as well as the control *Salmonella typhi* strains from Sulawesi were susceptible to all of the eight antibiotics assayed in this study and no drug resistance was encountered (Table 3). On basis of biochemical and antibiotic susceptibility profiling, the 82 strains from the Jatinegara study area and seven control strains from other geographic areas grouped together at very high similarity level, i.e., of 95%. A second small cluster comprised of six strains including four nontyphoidal *Salmonella* species strains and only two *Salmonella typhi* strains: especially the nontyphoidal *Salmonella* strains were more heterogeneous with respect to antibiotic susceptibility and clustered together at about 85% (data not shown).

Optimization of AFLP methods

Eight different primer combinations were compared for their discriminatory potential. For this purpose six strains from different geographic origin were used as a test set. It was concluded that primer set 7, AT-0 and set 8, C-A) had the best discriminatory power. Of note, primer set 5 (A-A) and set 1 (0-G) which have been described to be useful for discrimination between *S. typhi* strains in a previous study (19) did not allow sufficient differentiation in our system (Figure 2). To check the stability and reproducibility of banding patterns, we randomly picked one *Salmonella typhi* strain and one *Salmonella paratyphi* A strain and performed DNA isolation, restriction-ligation procedure and PCR five times. These strains produced consistent banding patterns: the banding patterns of the *Salmonella typhi* clustered at 84.3%, while the banding patterns of *Salmonella paratyphi* A clustered at 81.6%. The AFLP for all other isolates was done by using primer set 7 and 8. Thus, in the final experiments, strains were typed using these two primer sets.

Grouping of strains on the basis of their AFLP fingerprints

For the 82 *S. typhi* and 21 *S. paratyphi*, the number of fragments of the combined profiles generated with primer set 7 and 8 ranged from 119-233. Overall, the pattern of variation was strikingly low within each serovar (see Figure 3 for a representative selection of the strains). At a grouping level of 85%, *S. paratyphi* A, *S. typhi* and nontyphoidal *Salmonella spp* could be separated, while all species were linked together at of ~60%(Figure 3). Figure 4 and figure 5

Salmonella typhi

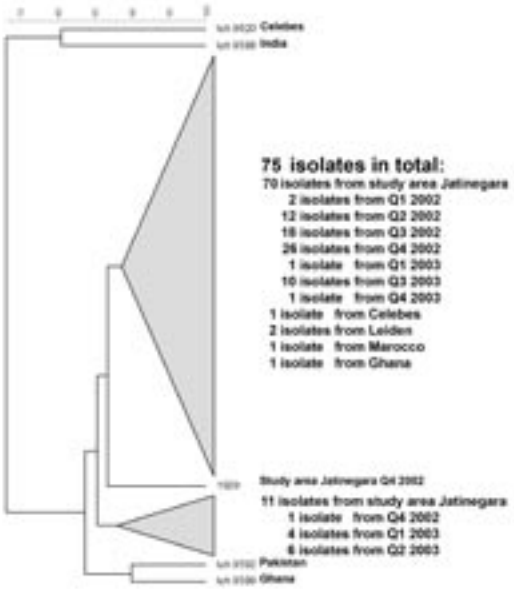


Figure 4. Clusters of *Salmonella typhi* in the study are shown in the figure together with the strains that were isolated from the study area later in 2003. Two isolates from Leiden are one isolate from LUMC and one isolate from Rijnland Hospital.

Salmonella paratyphi A

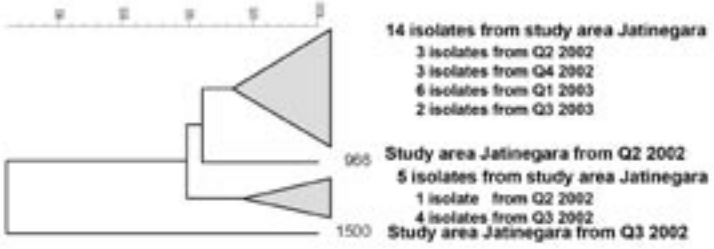


Figure 5. Clusters of *Salmonella paratyphi A* in the study are shown in the figure together with the strains that were isolated from the study area later in 2003.

Figure 6. The distribution of *Salmonella typhi* and *Salmonella paratyphi A* in the study area. The outlier strains are not marked in the map.

shows the grouping of all *Salmonella typhi* and *Salmonella paratyphi* A isolates analyzed in the study. At a cut off level of 85%, *Salmonella typhi* isolates from the study area could be divided into two groups and one single isolate, while four isolates from other geographic areas were linked with these strains at 73%. Of note, the major cluster of *S. typhi* (Figure 4) not only comprises strains from Jatinegara but also from other parts of Indonesia (Celebes) as well as isolates of other countries (Leiden, Marocco and Ghana) which are clustered together at 88%. *Salmonella paratyphi* A isolates could be divided into two groups and a single isolate grouping at 89% except for one isolate which is linked at ~76%. (Figure 5).

Characteristics of each group of Salmonella strains

Figure 6 shows the distribution of the *Salmonella* isolates in the study area as clustered by AFLP. There was one household in the area in which five cases of typhoid fever occurred; all *Salmonella* isolates from this household belonged to one group in the AFLP dendrogram (data not shown). In addition, there were three cases from the same RT (*Rukun Tetangga*, the smallest community unit in Jakarta) that also belonged to one AFLP.
Of note, age of the patient, days of fever before enrolment into the study, the number of days of inactivity and whether or not the patient was treated as ambulatory or hospitalized patients did differ for the *Salmonella* AFLP clusters identified.

Discussion

The main finding of this study is that *Salmonella typhi* and *Salmonella paratyphi* are highly homogeneous bacteria of which –at least in Jakarta– only a highly limited number of strains circulate. This strain homogeneity was evident from the almost identical biochemical and antibiotic susceptibility profiles of the isolates as well as the lack of diversity between isolates found on AFLP testing. Thus, at least in Jakarta, Indonesia, not even high resolution typing by AFLP does permit assessment of strain clustering and the analysis of transmission at the local level.
The study was initiated to assess the diversity and elucidate further possible routes of transmission of *Salmonella* strains in an endemic area in Indonesia. The microorganisms were collected within the framework of a risk factor surveillance study in which over a period of almost two years we enrolled ambulatory patients as well as hospitalized patients in the Jatinegara area of Jakarta (20). Because this area represents a small part of Jakarta only, we included various unrelated isolates from other parts of Indonesia, as well as other geographical regions. All isolates were recultured and identification given by the sources was confirmed.
Unlike in studies from other countries like India and Pakistan where multi-drug resistance

of *Salmonella* is an issue, the *Salmonella* isolates in our study were susceptible to the antibiotics assayed, representing the most commonly clinically used antibiotics for typhoid fever. Not only the antibiogram was highly similar among the isolates: variation in phenotypic (API-) profiles was similarly limited. Hence, tentative typing and clustering of isolates based on easily obtained biochemical profiles or the antibiogram to study clustering and transmission in the endemic area is not possible. Next, the AFLP method as performed in our laboratory (Leiden University Medical Center) by semi-automated laser fluorescence for fragment detection was optimized for *S. typhi* and *S. paratyphi* A. To assess the discriminatory power, isolates from different countries, and isolates from a different part of Indonesia were used as unrelated controls. Using eight combinations of primer sets, we concluded that the primer sets *Eco*RI+AT and *Mse*I primer set *Eco*RI+C and *Mse*I+A were the most discriminatory. Using these primers, we found that the strains were strikingly homogeneous as derived from the linkage levels, i.e., for *S. typhi* 73% and for *S. paratyphi* A 76%. The *Salmonella typhi* isolates from the study area as well as the *Salmonella paratyphi* A isolates clustered into two major groups only.

Salmonella typhi and *Salmonella paratyphi* A belong to the *Salmonella enterica* subspecies *enterica*. They are members of the O antigen serogroups, D1 and A, respectively (23). Multilocus enzyme electrophoretic studies have shown that the diversity of *S. typhi* and *S. paratyphi* A is limited which was assumed to be related to the high degree of host adaptation of these serovars (2). Sequence analysis of 3336 bp of seven housekeeping genes of a global collection of 26 *Salmonella typhi* isolates revealed only three polymorphic sites which allocated the isolates to four sequence types (24). Based on these findings it was estimated that *S. typhi* is a recent clone of approximately 50.000 years old. Although *Salmonella paratyphi* A and *Salmonella typhi* belong to different serogroups, they have genomes of highly similar gene content, including many pseudogenes (25). The finding that *Salmonella paratyphi* A contains fewer pseudogenes than *S. typhi* may indicate that the former may have arisen more recently than *S. typhi*, provided that accumulation of pseudogenes has proceeded at a similar rate in both serovars. The AFLP profiles from our study corroborated the homogeneity of *Salmonella typhi* and *Salmonella paratyphi* A strains as found in the cited studies (25). In line with the report that *Salmonella paratyphi* A is a more recent serovar (26) than S. typhi, the *Salmonella paratyphi* A strains show more similarity in banding profiles than the *Salmonella typhi* stains (i.e., 78%, SD 5% versus 72%, SD 6%).
Furthermore, there was no correlation between the AFLP grouping and morbidity, as indicated by age of the patients, need for hospitalization, days of fever or days of inactivity. This finding is in line with a previous study on bacterial isolates from Papua New Guinea that could not find a relation between particular *Salmonella typhi* assessed by AFLP with its virulence (19).

In conclusion, we set up and validated AFLP through automated laser fluorescence to study its use as a tool in the epidemiology of *S. typhi* and *S. paratyphi A.* The study was initiated after standard biochemical and antibiotic susceptibility profiling had proven to be of little value in clustering and distinguishing between clinical isolates in the Jatinegara study area. However, the strains isolated in the surveillance study were strikingly homogeneous in their AFLP profile also, although two major clusters (i.e., types) could be distinguished in both serovars. Given the limited variability no relevant correlation could be made between these two types and clustering of cases, transmission route, or disease patterns. Thus, the lack of variability between isolates of *S. typhi* (and *S. paratyphi*), a characteristic that appears to linked to these microorganisms in particular and that isn't confined to Jakarta, limits an in-depth analysis of strains circulating within the population and does not permit to assess strain transmission at the local level.

Acknowledgement

Financial support was provided by the Royal Netherlands Academy of Arts and Sciences (KNAW). We thank the physicians, nurses and technicians of the participating health centers in Jakarta for their cooperation: Mitra international hospital and microbiology lab, Budhi Asih, St. Carolus, Medistra and Persahabatan hospital, all puskesmas' in Jatinegara and local private practitioners.
We thank Wim Wannet from the National Institute of Public Health and Environment, the Netherlands (RIVM), Henk Smits from the Royal Tropical Institute, the Netherlands (KIT) and Mochammad Hatta from the Department of Microbiology (Hasanuddin University, Makassar, Indonesia), the Clinical Microbiology Department of Leiden University Medical Center and Rijnland Ziekenhuis (Netherlands) for providing *Salmonella* isolates.
We thank our research assistants in Jakarta, and Leo G. Visser and Henry A.G.H. van Asten for their contribution to the project.

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8

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

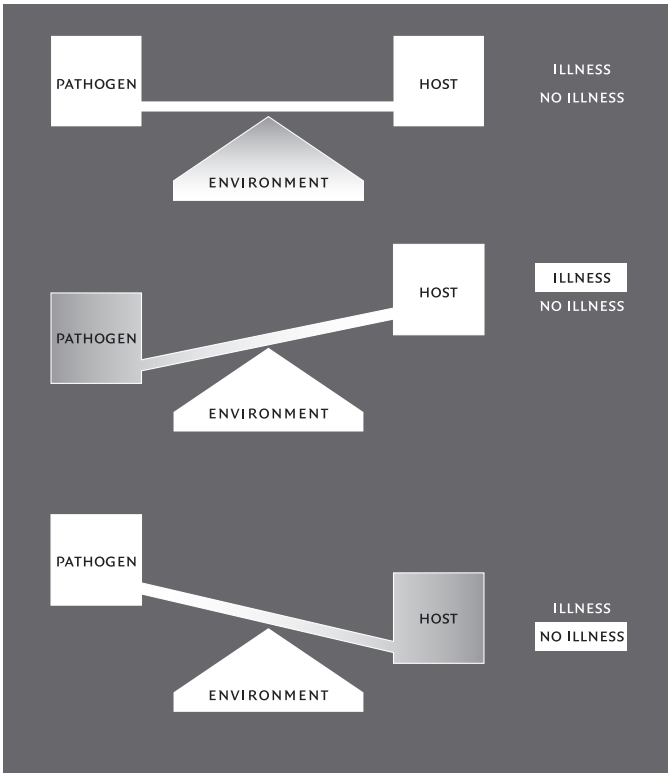
of the megacity of Jakarta, with health problems related to poverty, pollution, flooding, lack of sewage and human waste disposal, and lack of safe water and hygiene. Likely, just like in Western societies a century ago, solving Jakarta's environmental health problems will require commitment to pay for 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

Nederlandse samenvatting

Dit proefschrift beschrijft de wisselwerking tussen drie determinanten van de uitkomst van een buiktyfus- en paratyfusinfectie: de bijdrage van omgevingsfactoren en van genetische factoren van de menselijke gastheer in combinatie met eigenschappen van de bacterie. *Salmonella typhi* en *S. paratyphi* veroorzaken een ernstig ziektebeeld (aangeduid met respectievelijk buiktyfus en paratyfus), met koorts die meerdere weken kan aanhouden en, indien de patiënt niet behandeld wordt, een mortaliteit van circa 15% heeft. Naast de koorts, malaiseklachten en gewichtsverlies, wordt de infectie gekenmerkt door zweren van de dunne darm waaruit een darmbloeding of zelfs darmperforatie kan ontstaan.
Bij circa één op de tien patiënten treedt na schijnbaar herstel een "relapse", een tweede koortsepisode op. Hierin onderscheiden *S. typhi* en *S. paratyphi* zich van de vele andere

Salmonella bacteriën die meestal een "self-limiting" gastro-enteritis veroorzaken, of hooguit een ernstige ziekte veroorzaken bij patiënten met gestoorde afweer. *S. typhi* heeft nog een unieke eigenschap: deze bacterie kan alleen bij de mens een infectie veroorzaken. Een klein deel van deze patiënten, rond de vier procent wordt na infectie een chronische drager ("carrier"). Dit betekent dat ze vele jaren in de ontlasting *S. typhi* uitscheiden, en daarmee een bron van besmetting kunnen zijn voor andere mensen, vooral als ze betrokken zijn bij voedselbereiding ("Typhoid Mary") . Omdat buiktyfus alleen mensen infecteert, is het in principe mogelijk de infectieziekte uit te roeien, zoals eerder met pokken is gelukt, mits er voldoende bekend is over de wijze van overdracht. Al ruim 100 jaar weet men dat *S. typhi* en *S. paratyphi* bacteriën in de ontlasting van patiënten en dragers worden uitgescheiden en dat daardoor transmissie in de bevolking in stand wordt gehouden. De overdracht treedt op zolang patiënten persoonlijke hygiëne veronachtzamen en/of ontlasting in drinkwater of voedsel terecht kan komen door een defect waterleidingssysteem. In de ontwikkelde Westerse landen werd met de aanleg van riolering gescheiden van de drinkwatervoorziening, zorg voor persoonlijke hygiëne en de opsporing van dragers de faeco-orale transmissie van mens-tot-mens doorbroken en zijn buiktyfus en paratyfus tot importinfectieziekten geworden. In de rest van de wereld komt immers nog veel buiktyfus en paratyfus voor: jaarlijks treden naar schatting ruim 21 miljoen gevallen van buiktyfus en 5 miljoen gevallen van paratyfus op.
Ondanks het feit dat de omgevingsfactoren en aspecten van het menselijke gedrag, welke bijdragen aan de transmissie van beide infectieziekten, bekend zijn, althans in theorie, is in een ontwikkelingsland zoals Indonesië niet duidelijk welke van die factoren en aspecten de belangrijkste bijdrage leveren aan vóórkomen van de ziekten. Dergelijke kennis is echter onontbeerlijk om kosten-effectieve interventies op te stellen en uit te voeren, vooral in landen waar het budget voor gezondheidszorg nu eenmaal beperkt is. Om die zwakste schakel ('weak link') in de overdracht van *S. typhi* en *S. paratyphi* in kaart te brengen is in

Jakarta een bevolkingsonderzoek verricht.
Jakarta is een megacity met ruim 12 miljoen inwoners. In een oostelijk district van deze stad, Jatinegara, een gebied van 10.6 km^2 met circa 260.000 inwoners, werd gedurende anderhalf jaar bij patiënten die zich melden bij een van de lokale gezondheidscentra ('puskesmas') en drie of meer dagen koorts hadden een bloedkweek afgenomen. Hierbij bleek dat één op elke tien van zulke patiënten een buiktyfus- of paratyfusinfectie hadden. Karakteristieken van deze patiënten werden vergeleken met die van personen met koorts ontwikkeld door een andere reden dan buiktyfus- of paratyfusinfectie, en die van bijna 400 willekeurige, gezonde controles die in hetzelfde gebied woonden. Op die manier werd nagegaan welke risicofactoren in gedrag of omgeving van invloed waren op de buiktyfus en paratyfus. Ook konden deze groepen op immunologische en genetische eigenschappen vergeleken worden om na te gaan of er naast omgevingsfactoren ook andere verklaringen aan te wijzen zijn voor verschillen in gevoeligheid voor infectie of ernst van ziekte. Tenslotte werd onderzoek verricht naar eigenschappen van de bacteriën zelf: welke typeringsmethoden kunnen toegepast worden om te helpen bij de epidemiologische analyse van overdracht en verspreiding?

Omgevingsfactoren en buiktyfus en paratyfus in Jakarta

In het onderzoeksgebied Jatinegara werden 1019 patiënten geïncludeerd tijdens de surveillance periode van anderhalf jaar: 88 gevallen van buiktyfus, 26 van paratyfus en 905 patiënten met koorts door andere reden, de zgn. "fever controls". Deze patiënten én de gezonde controles werden thuis bezocht. Bij dit bezoek werd een vragenlijst ingevuld, en monsters van bloed, faeces en drinkwater verkregen. Uit de analyse bleek dat buiktyfus met name binnen huishoudens ('eet- en hygiëne unit') werd overgedragen, door personen in dat huishouden die recent een buiktyfusinfectie doorgemaakt hadden, hun handen niet wasten en waarmee de overige leden het eten deelden van één en hetzelfde bord. In Indonesië eten veel mensen met hun handen. In het huishouden van veel buiktyfuspatiënten ontbrak bovendien een toilet.

Het krijgen van een paratyfusinfectie bleek geassocieerd met eten buitenshuis, en met overstroming van het huis. Veel Indonesiërs eten buiten de deur, omdat daar een gevarieerd en goedkoop dieet wordt aangeboden. Veelal goedkoper dan wanneer zelf gekookt wordt. Drinkwater, zelfs drinkwater uit de waterleiding, is in Jakarta vaak faecaal verontreinigd. Omdat mensen gewend zijn hun drinkwater voor consumptie te koken, was de mate van verontreiniging van drinkwater niet geassocieerd met buiktyfus of paratyfus. Deze gewoonte is een wenselijk en noodzakelijk gedragselement, en het gunstige effect daarvan zou benadrukt moeten worden, naast maatregelen gericht op persoonlijke hygiëne, zoals het wassen van handen met zeep. Door middel van zulke relatief simpele gedragsinterventies

zou de transmissie belangrijk gereduceerd kunnen worden, zeker zolang de kostbare aanleg van riolering en een centrale drinkwaterinfrastructuur in de sloppenwijken nog op zich laat wachten.
Er werden in de huishoudens geen chronische dragers ("carriers") geïdentificeerd onder de individuen die het voedsel bereiden. De bijdrage aan de overdracht van buiktyfus door chronische dragers bleek dus geringer dan die als gevolg van transiente, tijdelijke faecale uitscheiding door personen die herstelden van een doorgemaakte infectie.

Met gegevens van ziektesurveillance gekoppeld aan optimale individuele diagnostiek door bloedkweken werd inzicht verkregen over het vóórkomen van buiktyfus en paratyfus: slechts 10% van de personen met langdurige koorts bleek buiktyfus of paratyfus te hebben. Omdat de dokters in het onderzoeksgebied (en elders in Jakarta) zelden bloedkweken verrichten beschouwen zij –tenminste buiten het Dengue seizoen– de meeste van deze personen als buiktyfuspatiënten en stellen een behandeling in met antibiotica. Opmerkelijk was dat paratyfus een kwart van de systemische Salmonella infecties veroorzaakte en klaarblijkelijk met een opmars bezig is, iets dat ook in andere Aziatische landen gesignaleerd is. Praktisch gezien betekent dit dat dure massavaccinatiecampagnes tegen buiktyfus niet langer een gewenste strategie vormen, omdat buiktyfusvaccins specifiek gericht zijn tegen buiktyfus en gevallen van paratyfus niet voorkómen.
Paratyfus, en in mindere mate ook buiktyfus, bleek geassocieerd met straatvoedsel. Om dit nader te duiden werd een cross-sectioneel onderzoek verricht onder 74 verkopers uit restaurants en 128 personen met voedselstalletjes of -karren. Veel van de geteste personen bleek geïnfecteerd met darmparasieten, en bij een belangrijk aantal werden salmonella uit de faeces gekweekt. Ook bij deze groep zouden relatief eenvoudige interventies de overdracht van bacteriën door straatvoedsel kunnen inperken, omdat handwashygiëne en het gebruik van zeep in afwaswater in gebreke bleven bij de straatverkopers. Ook moet meer aandacht geschonken worden aan veilige (gekoelde) opslag van voedsel.

Genetische gastheerfactoren en buiktyfus en paratyfus in Jakarta

Een tweede aspect van het onderzoek betrof de gastheerreactie op een infectie, een complex gebeuren waarin meerdere typen afweercellen nauw moeten samenwerken. Salmonella bacteriën overleven in macrofagen van lever, milt en beenmerg, de eerste lijn van afweer tegen de bacteriën die erin geslaagd zijn de darmwand te passeren. In ons onderzoek hebben we enkele facetten van dit eerste contact nader onderzocht.
Allereerst werd duidelijk dat *Salmonella typhi* het chloridekanaal op de enterocyt, de zogenaamde Cystic Fibrosis Transmembrane conductance Regulator (CFTR), gebruikt om aan te hechten aan de darmwand, een eerste essentiële stap bij het binnendringen. Een kleine verandering in het genetische materiaal (een genmutatie) dat codeert voor het CFTR

kan zeer schadelijk zijn en tot de ernstige longziekte cystic fibrosis (CF) leiden. In het laatste geval heeft de patiënt van beide ouders een afwijkend gen geërfd, en heeft de genetische verandering tot gevolg dat er in het geheel geen CFTR op de membraan van cellen in longen, darm, etc, aanwezig is. Hierdoor kan geen chloride (en daarmee water) uitgescheiden worden op de slijmvlkiesoppervlakten en ontstaat taaislijmziekte. Omdat CF een ernstige ziekte is en zeker vroeger altijd resulteerde in de dood van een patiënt op jeugdige leeftijd is het opmerkelijk dat het afwijkende gen zo vaak in de bevolking vóórkomt (tot bij 3-5%). Zou het zo kunnen zijn dat de afwezigheid van CFTR (of verlaging van de hoeveelheid CFTR bij dragers van een afwijkend gen) enige bescherming biedt tegen een ernstige infectieziekte zoals buiktyfus? Uit ons onderzoek blijkt dat de in het Westen karakteristieke CF-mutatie in Jakarta niet voorkomt. Wel bleken enkele specifieke genetische variaties in het CFTR-gen geassocieerd met buiktyfus. Ofschoon de oorspronkelijke hypothese niet getest kon worden door ontbreken van de karakteristieke CF-mutatie in Indonesië, kon wel aangetoond worden dat kleine veranderingen in CFTR geassocieerd waren met buiktyfus. Blijkbaar speelt het CFTR eiwit dus een essentiële rol in de pathogenese van buiktyfus, ofwel door te fungeren als aanlegsteiger van de bacterie in de darm, ofwel als essentiële co-factor bij het doordringen van de darmwandcellen.

Naast het CFTR werd de rol onderzocht van genen die coderen voor pro-inflammatoire cytokines en hun receptoren, zoals TNF-α, IFN-γ, IL-1α, IL-1β, IL-12, IL-18, TNFR1, IFN-γR1, IL-1R, CASP1 en CRP. Deze cytokinen en hun receptoren spelen een belangrijke rol bij de samenwerking tussen macrofagen, T-lymfocyten en Natural Killer cellen in de immuunreactie tijdens buiktyfus en paratyfus. De interpretatie van de uitkomsten maakt duidelijk dat genetische verschillen in aanmaak van deze cytokines geen rol spelen bij het feit of iemand vatbaar is voor ziekte of niet. Hooguit spelen eventuele verschillen hierin een rol bij de consequenties daarná: de ernst van de ziekte, die b.v. bepaalt of iemand moet worden opgenomen of niet.

Daarnaast werd nagegaan of single nucleotide polymorfismen (SNPs; veranderingen van enkele basenparen in het erfelijk materiaal DNA) in het PARK2/PACRG gen geassocieerd is met buiktyfus of paratyfus. Eerder was vastgesteld dat mutaties in deze genen individuen verhoogd vatbaar maken voor lepra, net als salmonella een intracellulair pathogeen micro-organisme. De eiwitten die door het PARK2/PACRG gensysteem gecodeerd worden zijn van belang voor poly-ubiquitinatie van eiwitten in het cytoplasma van de afweercellen. Het gaat hier om het van een ubiquitinelabel voorzien van eiwitten, waarna deze geïdentificeerd en afgebroken kunnen worden door het proteasoom ('stofzuiger') in het cytoplasma. Dergelijke mechanismen zijn van groot belang bij de gerichte afbraak van bacteriële eiwitten (toxines) die het functioneren van de cellen beïnvloeden. Van verschillende SNPs in de PARK2/PACRG gen was PARK2_e01(-2599) geassocieerd met buiktyfus, net als in lepra,

terwijl andere SNPs, PARK2_e01(-697), rs1333955 en rs1040079 dat niet waren. Verder onderzoek moet verhelderen hoe de "proteasome-ubiquitination pathway" *S. typhi* en *S. paratyphi* helpt te overleven in de macrofagen.

Bacteriële factoren en buiktyfus en paratyfus in Jakarta

S. typhi en *S. paratyfi* hebben veel overeenkomsten in hun voorkeur voor de mens en hun ziekmakende eigenschappen, maar zijn toch twee verschillende micro-organismen, met in Jakarta verschillende transmissieroutes. Binnen de twee bacteriën zelf zijn ook weer meerdere varianten, subtypes, te onderscheiden, mits er analyses worden gedaan die voldoende discriminerend vermogen hebben. Dergelijke analyses kunnen helpen meerdere gevallen aan een bron te koppelen, en zijn essentieel bij de epidemiologische analyse van onderzoeksgegevens. Zo zijn er verschillen in gevoeligheid voor antibiotica te onderscheiden, al zijn in Jakarta slechts zeer weinig bacteriën multidrug-resistent (MDR). Deze methode helpt niet bij het verder onderverdelen in "subtypes", al is het natuurlijk gunstig te kunnen constateren dat buiktyfus en paratyfus in Jakarta met relatief simpele antibiotica nog steeds adequaat behandeld kunnen worden. De meeste van deze antibiotica zijn goedkoop en voldoende voorradig in de meeste gezondheidscentra.

Ook onze andere methoden, zoals biochemische tests en AFLP, bleken "subtypes" niet te kunnen onderscheiden. De in Jakarta circulerende buiktyfus en paratyfus- stammen zijn dus bijzonder homogeen, wat wellicht weerspiegelt dat kleine veranderingen in genetisch profiel, zoals gedetecteerd door AFLP, de bacteriële fitness zodanig verminderen dat er niet langer een infectie optreedt. Andere mogelijke verklaringen zijn dat er een centrale gemeenschappelijke bron voor infectie aanwezig is (minder waarschijnlijk vanwege de spreiding van infecties in plaats en tijd), of dat de gekozen analysemethodes onvoldoende sensitief zijn als discriminatoire test. Nieuwe testen moeten dus ontwikkeld en gevalideerd worden die wel de lokale verspreiding van specifieke "subtypes" kunnen ophelderen, om daarmee specifieke overdrachtskenmerken in kaart te brengen, die kunnen helpen bestrijdingsplannen te verfijnen.

Conclusie

S. typhi en *S. paratyphi* infecties hebben in de ontwikkelde landen geen voet meer aan de grond, dankzij de verbeteringen in riolering, drinkwatervoorziening, hygiëne en voedselveiligheid. De afname van incidentie werd bereikt vóór de introductie van antibiotica of vaccinaties. Ook in Jakarta is vandaag de grootste gezondheidswinst niet zozeer te bereiken door het uitbreiden van de gezondheidszorg, maar door verbeteringen in de eerstgenoemde omgevings- en gedragsfactoren.

Ons onderzoek maakt duidelijk dat adequate surveillance en risicofactoronderzoek noodzakelijk zijn om effectieve interventieprogramma's te ontwerpen. Veranderingen in incidentie van de ziekten als gevolg van grote, dure infrastructurele projecten kunnen voorafgegaan worden door een belangrijke afname als gevolg van aandacht voor persoonlijk (handwas)hygiëne, zowel in huishoudens als in de commerciële voedselbereiding. Er bestaat een grote behoefte aan nieuwe diagnostische testen (of introductie van bestaande bloedkweektechnologie) omdat een empirische behandeling met antibiotica van alle koortspatiënten ('verdenking buiktyfus') een grove overbehandeling inhoudt, en op termijn multi-drug resistentie induceert. Eventuele massavaccinatie-campagnes zullen de toename van paratyfus niet afremmen.

Op individueel niveau maakt niet zozeer de cytokine-reactie op infectie uit of iemand vatbaar is voor buiktyfus of paratyfus, maar blijkt de entree een bepalende factor met een belangrijke rol van CFTR hierin. Pas daarna komen cytokines in het spel, alsmede mutaties in het *PARK2/PACRG* gen, die een tolerant klimaat in de afweercellen creëren dat bacteriële overleving mogelijk maakt.

Over omgeving en gastheer moge meer duidelijk geworden zijn, maar de eigenschappen van de bacterie blijven nog onderbelicht, vooral omdat de juiste analysemethode ontbreekt om subtypes te differentiëren om daarna die kennis te kunnen koppelen aan specifieke risicofactoren. Wel weten we dat de behandelingsopties (nog) ruim voorhanden zijn, omdat multi-drug resistentie in Jakarta, in tegenstelling tot veel andere Aziatische landen, praktisch afwezig is. Mijn hoop is dat dit proefschrift een bijdrage levert aan het inzicht in buiktyfus en paratyfus, zowel wat de bestrijding ervan in Jakarta aangaat, als wat betreft de complexe communicatie over en weer tussen bacterie, omgeving en mens.

Pembahasan Umum

Tesis ini menggambarkan peran dari faktor lingkungan dan genetik inang serta karakteristik dari patogen sebagai determinan interaktif dari terjadinya demam tifoid atau paratifoid. Ahli epidemiologi JR Paul (Clinical Epidemiology, 1966) menggunakan perumpamaan dalam Matius 13:3-8, menyatakan bahwa penyakit infeksi dapat dianggap sebagai triad " bibit, lahan dan iklim". Pada demam tifoid, *Salmonella typhi* dan *Salmonella paratyphi* adalah bibitnya, inang dan lingkungan adalah lahan dan iklimnya. Ketiganya secara bersama-sama menentukan kemampuan patogen untuk berkembang di dalam populasi. S. *typhi* dan S. *paratyphi* menyebabkan infeksi sistemik yang berat hingga berminggu-minggu sehingga merupakan patogen khusus dalam *Salmonellae* yang sebagian besar hanya menyebabkan gastro-enteritis yang akan sembuh sendiri, atau menyebabkan bakterimia dan/atau infeksi fokal yang menyerang secara khusus individu dengan kelainan imunologi. *Salmonella typhi* menjadi unik karena hanya mempunyai satu inang, yaitu manusia, sementara S. *paratyphi* juga dapat menyerang ternak. Sebagian kecil dari penderita tifoid juga dapat menjadi karier kronik, yaitu mereka mengekskresikan bakteri di dalam tinja untuk bertahun-tahun tanpa ada gejala. Karier kronik dan ekskresi sementara oleh pasien merupakan satu-satunya sumber dari penularan S. *typhi*. Walaupun rantai penularan fekal-oral S. *typhi* dan S. *paratyphi* telah banyak dipelajari, aspek khusus dalam situasi tertentu yang merupakan elemen kritis dalam penularan belumlah banyak dipelajari. Pengetahuan terinci untuk determinan penyakit ini, penting dalam merancang intervensi publik yang efektif. Karena itu perspektif mengenai demam tifoid dan paratifoid tersebut menjadi dasar dalam tesis ini serta penelitian penyakit demam tifoid dan paratifoid di Jatinegara, Jakarta untuk memberikan bukti empiris terhadap kemungkinan pengendalian demam tifoid dan paratifoid.

Faktor lingkungan dan demam tifoid serta paratifoid di Jakarta

Kebanyakan dari kota-kota yang tumbuh pesat berada di negara berkembang. Jakarta dengan populasi lebih dari 12 juta orang merupakan salah satu dari 10 wilayah perkotaan terbesar di dunia. Dalam dekade ke depan, populasi Jakarta diramalkan tumbuh sepertiga dan akan melampaui megapolis seperti Kalkuta dan Buenos Aires. Kecamatan Jatinegara yang menjadi daerah penelitian terletak di Jakarta Timur dan mempunyai populasi 300.000 orang yang hidup dalam area 10,6 km^2. Wilayah urban ini dipilih karena sebagian dari wilayah ini, misalnya Kelurahan Kampung Melayu, merupakan tipikal daerah pemukiman kumuh dengan infrastruktur yang buruk dibatasi oleh sungai di dalam kota. Di wilayah ini, masalah kesehatan yang ada terkait dengan kemiskinan, tidak tersedianya sistem pembuangan limbah manusia dan air sehat, serta infrastruktur yang buruk. Buruknya sistem pengairan dan pembuangan limbah serta fakta bahwa sebagian Jatinegara terletak

di bawah permukaan laut menyebabkan banjir tahunan pada daerah bantaran sungai yang padat. Bagian lain dari Kecamatan Jatinegara adalah daerah yang cukup baik dilengkapi dengan sistem pengairan serta pembuangan limbah manusia yang lebih tertata serta dihuni oleh populasi kelas menengah. Dengan demikian Kecamatan Jatinegara ini mempunyai lingkungan dan populasi dari kelas sosial ekonomi bawah dan menengah serta mewakili spektrum permasalahan air sehat, sistem pembuangan limbah, infrastruktur, masalah banjir dan padatnya penduduk di Jakarta.

Pada beberapa bagian di Jakarta, sanitasi yang buruk dan lingkungan yang kotor merupakan masalah kesehatan yang serius. Sayangnya, hanya sedikit penelitian yang memperdalam hal ini. Penelitian mengenai penularan demam tifoid dan paratifoid yang disebarkan melalui makanan dan minuman pada **Bab Dua**, menemukan bahwa kebanyakan dokter melakukan diagnosis berlebih penyakit ini sampai sepuluh kali karena mereka jarang melakukan kultur darah yang akan memastikan atau menyangkal diagnosis. Dengan demikian surveilans populasi akan menguntungkan dalam *insight* dan peningkatan kemampuan diagnosis klinis individual. Dalam area Jatinegara, ditemukan faktor resiko utama untuk penyebaran demam tifoid terkait dengan rumah tangga, seperti adanya kasus baru dalam rumah dan berbagi makanan dari piring yang sama, terutama di dalam keluarga yang tidak menggunakan sabun untuk mencuci tangan dan tidak mempunyai jamban yang layak.

Oleh karena terus berlanjutnya dampak dari krisis ekonomi Asia pada tahun 1997, semakin banyak populasi yang mengandalkan asupan makanan dari penjaja makanan. Seperti yang diuraikan dalam **Bab Dua** dan **Bab Tiga**, fenomena ini berperan dalam kenaikan relatif penyebaran *S. paratyphi*, patogen penyebab demam paratifoid, bila dibandingkan dengan *S. typhi*. Faktor lainnya yang dihubungkan dengan demam paratifoid adalah kebanjiran. Pola penularan yang berbeda antara *S. typhi* dan *S. paratyphi* menyimpulkan bahwa penanganan tunggal masalah kesehatan masyarakat untuk mengendalikan demam enterik (demam tifoid dan paratifoid) patut dipertanyakan. Temuan di atas mengilustrasikan kebutuhan untuk peningkatan surveilans penyakit secara keseluruhan dan diagnosis klinis individual sebelum melakukan kampanye mahal dalam mengatasi masalah kesehatan masyarakat. Untuk mengatasi masalah kesehatan lingkungan di Jakarta akan menyebabkan komitmen bagi pendanaan penelitian kesehatan masyarakat melalui survei, pemantauan dan karakterisasi dari masalah serta pendanaan infrastruktur (seperti air sehat, sanitasi dan sistem pembuangan limbah) dibutuhkan untuk mengatasi masalah ini. Di samping intervensi terhadap infrastruktur yang mahal serta membutuhkan masukan dari berbagai institusi, intervensi sederhana dalam kebiasaan higiene penduduk seperti mempromosikan cuci tangan yang adekuat serta mendidihkan air minum juga harus ditekankan dan dapat memberikan dampak segera terhadap penyebaran dari berbagai penyakit infeksi, termasuk demam tifoid. Sebagai contoh, sebagaimana telah diuraikan literatur medis dan juga disimpulkan dalam penelitian kami, pasien dengan demam tifoid atau paratifoid

akan mengeluarkan bakteri dalam tinja mereka berminggu-minggu sampai berbulan-bulan setelah episode akut. Dengan demikian, kombinasi dari ekskresi bakteri jangka panjang, pencucian tangan sebelum mengolah makanan atau makan yang tidak adekuat, serta berbagi makanan dari piring yang sama akan memfasilitasi penularan di dalam rumah tangga. Walau demikian, hal ini dapat dicegah dengan meningkatkan level higiene perorangan dan higiene mengolah makanan. Sebagai catatan, peningkatan relatif kasus demam paratifoid juga mempertanyakan kebutuhan akan imunisasi massal dengan vaksin yang hanya akan memberikan perlindungan sementara terhadap *S. typhi*.

Konsisten dengan peran penjaja makanan dalam menyebarkan pathogen, penelitian mengenai hal ini dalam **Bab Tiga** menunjukkan dalam suatu waktu tertentu, satu dari dua puluh lima penjaja makanan mengekskresikan bakteri *Salmonella* dalam tinjanya. Dalam pengamatan kami, pengolah makanan yang dijajakan di jalan mempunyai kebiasaan mencuci tangan yang lebih buruk serta lebih sering menjamah makanan secara langsung dibandingkan dengan pengolah makanan di restoran. Pada pemeriksaan tinja, banyak dijumpai infestasi parasit usus pada penjaja makanan ini. Hal ini juga menguatkan kesimpulan buruknya higiene pengolahan makanan. Terlebih, kami juga menemukan sampel air yang dipakai untuk minum, mencuci piring serta es yang dipakai untuk mencampur minuman seringkali mengandung bakteri *coliform*, suatu marker yang umum dipakai untuk kontaminasi tinja. Dengan demikian, berbagai faktor yang terkait higiene perorangan dan higiene pengolahan makanan berperan untuk transmisi penyakit yang disebarkan melalui makanan atau minuman termasuk penyakit indikator yang menjadi fokus penelitian ini, demam tifoid dan paratifoid.

Banyak dari faktor risiko penyebaran penyakit yang telah disebutkan di atas dapat dihentikan dengan intervensi sederhana dan murah terfokus pada penjaja makanan, mis, instruksi untuk mencuci tangan dengan baik serta higiene mengolah makanan yang lebih baik, penggantian air mencuci piring yang lebih baik dan penggunaan sabun cuci di dalam air untuk mencuci piring. Dengan demikian usaha pencegahan penularan ini perlu dilakukan bersama. Perhatian juga harus diberikan kepada penjaja air keliling. Seringkali air yang dijajakan ini didapat dari pipa perusahaan air minum yang bocor atau ilegal, walaupun air ini tetap aman kalau telah dididihkan. Perhatian juga perlu diberikan kepada es untuk konsumsi yang seharusnya dibuat dengan mendinginkan air yang telah dididihkan untuk mengurangi risiko penyebaran penyakit yang ditularkan oleh air. Secara keseluruhan, diperlukan kendali mutu menyeluruh terhadap makanan dan minuman di Jakarta.

Faktor genetik inang serta demam tifoid dan paratifoid di Jakarta

Setelah memasuki inang melalui makanan atau minuman yang terkontaminasi, *S. typhi* dan *S. paratyphi* harus menghindari mikroorganisme lain untuk bersaing dalam mendapatkan

makanan dan tempat perlekatan pada mukosa, mendapatkan tempat memasuki tubuh, menghindari mekanisme pertahanan non-spesifik dari inang, menemukan tempat uniknya di dalam sistem fagosit mononuclear, mampu bertahan dan bereplikasi, serta pada akhirnya keluar dari inang dan disebarkan ke inang lain yang rentan.
Banyak dari fungsi ini tergantung dari protein inang yang terdapat dalam berbagai bentuk polimorfisme di dalam populasi. Dengan demikian, tergantung dari latar belakang genetiknya, seseorang dapat bervariasi dalam faktor-faktor ini, sebagaimana variasi dalam golongan darah. Dalam mempelajari faktor genetik inang dalam penyakit infeksi ini, penting dibedakan faktor-faktor yang mengontrol proses mendapatkan penyakit, seperti faktor-faktor yang terkait dengan kerentanan terhadap demam tifoid dan paratifoid, serta faktor-faktor yang menentukan berat-ringannya suatu penyakit setelah bakteri berhasil masuk ke dalam tubuh dan menemukan tempat khususnya. Untuk menjawab pertanyaan pertama, seluruh kasus dalam populasi tertentu harus dikumpulkan baik yang dirawat di rumah sakit maupun tidak, dan variasi di dalam gen kandidat yang relevan dianalisa dengan menggunakan kelompok kontrol dalam masyarakat yang tidak menderita penyakit tersebut. Untuk menjawab pertanyaan kedua, kasus-kasus, baik yang dirawat di rumah sakit atau yang diobati sebagai pasien rawat jalan, harus dianalisa derajat berat-ringannya perjalanan penyakit yang diderita.
Peran dari protein *pro-inflammatory* seperti TNF–α, IFN–γ, IL–1α, IL–1β, IL-12, IL-18, TNFR1, IFN-γR1, IL-1R, CASP1 dan CRP dalam menginduksi ekspresi berbagai gen dan sintesa dari beberapa protein yang dapat menginduksi perubahan radang akut dan kronik telah lama diketahui. Walau beberapa dari polimorfisme ini telah dilaporkan berasosiasi dengan berbagai penyakit infeksi, kami tidak menemukan asosiasi dari polimorfisme ini dengan demam tifoid atau paratifoid (**Bab Empat**). Sebagai contoh, sebuah laporan dari Vietnam mengasosiasikan alel *TNFA-308*A* dengan kerentanan terhadap demam tifoid. Di Indonesia, prevalensi dari polimorfisme –238 and –308 di daerah promoter dari gen yang mengkode untuk TNF–α pada pasien dengan demam tifoid dan paratifoid tidak berbeda dari kontrol masyarakat yang dipilih secara acak. Perekrutan pasien demam tifoid yang dirawat di rumah sakit di Vietnam dibandingkan dengan perekrutan pasien demam tifoid dan paratifoid yang didapat dari penelitian surveilans berbasis masyarakat di Jakarta mengisyaratkan alel *TNFA–308*A* khususnya mungkin lebih berperan dalam menentukan perjalanan penyakit dan berat-ringannya perjalanan penyakit yang hingga membutuhkan perawatan rumah sakit, namun tidak menentukan kerentanan untuk mendapatkan demam tifoid dan paratifoid. Jelaslah, bahwa variasi gen ini lebih berperan dalam menentukan berat-ringannya perjalanan penyakit seseorang setelah terinfeksi, dibanding dengan menentukan apakah seseorang akan menjadi sakit setelah terpapar. Beberapa dari polimorfisme nukleotida tunggal (SNPs) lainnya dalam gen *pro-inflammatory* yang dipelajari mempunyai frekuensi alel minor yang sangat rendah, misalnya, dua SNPs di *IL1R* tidak dijumpai dalam populasi

penelitian ini, baik pada kasus maupun kontrol. Dengan demikian, untuk memperoleh daya statistik yang memungkinkan terdeteksinya hubungan dari SNPs ini dengan demam tifoid, dibutuhkan jumlah sampel populasi yang lebih besar, baik kasus maupun kontrol.

Disamping gen-gen *pro*-inflammatory, polimorfisme lainnya pada gen inang menarik untuk dipelajari sehubungan dengan kerentanan atau berat-ringannya penyakit demam tifoid yang diderita. Salah satu kompleks gen yang menarik adalah *PARK2/PACRG*, diuraikan dalam **Bab Lima**. Polimorfisme *PARK2/PACRG* telah dihubungkan dengan penyakit kusta, sebuah patogen intraseluler lain yang mempunyai kemiripan dengan *Salmonella* dalam beberapa aspek reaksi imun inang. Produk gen dari *PARK2/PACRG* mempunyai peran dalam *polyubiquitination* dan degradasi protein oleh proteasom. Jalur ini sering berperan dalam mengatasi protein bakteri yang bersifat toksik dan mengganggu fungsi normal dari sel, dengan memecahnya menjadi molekul yang tidak berbahaya. Dari keempat SNPs pada daerah *PARK2/PACRG*, *PARKS_e01(–2599)* ditemukan mempunyai hubungan dengan demam tifoid, sementara ketiga polimorfisme lainnya, *PARK2_e01(–697)*, *rs1333955* dan *rs1040079* tidak berhubungan dengan demam tifoid. Secara kebetulan, *PARK2_e01(–2599)* juga merupakan polimorfisme yang mempunyai asosiasi paling kuat dengan penyakit kusta. Sampai saat ini belum jelas diketahui apakah jalur proteasom-ubiquitin berperan dalam infeksi *S. typhi* dan *S. paratyphi*. Walau demikian, beberapa hipotesa telah disusun berdasarkan model *in vitro* yang menghubungkan jalur ini ke mekanisme penghindaran dari *Salmonella*. Model pertama membuktikan bahwa invasi *Salmonella* ke dalam sel epitel dari inang membutuhkan aktivasi Cdc-42 dan Rac1 yang reversibel oleh protein bakteri yang dikenal sebagai SopE dan SptP. Stabilisasi SopE dengan menghambat proteasom akan mencegah pemulihan sel setelah infeksi bakteri sehingga memungkinkan bertahannya lingkungan yang menguntungkan bakteri untuk bereplikasi atau menghindari mekanisme pertahanan inang. Penelitian kedua menemukan bahwa strain *Salmonella* non-virulen berinteraksi dengan epitel manusia untuk mereduksi sintesa dari molekul efektor peradangan yang dicetuskan oleh berbagai stimulus *pro-inflammatory*. Reduksi ini ditimbulkan oleh fosforilasi IκB–α dan reduksi dari *polyubiquitination* IκB–α. Mekanisme ini penting dalam interaksi antara *Salmonella* dengan sel inang sehingga, perubahan kecil dari mekanisme ini dapat menjelaskan asosiasi dari polimorfisme *PARK2/PACRG* dengan demam tifoid dan paratifoid. Untuk menjelaskan konsekuensi patofisiologi yang tepat dari polimorfisme *PARK2/PACRG* dalam infeksi *Salmonella typhi* dan *Salmonella paratyphi*, diperlukan suatu penelitian dengan jumlah sampel yang lebih banyak.
Sebuah molekul yang menarik dalam infeksi tifoid dan paratifoid adalah *Cystic Fibrosis Transmembrane conductance Regulator* (CFTR). CFTR adalah sebuah kanal klorida yang terpengaruhi (biasanya tidak ada) pada pasien dengan *cystic fibrosis*. Percobaan *in vitro* mengisyaratkan *S. typhi* menggunakan protein CFTR sebagai tempat masuk di dalam

usus, yaitu untuk mendapatkan perlekatan kepada sel mukosa gastrointestinal. Jelaslah, perlekatan ini mendahului invasi dari sel mukosa, dan kemampuan untuk melekat ini penting untuk memulai demam tifoid. Hal ini menimbulkan hipotesis menarik, yaitu demam tifoid mungkin merupakan hal yang menyebabkan bertahannya mutasi CFTR di dalam populasi, yaitu dengan memberikan ketahanan lebih terhadap penyakit yang berbahaya pada masa kanak-kanak kepada karier heterozigotnya (3% populasi), karena karier hanya mengekspresikan separuh dari jumlah normal CFTR pada sel mebran (diuraikan pada **Bab Enam**). Pada analisa terhadap mutasi CFTR yang paling umum ΔF508 (mutasi yang dijumpai pada 30-75% populasi di Eropa Barat), tidak dijumpai adanya kasus, kontrol demam, maupun kontrol acak pada komunitas yang mempunyai mutasi ini. Penelusuran lebih lanjut mengisyaratkan bahwa *cystic fibrosis* merupakan penyakit yang jarang di Indonesia. Karena itulah, polimorfisme tambahan dalam gen CFTR harus diidentifikasi dan diteliti dalam hubungannya dengan demam tifoid dan paratifoid. Sebuah polimorfisme dalam bentuk jumlah pengulangan CA di *microsatellites* IVS17bCA dan IVS8CA di daerah intron ditemukan pada populasi penelitian dalam frekuensi memadai untuk analisa secara bermakna. Pada IVS17bCA, pengulangan 13 CA merupakan alel dominan dengan prevalensi 94%, sehingga marker ini tidak cocok (kurang polimorfik) untuk diferensiasi di antara kelompok-kelompok tersebut. *Microsatellite* kedua yang dipelajari, IVSbCA, mempunyai 2 alel utama 181(CA_{16}) dan 183 (CA_{17}) yang ditemukan mempunyai efek perlindungan terhadap demam tifoid, bila dibandingkan dengan alel lain pada marker ini. Penemuan ini mengisyaratkan bahwa walaupun mutasi ΔF508 tidak dapat ditemukan pada populasi penelitian sehingga hipotesa mengenai hubungan protein ini dengan demam tifoid tidak dapat disangkal atau dikonfirmasi, protein CFTR memang memainkan peran dalam infeksi *Salmonella typhi*. Pada penelitian lanjutan, hubungan antara alel 181(CA_{16}) dan 183(CA_{17}) dan pengurangan risiko untuk mendapatkan demam tifoid dapat dipelajari secara *in vitro* dengan menganalisa keseluruhan protein CFTR pada kasus-kasus spesifik ini, baik dengan melakukan *sequencing* maupun dengan menentukan gangguan fungsional (misalnya dengan mengukur kemampuan melekat dan masuknya *S. typhi* ke dalam sel dengan CFTR $^{-/-}$).

Dengan mempertimbangkan data genetik di atas, tampaknya kerentanan seseorang terhadap infeksi *S. typhi* kurang terkait dengan respon *pro-inflammatory* terhadap bakteri, tetapi lebih kepada hasil interaksi *S. typhi* dengan isi usus dan sel mukosa serta internalisasinya. Sebuah hipotesa dapat disusun, yaitu apakah setelah sebuah inokulum efektif masuk ke dalam tubuh, reaksi imun akan beraksi sesuai programnya dan walaupun terkait dengan berat-ringannya perjalanan penyakit, respon peradangan ini tidak menentukan apakah seseorang akan menjadi demam atau tidak (yaitu mendapatkan demam tifoid atau tidak), walaupun waktu yang dibutuhkan untuk menunjukkan gejala demam ini berbeda-beda. Jelaslah bahwa internalisasi dari sebuah inokulum yang efektif dipengaruhi

oleh beberapa faktor, termasuk polimorfisme pada CFTR dan kemampuan sel epitel untuk membatasi atau memprosmosikan pertumbuhan intraseluler dan pelintasan transmukosal dari *S. typhi*, sebagaimana diisyaratkan antara lain oleh peran polimorfisme *PARK2_e01(–2599)* pada gen *PARK2/PACRG*.

Laporan mengenai rendahnya prevalensi dari *cystic fibrosis* di Indonesia dan tidak dijumpainya mutasi ΔF508 menggarisbawahi potensi perbedaan faktor inang di antara berbagai kelompok etnik. Penelitian mengenai genetik, terutama mengenai variasi dari polimorfisme spesifik di populasi Indonesia sendiri masih sangat terbatas, sementara kebanyakan penelitian dilakukan di negara-negara Barat. Frekuensi dari polimorfisme pada penelitian-penelitian ini tampaknya berbeda dari populasi penelitian kami di Indonesia, sehingga penelitian lanjutan terhadap berbagai variasi polimorfisme yang terkait dengan berbagai penyakit infeksi umum di Indonesia menjadi sangat menarik. Karena beberapa polimorfisme mempunyai frekuensi yang sangat rendah, penelitian-penelitian ini seyogyanya mengikutsertakan kasus dan kontrol dalam jumlah besar sehingga didapatkan daya statistik yang cukup untuk memperoleh suatu asosiasi antara faktor genetik inang dengan kerentanan atau perjalanan penyakit dari suatu penyakit.

Faktor bakteri dan demam tifoid serta paratifoid di Jakarta

Salmonella typhi yang saat ini beredar telah diperkirakan merupakan klon yang baru berusia sekitar 50.000 tahun. Penemuan bahwa *S. paratyphi* A mengandung lebih sedikit pseudogen dibanding dengan *S. typhi* mengisyaratkan bahwa *S. paratyphi* hadir kemudian, dengan berasumsikan akumulasi dari pseudogen pada kedua serovar mempunyai kecepatan yang sama. Pada **Bab Tujuh** kami melakukan validasi dengan metode AFLP untuk mempelajari hubungan dari isolate *S. typhi* dan *S. paratyphi* dari hasil penelitian kami. Hasil analisa ini menunjukkan sedikitnya variasi antara isolat bakteri yang berhasil dikumpulkan selama periode dua tahun penelitian ini. Profil AFLP ini juga bersesuaian dengan penelitian sebelumnya yang menunjukan bahwa *Salmonella paratyphi* A bahkan lebih homogen dibandingkan dengan *Salmonella typhi* (tercermin pada tingkatan pengelompokkan 89% vs 85%). Disamping menentukan profil AFLP, isolat *S. typhi* dan *S. paratyphi* A juga dikelompokkan berdasarkan kerentanan mereka terhadap berbagai antibiotik yang berbeda serta berdasarkan profil biokimiawinya. Hasil analisa dari strain berdasarkan zona inhibisi antibiotik pada metode difusi cakram dan sifat-sifat biokimiawi ini lagi-lagi tidak memperlihatkan perbedaan bermakna di antara isolat-isolat ini, menunjukkan bahwa tidak hanya analisa AFLP, tapi juga pemeriksaan standar fenotip dari strain hanya mempunyai peran yang kecil untuk analisa kejadian luar biasa penyakit ini.
Salmonella typhi yang *multi-drug resistant* merupakan masalah kesehatan yang serius di berbagai benua Asia, termasuk Vietnam, Pakistan dan India. Walau demikian, kami hanya

menemukan beberapa strain yang resisten terhadap antibiotik pada penelitian kami ini. Dengan demikian skema pemberian antibiotik untuk mengobati demam tifoid dan paratifoid di Indonesia masih cukup luas dan mencakup antibiotika lini pertama seperti ampisilin dan amoksisilin. Absennya *S. typhi* yang *multi-drug resistant* di Indonesia mungkin terkait dengan isolasi geografis yang ada. Indonesia merupakan negara kepulauan dengan batas negara kebanyakan berupa lautan yang tidak dapat dihuni, dengan perkecualian di Kalimantan yang berbatasan dengan Malaysia Timur di bagian Utara, Papua yang berbatasan dengan Papua New Guinea di bagian Timur, serta Timor yang berbatasan dengan Timor Leste di bagian Utara. Di Malaysia Timur dan Papua New Guinea, *Salmonella* dengan *multi-drug resistant* belumlah merupakan masalah, sementara data mengenai *Salmonella* yang *multi-drug resistant* di Timor Leste sampai saat ini belum ada. Karakteristik geografis yang spesifik untuk Indonesia ini yang mungkin merupakan hambatan bagi penyebaran strain *Salmonella* dengan *multi-drug resistant* dari negara seperti Vietnam, India atau Pakistan, terutama dalam kaitannya dengan tatanan epidemiologi dimana penyebaran bakteri terutama terjadi di antara anggota keluarga atau karier kronik yang tidak terpapar dengan antibiotik. Transportasi udara dari dan menuju negara-negara dengan risiko tinggi saat ini masih merupakan moda yang mahal sehingga belum merupakan ancaman bagi tersebarnya strain *multi-drug resistant* ini. Penyebaran *S. typhi* di dalam rumah tangga, berasal dari anggota keluarga yang dalam tahap penyembuhan dan karier kronik dan bukan dalam tatanan rumah sakit atau kejadian luar biasa skala luas juga mendukung kesimpulan di atas. Kebanyakan orang dalam area penelitian pergi ke unit kesehatan lini pertama dimana pemberian antibiotik seringkali dilakukan tanpa melalui uji sensitivitas antibiotik; antibiotik yang diberikan umumnya adalah kloramfenikan atau kotrimoksasol. Banyak orang seringkali tidak mendapatkan pengobatan yang memadai disebabkan masalah keuangan dan hanya mendapatkan antibiotik untuk waktu yang singkat. Mengingat banyaknya kemiripan antara pelayanan kesehatan di Indonesia dengan negara-negara dimana *S. typhi* yang *multi-drug resistant* banyak dijumpai, serta belum adanya pemahaman yang baik mengapa strain tersebut masih jarang dijumpai di Indonesia, penyebaran *Salmonella* yang *multi-drug resistant* (juga bakteri lainnya) tetap merupakan ancaman dalam masa yang akan datang. Untuk itu pengobatan demam tifoid dan paratifoid yang adekuat dengan antibiotik yang sesuai haruslah dipromosikan oleh para dokter di Indonesia.

Secara umum, isolat *Salmonella typhi* dan *Salmonella paratyphi* A dari Jatinegara sangatlah homogen. Sifat homogen ini selain tampak pada karakteristik fenotip sebagaimanan ditunjukkan dalam profil biokimia dan sensitivitas terhadap antibiotik juga tampak dalam karakteristik genotip melalui analisa AFLP. Sifat homogen ini mungkin merupakan cerminan dari tingkat adaptasi serovar-serovar ini terhadap inang yang sangat tinggi, sehingga hanya memungkinkan variasi yang kecil sebelum bakteri kehilangan kemampuannya untuk menginfeksi manusia. Sebagai alternatif, kurangnya variasi ini

mempunyai penjelasan lain dengan kemungkinan yang kecil, seperti adanya sumber infeksi tunggal bersama (kemungkinan ini kecil karena pengambilan sampel dilakukan selama dua tahun dan kasus-kasus tersebar secara acak di dalam area penelitian). Walau demikian, penjelasan yang lebih mungkin adalah *typing* dari *Salmonella typhi* dan *Salmonella paratyphi* membutuhkan teknik molekuler baru untuk analisa strain sehingga dapat menghasilkan perbedaan antar isolate. Salah satu metode yang cukup menjanjikan adalah dengan memakai *pulse-field gel electrophoresis*.

Catatan penutup

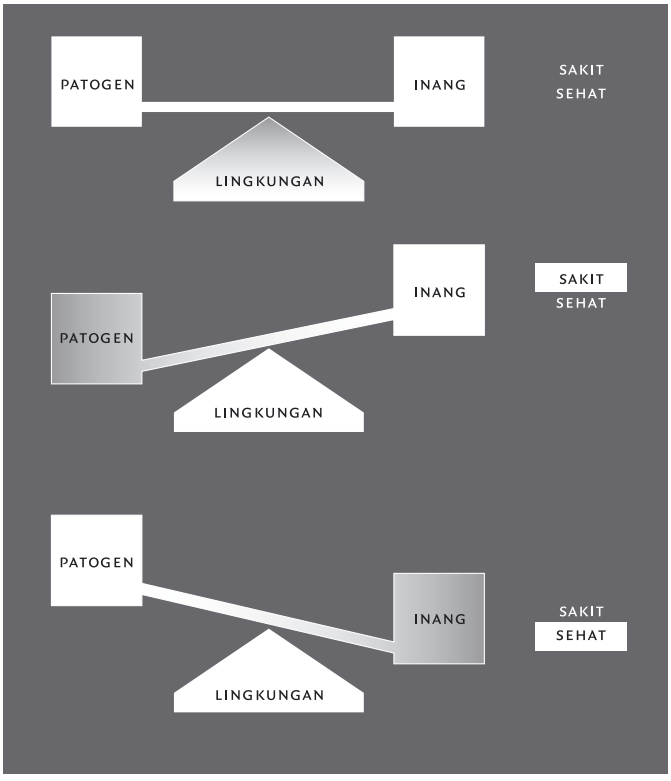
Salmonella dahulu dianggap sebagai ancaman bagi masyarakat. Di negara Barat yang saat ini telah maju, demam tifoid mempunyai prevalensi tertinggi di akhir abad ke sembilan belas pada akhir periode urbanisasi dan industrialisasi. Seperti juga tifus, demam tifoid danggap sebagai penyakit yang terkait dengan kurangnya higiene dan kotor. Situasi ini mengalami perbaikan dengan diperkenalkannya sistem pembuangan limbah kota dan kesadaran akan pentingnya higiene pribadi dan makanan, bahkan sebelum vaksinasi dan antibiotic diperkenalkan. Pembangunan sistem pengolahan air terpusat dan distribusi air minum ke sebagian besar dari kota secara paradoks menimbulkan beberapa kejadian epidemi demam tifoid yang menyebar melalui air minum, karena pada masa awal tersebut, hanya sedikit tindakan yang dilakukan untuk mencegah kontaminasi tinja ke dalam sumber air, penampungannya serta pipa distribusinya. Penyaringan dan klorinasi air mengatasi masalah ini. Kemudian, masalah karier kronik demam tifoid dikenali dan diatasi dengan melakukan penyelidikan kejadian luar biasa serta melakukan pengobatan individual pada sasaran-sasaran tertentu. Selama abad berikutnya, tindakan-tindakan ini praktis telah mengelimasi demam tifoid sebagai penyakit endemik di negara-negara Barat. Sebagai catatan, mulainya pemakaian antibiotik dan vaksinasi hanya mempunyai peran yang kecil dalam proses ini. Menarik bahwa faktor-faktor tadi saat ini juga dijumpai di Jakarta. Hasil temuan saat ini mengenai adanya kenaikan relatif dari prevalensi demam paratifoid dibanding demam tifoid tampaknya merupakan cerminan perubahan situasi sosial ekonomi dan meningkatnya konsumsi sumber makanan murah dari penjaja makanan, sehingga menggarisbawahi isu bahwa prevalensi demam tifoid dan paratifoid mencerminkan tahapan perkembangan dalam masyarakat, yang dalam hal ini megapolis Jakarta, dengan berbagai masalah kesehatan yang terkait dengan kemiskinan, polusi, banjir, tidak memadainya sistem pembuangan limbah dan kotoran manusia, serta kurangnya higiene dan air sehat. Tampaknya, seperti juga yang terjadi di masyarakat Barat abad yang lalu, penyelesaian masalah kesehatan lingkungan di Jakarta akan membutuhkan komitmen untuk mendanai surveilans penyakit, pendirian fasilitas kesehatan primer untuk pengobatan dan vaksinasi individu-individu tertentu, misalnya anggota keluarga yang berisiko karena ada yang mengekskresikan S. *typhi* dalam tinjanya, pendidikan kesehatan dan higiene di sekolah-

sekolah, serta dana yang besar untuk pembangunan infrastruktur yang dibutuhkan untuk mengatasi masalah-masalah di masa yang akan datang. Karakteristik daerah penelitian seperti tingkatan sosial-ekonomi, kondisi tempat tinggal dan perilaku higiene masyarakat juga dapat dijumpai pada berbagai megapolis dengan masalah urbanisasi di negara-negara berkembang.
Pada tingkatan individu, faktor genetik inang tampaknya berperan dalam resistensi terhadap demam tifoid dan mungkin unik untuk populasi yang diteliti. Dengan demikian, walaupun gen sitokin mungkin mengendalikan intensitas reaksi radang setelah masuknya *S. typhi* dan *S. paratyphi* ke dalam tubuh, sehingga menentukan berat-ringannya perjalanan penyakit, mereka tampaknya tidak memegang peranan dalam menentukan kerentanan seseorang terhadap suatu penyakit ketika individu tersebut terpapar dengan suatu patogen. Kontak awal dengan bakteri pada sel mukosa inang mempunyai peran utama dalam proses inisialisasi penyakit dan temuan kami membuktikan bahwa polimorfisme pada gen CFTR, terutama mengkode tempat penempelan bakteri di dalam usus, dapat dihubungkan dengan kerentanan terhadap demam tifoid dan paratifoid. Sebagai konsekuensi, kerentanan seseorang untuk mendapatkan penyakit atau untuk mempunyai perjalanan penyakit yang berat akan mempengaruhi kemungkinan penyebaran demam tifoid dan paratifoid ke anggota masyarakat lainnya, misalnya bila beberapa individu mempunyai kecenderungan menjadi karier kronik demam tifoid, atau mengeluarkan jumlah besar bakteri melalui tinja. Selanjutnya, beberapa strain *Salmonella* yang prevalen di daerah penelitian adalah unik. Sebagaimana dilaporkan di negara lain, kami menemukan tingkat homogenitas *S. typi* dan *S. paratyphi* yang tinggi. Hal penting lainnya, prevalensi *multi-drug resistant* yang masih rendah di daerah Jatinegara (tidak seperti yang dilaporkan oleh penelitian di negara berkembang lainnya) memungkinkan pengobatan pasien demam tifoid dengan antibiotik kotrimoksasol yang mempunyai sifat mudah diserap melalui usus sehingga dapat diberikan secara oral dan memungkinkan pasien berobat secara rawat jalan.

Dalam pengendalian demam tifoid dan paratifoid di daerah endemik seperti Jakarta, analogi interaksi inang-patogen dengan lingkungan dalam konsep suatu keseimbangan dapat membantu. Situasi di Jakarta saat ini dapat dianggap dalam ilustrasi model I, dimana patogen dan faktor inang dalam interaksinya dengan lingkungan berada dalam keseimbangan sehingga timbul endemisitas dari demam tifoid dan paratifoid. Keseimbangan ini dapat terganggu dengan mudah serta merugikan inang dan menguntungkan sirkulasi bakteri, misalnya dengan hadirnya klon baru bakteri yang lebih virulen, atau terjadi banjir dan kontaminasi air minum serta makanan dengan patogen. Hal ini akan menyebabkan timbulnya kejadian luar biasa di masyarakat. Situasi ini diilustrasikan dalam model II. Untuk mengatasi penyebaran penyakit, maka model III harus dituju, dimana perubahan lingkungan menguntungkan bagi pengendalian patogen dan

menguntungkan inang manusia.

Walaupun banyak perubahan lingkungan dan infrastruktur merupakan hal yang kompleks dan mendasar, beberapa intervensi sederhana dan praktis dapat dianjurkan serta akan mengurangi beban penyakit beberapa patogen enterik di Jakarta secara cepat. Intervensi tersebut antara lain dengan pengobatan adekuat dari kasus untuk menghindari timbulnya strain *multi-drug resistant*, kontrol terhadap lingkungan bagi keuntungan inang, misalnya dengan edukasi praktek higiene pribadi di sekolah dan penerapan higiene pribadi dan pengolahan makanan di antara penjaja makanan (karena penjaja makanan merupakan sumber nutrisi bagi banyak orang di dalam masyarakat), serta bila pendanaannya memungkinkan, dengan penyediaan sarana sanitasi yang adekuat dan air sehat ke rumah-rumah yang belum mempunyai fasilitas ini. Di sisi lain, dari sudut pandang inang manusia, identifikasi individu yang rentan terhadap penyakit dan mempunyai kecenderungan untuk berkembang menjadi karier kronik serta imunisasi anggota keluarga yang mempunyai kontak dengan kasus demam tifoid dan paratifoid akan mengendalikan lebih jauh penyebaran penyakit ini.



I. Patogen, inang dan lingkungan dalam keseimbangan

II. Patogen menjadi lebih virulen atau inang menjadi lebih rentan dan faktor lingkungan berubah ke arah merugikan bagi inang

III. Perubahan faktor lingkungan dan pengendalian patogen ke arah menguntungkan bagi inang

Curriculum Vitae

Soegianto Ali was born on April 11th, 1967 in Jakarta, Indonesia.
In 1984, he finished his education from Ricci Catholic School, Jakarta, after which he studied medicine at the University of Indonesia, Jakarta. He graduated as MD in 1990.
As part of the Civil Service Obligation he worked from 1991 to 1993 as head of a primary health care center in West Malaka district, Belu County in Timor Island. Back in Jakarta, he took up a position at the Faculty of Medicine of the Catholic University of Atma Jaya, as research assistant in a Hepatitis project. This concerned a 3-years research project in collaboration with the Catholic University of Leuven, Belgium. After the project, he joined the Department of Internal Medicine, Atma Jaya hospital in 1996. From 1998 to 2000, he received a scholarship from the Catholic University of Leuven and joined the Department of Hepatology at the Gasthuisberg University Hospital. Here, he worked on the TT Virus and received the Masters degree in Medical Science (head: Prof. Dr. S.H. Yap and Prof. Dr. Johan Fevery).
Back in Jakarta again, he joined the KNAW-SPIN typhoid fever project in 2000, and together with Albert Vollaard worked under supervision of Prof. Dr. J.T. van Dissel (Department of Infectious Diseases, Leiden University Medical Center), Prof. Dr. Suwandhi Widjaja and Prof. Dr. Charles Surjadi (both from the Catholic University of Atma Jaya). The typhoid fever project concerned a field study in the Jatinegara district of Jakarta; this part was conducted from 2001 until 2003. In this period, he received training in The Netherlands, and following completion of the field study he joined the laboratory of the Department of Infectious Diseases in Leiden to perform additional measurements on samples collected in the Jakarta field study, under supervision of Dr. Esther van de Vosse and Prof. Dr. J.T. van Dissel. Since 2005, he is back in Indonesia and joined the teaching staff at the Department of Medical Biology (head: Dra. Hj. Afni Zahara), Atma Jaya Catholic University. He has now become a member of the Medical Education Unit of Faculty.