Typhoid and paratyphoid fever in Jakarta, Indonesia. Epidemiology and risk factors

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Contents

Pursue him to his house, and pluck him thence;

Lest his infection, being of catching nature,

Spread further.

William Shakespeare, Coriolanus, act iii, *scene* 1

General introduction

Typhoid and paratyphoid fever – together often called enteric fever – constitute a serious health threat worldwide. In developing countries 21 million patients suffer from typhoid fever annually and more than 200 000 typhoid fever patients die every year.[1] Paratyphoid fever is also a global health burden, but its incidence is about ten times less than typhoid. Most Dutch physicians will deal sporadically with enteric fever patients, because enteric fever is virtually non-existent in the Netherlands since more than half a century. In fact, many of the cases in hospitals in the Netherlands are travelers coming from Indonesia. At the turn of the 19th century the picture in the Netherlands was quite different. Incidence rates of more than 50/100 000 population-year were reported, that rapidly declined to 0.2/100 000 population-year in 1967 due to improvements in drinking water supply, pasteurization of milk and identification of chronic carriers.[2] In the Indonesian archipelago (para)typhoid fever is still an endemic disease. In consequence, studies were needed to understand the reason for its frequent occurrence. An increased understanding could lead to better and cost-effective control strategies implemented by public health authorities.

The presented compilation of articles in this PhD-thesis has a specific focus on Indonesia, because in a scientific collaboration Indonesian and Dutch physicians – including the author – participated in a typhoid fever research project in Jakarta from February 2001 until October 2003.

Typhoid fever

Bacterial aspects

Bacterium: *Salmonella enterica* serotype Typhi (*S. typhi*) is a Gram-negative rod and a member of the *Enterobacteriaceae*. In the 19th century several infectious diseases were dubbed "typhus". "Typhos" in Greek means smoke, in which resonates both the delirious state commonly observed in typhoid fever, and the miasmatic theory, i.e., "malicious vapours as cause of disease", that dominated conceptual thinking about the origin of febrile illnesses in those days. The dispute on the cause of the specific and lethal variant "typhus abdominalis" or "typhoid fever" was only settled in 1880 with the discovery of the bacterium responsible for infection by three independent investigators: Eberth, Klebs and Koch. The genus derives its name however from another investigator, Salmon, who together with Smith discovered a related serotype in 1885: *Salmonella choleraesuis*. After the initial discovery of bacteria in intestinal tissues followed the isolation of bacteria in stools, urine and blood, explaining the pathogenesis and transmission of the disease. Robert Koch

deserves the credit for being the first to describe the concept of convalescent or even "healthy" carriers in the transmission of typhoid fever.[3]

Antigen structure: Bacteria of the Salmonella genus and other related organisms share several antigens with *S. typhi*. Relatively *S. typhi*-specific antigenic features are the somatic lipopolysaccharide antigens O9 and O12, protein flagellar antigen Hd and the polysaccharide capsular antigen Vi. Vi-negative strains have been described [4] and also a distinct flagellar antigen Hj was detected in circulating strains in Indonesia.[5]

Genetics: In 2001 the complete genome sequence of a *S. typhi* strain was determined and published in Nature [6], which elucidated many individual features of this highly host-adapted bacterium. A remarkable colinearity with genomes of *E.coli* and *S. enterica* serotype Typhimurium was observed, which led to the assumption that *S. typhi* is a "recent" offspring of an ancestral *E. coli*.[7] Two major differences have been observed: 11 large insertions unique for *S. typhi* that are called salmonella pathogenicity islands (SPI), combined with multiple smaller insertions scattered in the genome, and 204 so-called pseudogenes. The acquired insertions are important for the survival, host-specificity and pathogenicity of *S. typhi* in man. The pseudogenes, of which interestingly more than half are inactivated by mutations, are ancestral genes that presumably have lost their relevance for bacterial survival in a wide variety of hosts, because of the adaptation of *S. typhi* to the human host only.

Strain typing: *Salmonella* family members can be distinguished by the agglutination characteristics of members as was first described for *S. typhi* by Widal in 1896. With the discovery of antibiotics and consequent rise of antibiotic resistance of strains in the 1960s, also different strains within the *S. typhi*-group could be distinguished using antibiotic susceptibility tests. The introduction of (bacterio)phage-typing of *S.typhi* has been helpful in epidemiological surveillance and has been refined in recent years by the use of pulsed field gel electrophoresis, ribotyping and amplified fragment length polymorphism fingerprinting.[8-12]

Infective dose: In optimal conditions *S. typhi* undergoes division in less than half an hour. Prior multiplication of bacteria in the intestine is not a necessary step in the development of typhoid fever.[13] Therefore, the ingested dose is the decisive momentum in the infection. Experiments in the 1960s demonstrated the required dose for infection: at least 1000 bacteria.[14] High numbers of ingested bacteria resulted in higher attack rates implicating a linear dose-response curve with respect to the logarithmic dose, starting with attack rates of 10-20% at a dose of 10^3 organisms. The inoculum-size is also associated with the length of the incubation period, as was illustrated by the longer incubation periods in waterborne outbreaks of typhoid fever. The dilution and lack of growth of bacteria in water result in lower bacterial concentrations.[14] The intensity of excretion in carriers is variable, but up to 450 million organisms per gram faeces have been determined in the

stool of a paratyphoid fever carrier.[13] Excreted bacteria in water do not multiply, but can survive for substantial periods depending on the temperature and amount of organic matter in water. In sewage survival of at least 2 weeks is reported.
In food, however, bacteria can multiply to high numbers and subsequently overcome acquired immunization due to prior infection or the protective effect of vaccination.[15] Milk, (ice)cream, meat products, salads and coconut milk are good culture media and before pasteurization dairy products were often implicated in typhoid transmission. Direct person-to-person spread of bacteria is rare, but transmission in homosexual contact is documented.[16]

Pathogenesis

General life cycle: The mapping of the genome of *S. typhi* has been essential for the growing understanding of the unique host-adaptation of the bacterium: humans are the only host. Next to this host-specificity another feature is characteristic of *S. typhi*: its ability to survive and even multiply in the human host inside the macrophages that are responsible for the first line of defense against invaders. Even so, the roadmap of infection should begin in the gastro-intestinal tract after ingestion of a sufficient number of bacteria in food or water, i.e., the minimum infective dose.[14] Gastric acid is the first barrier to overcome and a reduced production of gastric acid, for example due to antacids, *Helicobacter pylori* gastritis, chronic atrophic gastritis or gastrectomy, might understandably lead to an increased susceptibility for disease by allowing the passage of high numbers of *S. typhi*, as is explained in the fifth chapter of this thesis.[17]
Inside the small intestine *S. typhi* bacteria attach to intestinal cells. Both enterocytes and microfold- or M-cells overlying the Peyer's patches are the porte-d'entrée of bacteria into the circulation of the human host. The *S. typhi*-specific interaction with the enterocytes depends on the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) on the surface of the enterocytes.[18] CFTR interacts with bacterial LPS and factors from *S. typhi* are able to upregulate the CFTR levels on the enterocytic membrane leading to enhanced bacterial ingestion and submucosal translocation.[19] The type III secretion apparatus of the bacteria, encoded within SPI-1, injects signaling components into the enterocytes in order to modify the cytoskeletal and vacuolar organization of the host cell to trigger invasion.[20] Passage through the intestinal mucosa in membrane-bound vacuoles enables *S. typhi* to reach the lymphatic circulation in the lamina propria and the draining mesenterial lymph nodes. After reaching the blood circulation via the thoracic duct the bacteria are filtered from the circulation and sequestered inside the phagocytic cells of the liver, spleen and bone marrow. On the SPI-2 pathogenicity island of *S. typhi* the SpiC gene encodes the inhibition of phagosome-lysosome fusion, which enables *S. typhi* to

survive and even multiply inside the macrophage. After an incubation period of 6-21 days secondary dissemination could occur causing disease symptoms associated with systemic infection and also re-infection of the Peyer's patches due to excretion of bacteria in bile. This re-infection could result in ulceration and necrosis of the previously primed Peyer's patches culminating in intestinal hemorrhage or perforation.[21] However, not all subjects infected with *S. typhi* develop symptoms, because the eventual outcome is influenced by interacting factors related to the bacterium, the host and antimicrobial agents.

Bacterial factors: An increase in the ingested dose leads to a higher attack rate and shorter incubation period.[14] However, the total number of bacteria ingested seems not to be associated with the severity of disease, suggesting an on-off mechanism of disease instead of an dose-response curve as found in other salmonelloses.[22] An increased virulence of the bacteria as determined by the presence of the Vi-antigen and mutations resulting in fluoroquinolone resistance [23] was found to be associated with severe typhoid.

Host factors: Typhoid in young children may follow a mild course.[24,25] Following the roadmap of infection multiple sites can be identified where insufficiencies in the defense mechanisms could lead to increased susceptibility or severity of disease. A decreased gastric acid production or gastrectomy has already been mentioned. Other factors are related to the immune response, because *S. typhi* induces macrophages to produces cytokines. The cytokine-mediated signaling of immune cells is responsible for clinical manifestation of typhoid fever such as fever, altered consciousness, hepatic dysfunction, renal failure, intestinal necrosis, thrombosis and shock. In some patients an increased production of proinflammatory cytokines (TNF-α, IL-1β and IL-6) and cytokine antagonists (IL-1 receptor antagonist and soluble TNF-α receptor) has been demonstrated.[26,27] Consequently, circulating cytokine levels are associated with severity and response to treatment. [28] The acute stage of typhoid fever results in depressed TNF-α and IL-1β release and consequently in delayed recovery.[29]

Polymorphisms in the genes encoding the nRAMP (natural-resistance-associated macrophage protein) are not associated with resistance to typhoid, even though in murine models this mechanism proved to be important for bacterial survival.[30]

The influence of genes of the major histocompatibility complex class II and III loci, encoding TNF-α and lymphotoxin-a, on typhoid fever susceptibility has been studied and associations of different haplotypes with disease susceptibility and resistance were demonstrated. [31,32] Future studies will examine whether the genetic polymorphisms associated with increased susceptibility to other salmonelloses play a role in typhoid fever as well.[33]

The presence of anti-*S. typhi* antibodies does not prevent previously infected individuals from recurrence of infection when they are challenged with high inocula.[34,35]

Carrier state: The gall bladder could be invaded after the secondary dissemination of bacteria as discussed above. *Salmonella* bacteria are capable of survival in gall and could

turn into permanent inhabitants of the gall bladder in case of favorable conditions, such as stones. Their ability to produce a biofilm might help them to evade the immune system.[36] Four percent of patients with acute infections, most of them female patients and especially in presence of gall stones, continue to excrete bacteria for prolonged periods of time. The continuous excretion of bile soiled with bacteria is the likely mechanism required to permit the survival of *S. typhi* in the human population, because during many years the carriers may constitute a potential source of infection for immunologically naive humans. The first identified carrier in the USA was Mary Mallon, the infamous cook in New York, better known as 'Typhoid Mary'. After causing several micro-epidemics in New York in the beginning of the 20th century, she was quarantined for life in a tuberculosis colony on North Brother Island until she died in 1938 from a non-related stroke. Chronic typhoid and paratyphoid fever carriers have an increased risk of cancer of the gallbladder and biliary tract.[37-40] However, this risk may be confounded by the association of gallstones and malignancies of the hepatobiliary tract.

Diagnosis

Culture: Diagnosis of typhoid fever requires culture of bacteria in bone marrow, blood (i.e., in the first week and lower chance of recovery from blood in the second to third week, sensitivity 60-80 percent), stool (i.e., end of first week with highest number of bacteria in second week), bile [41], urine (positive in a quarter to one-third of cases in the first weeks) and rose spots.[42] The bacterial loads in humans are low: in blood 1 bacterium per mL was measured of which 66% lies inside phagocytic cells, whereas in bone marrow 10 bacteria per mL were isolated.[23,43] However, the ratio of bacteria in blood versus bone marrow depends on the duration of illness; in the first week of illness this ratio is approximately 1, but later in illness the likelihood to isolate bacteria from bone marrow is greater than from blood, especially after antibiotic treatment.[44]

Serology: After the initial discovery of the agglutination of bacteria in blood of infected patients in 1896 by Widal, little progress in serologic diagnosis has been made. The simplicity of the Widal test has been hard to match even though the limitations of this test became apparent in endemic regions.[45,46] Major drawbacks for the use of the Widal test are: false-positivity in healthy individuals living in regions of endemicity, cross-reactivity with other *Enterobacteriaceae,* the choice of a cutoff titre signifying acute infection, particularly low sensitivity in the first week of infection, reduced sensitivity after antibiotic treatment, false-positivity after immunization with attenuated strains and differences in antigen preparation or laboratory standards. Efforts have been made to develop new simple serodiagnostic methods to replace the Widal test and some of them have been evaluated in clinical setting. The Typhidot and Typhidot-M (Malaysian Biodiagnostic Research) is

a dot enzyme immunoabsorbent (dotEIA) assay which detects antibodies to a presumably S.*typhi* specific antigen – an outer membrane protein of 50 kD – and has been tested in case-control studies.[47-49] A recent, innovative rapid-test, Tubex (distributor IDL, Sweden), which allows detection of antibody production to O9-somatic antigen has been compared to the Widal test and showed a better sensitivity.[50,51] A non-commercial prototype dipstick assay for the detection of IgM-antibodies against S.*typhi* was developed by the Dutch Royal Tropical Institute and tested.[52,53]
Newer methods using PCR were not very useful, since it only reached sensitive levels with 10 bacteria per mL, whereas bacterial numbers in blood of patients are frequently lower.[54] A sensitivity of 75% and specificity of 92% for detection of chronic carriers using Vi antibody titers of 1/160 were found in a study in Chile.[55]

Clinical presentation

Symptoms of disease: After replication inside the macrophages in spleen, liver, bone marrow and Peyer's patches during the incubation period of 6-21 days, S. *typhi* bacteria are released from these cells and the dissemination is accompanied by progressive fever, chills, headache, malaise, anorexia, nausea, abdominal discomfort, a dry cough or myalgia.[4] The onset of illness after the dissemination is usually insidious with a characteristic stepladder increase of fever reaching 39-40^{0}C after 5 days. Consequently, prolonged fever is commonly the presenting symptom in health care facilities. Although gastro-intestinal symptoms such as abdominal discomfort, diarrhoea or constipation may occur in patients, absence of gastro-intestinal symptoms is common in typhoid fever. The latter was demonstrated in a diarrheal diseases surveillance in Jakarta where in only 0.3% of the acute diarrhoeal patients S. *typhi* was isolated in stools.[56] Also so-called pathognomonic symptoms such as relative bradycardia, rose spots appearing at the end of the first week of illness or a coated tongue are frequently absent. From the observations of physicians in the pre-antibiotic era several stages in the course of typhoid fever could be distinguished.[13]
After the initial week showing increasing fever and malaise, the second week is characterized by apathy, anorexia, abdominal discomfort, increased weakness and continuous high fever. In the second week splenomegaly and hepatomegaly become prominent. This may culminate in the feared typhoid state or "toxic typhoid" in the third week in which the patient becomes increasingly lethargic. In this week also the complications of gastro-intestinal bleeding from necrotized Peyer's patches or perforation could occur. The latter were responsible for the mortality rates of 10-24% in the pre-antibiotic era.
If the typhoid patient survived the first 3 weeks a gradual decrease of fever could be observed in the 4th week.
Physical examination of typhoid fever patients should include inspection of the tongue,

the skin to detect rose spots and abdominal palpitation to detect hepato- or splenomegaly. Laboratory examination shows normal to reduced leukocytes.[57]
Although all above-mentioned symptoms could occur in typhoid fever, most decisive for the development of symptoms and complications is the delay of antibiotic treatment. Development of symptoms could also be age-related and in literature severity of disease is assumed to be less in young children.[58] Symptomless infection in children has also been demonstrated by sampling of stools and blood.[24] Recent reports also mention a higher virulence of MDR-strains causing higher bacterial loads in blood and bone marrow, a more pronounced clinical presentation of typhoid fever and increased mortality.[23,59]
Complications: Three complications of typhoid fever are well known: relapse (in about 10% of typhoid fever patients), haemorrhage (in up to 10% of patients) and perforation (in 0.7-4.7%).[13]
Unfortunate patients may experience a relapse of fever after initial recovery. This second fever episode or relapse of typhoid fever is usually less severe and results from a secondary outburst of *S. typhi* bacteria from the bone marrow. The fever-free interval between the two episodes can range from 8 to 40 days.[60] Treatment of typhoid fever with chloramphenicol did not markedly lower relapse rates.
Ulceration of Peyer's patches could result in erosion of an enteric blood vessel and subsequent intestinal haemorrhage. The most serious and life threatening complication is perforation of the intestinal wall of the terminal ileum, which requires surgical intervention and treatment of peritonitis.
Several other sites of infection than the Peyer's patches, spleen or liver are documented in typhoid patients. Since antibiotic treatment became available most of these complications are not seen nowadays. 'Pneumo-typhoid' may occur due to *S. typhi* infiltration of the lungs. Myocarditis is regarded to occur quite often. Christie mentions a study describing evidence of myocarditis in 12.6% of patients examined post-mortem. Other infrequent complications such as pyelonephritis ('nephro-typhoid'), meningitis and periostitis have been described in less than 2% of typhoid fever patients.[13]
Nowadays, the case-fatality rate of typhoid fever is less than 1% and is predominantly influenced by delay in instituting effective antibiotic treatment.[4]

Treatment

Antibiotic treatment: One year after chloramphenicol was isolated from the *Streptomyces venezuelae* from soil in Venezuela and a compost heap in Illinois this new antibiotic proved to reduce typhoid mortality dramatically [61], making 1948 the starting point of a new stage in the symbiosis between humans and *S. typhi*. The widespread use inevitably led to development of antibiotic resistance in the 1970s in many endemic countries.[62] Towards the

end of the 1980s this *Inc*Hi plasmid-encoded antibiotic resistance involved ampicillin and co-trimoxazole as well and these strains were dubbed multidrug-resistant (MDR). The fluoroquinolones gave temporary relief for the next decade until from 1993 on nalidixic acid resistance and low-level resistance to fluoroquinolones was reported in Vietnam, Pakistan and Tajikistan with an inferior clinical response or even treatment failure.[63-67] Interestingly, this trend is also observed for other *Enterobacteriaceae*.[68] Multi-drug resistance could originate from clonal dissemination of individual resistant strains or transfer of plasmids to multiple strains.[69-71]

Currently several antibiotics are used for treatment of typhoid fever. In areas such as Indonesia where *S.typhi* is susceptible to the standard first-line antibiotics, i.e., chloramphenicol, cotrimoxazole and ampicillin, these cheap drugs provide adequate treatment (our study).[72,73] Interestingly, reappearance of susceptibility to chloramphenicol has been observed in regions where earlier resistance was common.[74-76] In other regions where the prevalence of multidrug resistance was high, fluoroquinolones are the recommended treatment.[77] In case of decreased susceptibility for fluoroquinolones treatment with intravenous third generation cefalosporines or azitromycin is the last refuge [78] until typhoid fever might once again regain its well-known mortality and morbidity rates from the past. Evaluation of the effects of the mentioned antibiotics should include several parameters: reduction of mortality and complications, toxicity of the administered antibiotic, required duration of treatment, fever clearance, low faecal carriage rates at the end of treatment to limit spread by convalescent cases, and the prevention of relapse.[4]

Chloramphenicol: With the introduction of chloramphenicol mortality rates dropped dramatically to 2% from earlier rates of 10-24%, but interestingly relapse and carrier rates after treatment for 2 weeks were not influenced by treatment. Defervescence occurs on average on the 5th day of treatment. Relapse and faecal carriage rates at the end of treatment are 5.6 and 5.9%, respectively. In especially Caucasians irreversible aplastic anaemia has occasionally been observed which led to the abolition of chloramphenicol for the treatment of typhoid fever in developed countries.

Beta-lactam antibiotics: Ampicillin and amoxicillin have similar fever clearance rates of 6.4 days and also 2 weeks of treatment are advised. Relapse and fecal-carriage rates are 2.2 and 4.1%, respectively.[4] These drugs are considered safe for the treatment of pregnant typhoid fever patients.[79]

Cotrimoxazole: Recent surveillance data in the SENTRY Program [80] demonstrated that *S. typhi* retained 94.9-100% susceptibility to cotrimoxazole worldwide. Although the duration of treatment is equal compared to chloramphenicol, fewer capsules are required for treatment and patient's adherence to treatment might consequently be stimulated. Fever clearance time is roughly equal to chloramphenicol, but relapse and fecal-carriage rates at the end of treatment are slightly lower: 1.7 and 3.5%, respectively.

Fluoroquinolones: Until low-level resistance to fluoroquinolones was noticed these drugs seemed a gift from pharmaceutical heaven. Shorter fever clearance times of 2-4 days and lower relapse rates were observed that could be related to the good penetration quality into macrophages.[4] The good penetration in bile resulted in reduced periods of faecal carriage of convalescent carriers. Even short courses of 5-7 days or less appeared to suffice for treatment.[81,82] Long discussions about the toxicity of fluoroquinolones on cartilage formation in young children, as was observed in animal tests with beagle dogs, have resulted in the cautious introduction of these effective antibiotics in the treatment of typhoid fever (and other febrile illnesses) in children. After several studies it became clear that in humans cartilage toxicity or growth impairment is not associated with fluoroquinolone treatment.[83,84] The antibiotic susceptibility of *S. typhi* is still very different from that of serotypes such as *Salmonella typhimurium* DT 104, which contains chromosomally-encoded multi-drug resistance.[80] However, full resistance to fluoroquinolones has already been noticed [85] and remains a frightening scenario, since the expensive intra-venous alternatives might be one bridge too far for treatment of typhoid fever in poor countries.

Cephalosporines: Resistance to extended-spectrum cephalosporins has been reported in *Salmonella typhimurium*, but prevalence is low (max. 1.2%).[80,86,87] In *S. typhi* strains resistance to ceftriaxone is very rare.[88] The fever clearance time of one week with ceftriaxone and cefixime is somewhat slower than with fluoroquinolones. Rates of treatment failure were 5-10%, relapse rates were 3-6% and fecal-carriage rates less than 3%.[89-91]

Azitromycin: For the macrolide azitromycin cure rates of 95% have been reported after 5-7 days of treatment. Fever clearance occurred after 4-6 days of treatment and both relapse and fecal carriage rates were less than 3 percent.[91-94]

Treatment of chronic carriers: Since *S. typhi* bacteria reside in the gallbladder or bile ducts of chronic carriers, good penetration of antibiotic agents in bile is required. Prolonged courses of ampicillin or cotrimoxazole of 3 months have been tried [4], but shorter courses of ciprofloxacin 750 mg b.i.d. during 28 days yielded better cure rates of 92%.[95] In presence of gallstones cholecystecomy is recommended.

Epidemiology

Global incidence: WHO's estimates on the incidence of typhoid fever (21.7 million cases annually)[1] are seriously hampered by the incompleteness of epidemiological data from developing countries. Evidence for increased typhoid susceptibility in HIV-positive individuals is conflicting [4,96] but major outbreaks of disease in Africa might occur. As was clearly shown by the eradication of typhoid fever in developed countries by the introduction of safe water supply and adequate sanitary provisions [2,97,98], the end of the symbiosis of *S. typhi* and man may be near providing that developing countries tackle water supply

and human waste disposal efficiently.

Risk factors: Two scenarios that require risk factor analysis can be defined. First, outbreaks demand the quick determination of sources of infection to prevent further spread.[99-102] Second, in endemic regions an assessment of the role and weight of all contributing risk factors is needed to focus the scarce resources on the most prominent factors. Several studies have been carried out in (South-East) Asia to describe the epidemiology of typhoid fever. Independently associated risk factors suggesting waterborne transmission were: drinking water at the work-site [103]; drinking of non-boiled spring water [104]; drinking of tap water [100]; drinking water from other sources than the municipal water network [105] and drinking of non-boiled water or water from outside taps.[99] Independently associated risk factors suggesting food borne transmission were: consumption of ice-cream [103,106]; eating food from roadside cabins in summer months [103] and eating from food stalls. [107] Other independently associated risk factors were: taking antimicrobials in the 2 weeks preceding the onset of symptoms [103]; crowded living conditions, poor kitchen hygiene and poor garbage handling [100]; recent contact with typhoid fever and low economic level [108]; poor hand washing hygiene [17,105,107]; living in houses with open sewers, and being unemployed or having a part-time job [105] and being a single student, washing clothes, and living in larger households.[107]

Although these studies gave insight on predominant local routes of transmission of typhoid, e.g., piped water or other sources of water, street food, poor hygiene and low socio-economic status, the methodology of the studies differed to a large extent. Most cases were included in hospitals, but different inclusion criteria were used, i.e., blood culture confirmation, clinical suspicion or serological tests. Also the selection methods of the control-groups were diverse: matched hospital controls with or without fever and/or matched neighbourhood controls, which might have influenced the outcome of the risk estimates for typhoid fever in these studies.

Prevention strategies

Public health interventions: The introduction of drinking water treatment and construction of water mains to reduce the possibility of contact of human waste with drinking water in the beginning of the 20th century in the US and Europe did most for the reduction of the incidence of typhoid fever.[97,109] Ironically, connection to water mains also opened the opportunities for massive typhoid outbreaks when central contamination of drinking water sources occurred.[99] The initiation of governmental public health initiatives to track down chronic carriers to isolate them from food preparation did the rest for the containment.[2,110] Antibiotic treatment was introduced after most improvements in public sanitation were achieved and helped to reduce patient suffering and to eliminate the role of

chronic carriers in developed countries. In many developing countries the quality of drinking water, sanitation and public health facilities is poor and transmission of typhoid is hard to reduce.

Vaccination: An important interim regime might be immunization as long as water supply and sanitation are inadequate, especially in the case of epidemics of fluoroquinolone-resistant strains.[111] Heat-killed whole cell bacteria were used for control of epidemics since 1900. Introduction of heat-phenol killed and acetone-dried whole cell vaccines in the 1960s demonstrated a protective efficacy of 51-66% and 79-88%, respectively.[112] Considerable decreases of typhoid incidence and the appearance of herd-immunity have been documented.[113] The growing dissatisfaction with frequent systemic side-effects resulted in the introduction of live, attenuated mutants, such as oral vaccine Ty21a, with 50-90% protective efficacy. However, the elaborate 3 dosage-regime and possible risk of infection in AIDS patients gave way to the most commonly used single-injection Vi-vaccine with 55-75% protection for at least two years.[112] The current development of a Vi-vaccine conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A (Vi-rEPA) has shown promising results in prevention of (severe) disease and stimulation of antibody response also in children less than 2 years of age.[114]

Paratyphoid fever

Paratyphoid fever is caused by *Salmonella paratyphi* A, B (*S. schotmuelleri*) or C (*S. hirschfeldii*). The incidence of paratyphoid fever caused by one of these 3 bacteria seems to be geographically determined. In the Netherlands *S. paratyphi A* was very infrequently diagnosed and most notably among immigrants or sailors in the first half of the 20th century, whereas *S. paratyphi B* was endemic.[2] Also Christie referred mostly to the latter infection in the section on typhoid and paratyphoid fever in his excellent book.[13] In developing countries *S. paratyphi A* infections are more frequently diagnosed.[115]

Paratyphoid fever in enteric fever: Enteric fever is caused in 5-15% by paratyphoid bacteria.[116] Recent reports from India, Nepal and also our study in Jakarta show a relative increase of enteric fever caused by paratyphoid fever due to *S. paratyphi A*.[117-119] Whether the growing importance of paratyphoid fever is due to a worldwide downward trend of typhoid fever [1] and a consequent proportional increase of paratyphoid fever or due to an absolute increase in the incidence of paratyphoid fever, is not clear. Most likely is that changes in risk factors for disease, e.g., by improvement of drinking water or sanitary provisions, could have decreased the relative burden of typhoid fever compared to that of paratyphoid fever. Also, since paratyphoid fever is mostly transmitted by food, the growing dependency of the urban population in the developing world on street food may have contributed to some extent.

Transmission: Paratyphoid fever is usually a human disease with a human source, but rare infections of *S. paratyphi B* in cows have been described.[120]
Symptoms: Paratyphoid fever caused by *S. paratyphi B* has a milder course than typhoid fever. Also, symptomless excreters are thought to be commoner than in typhoid fever.[13] In systemic infection duration of fever is shorter and occurrence of complications is less. Paratyphoid fever could also cause gastro-enteritis-like symptoms, comparable to other non-typhoidal *Salmonella* infections.[121] Infection with *S. paratyphi A* could have the same clinical course as typhoid fever as was demonstrated in our study as well.[119]
Treatment: In contrast to typhoid fever standard antibiotics mostly suffice for treatment of paratyphoid fever. However, an increase in the prevalence of MDR-*S. paratyphi* strains - even to nalidix acid - has recently been reported.[115,122,123]
Vaccination: In the whole cell vaccines that contained killed bacteria also *S. paratyphi* A and B were included. The later typhoid vaccines – parenteral Vi and oral Ty21a – did not include cross-linking antigens, with the exception of Vi, that is shared by *S. typhi* and *S. paratyphi C*. Whether vaccines are needed for the control of the spread of paratyphoid fever [116] or programs to improve food safety and preparation hygiene, should be decided after determination of the incidence rates of paratyphoid fever by use of local surveillance data.

Typhoid and paratyphoid fever in Indonesia

Typhoid fever is endemic in Indonesia. A vaccination trial in Sumatra established an incidence of typhoid fever of 810/100 000 population-year in the placebo group.[124] The same study found an incidence of paratyphoid fever of 189/100 000 population-year. In a surveillance-study in Jakarta *S. typhi* was responsible for a small percentage of diarrheal episodes in patients (0.3%), but gastro-intestinal symptoms are not the predominant clinical symptoms in typhoid fever [56] (this thesis). High rates of faecal carriage of non-typhoidal *Salmonella* species of up to 8% have been detected, but *S. typhi* was not isolated in the screened healthy population.[125]
In contrast to other Asian countries *S. typhi* strains in Indonesia are susceptible to most first-line standard drugs. [72,73] Several studies have been done to determine the heterogeneity or clustering of *S. typhi* strains among countries in Southeast Asia, that could explain why Indonesian typhoid fever patients seem to suffer more frequently from neuro-psychiatric manifestations and higher mortality rates than patients in other countries.[126] Evaluation of variable-number tandem repeat profiles of isolates by use of Multiplex PCR showed that most of the isolates in one country were different from the isolates from all other countries, and that a high level of heterogeneity could be observed among isolates from within a country.[8] Evaluation of isolates by use of pulsed field gel

electrophoresis demonstrated that identical or very similar PFGE patterns are shared by isolates from Indonesia, Malaysia and Thailand. Due to migrant workers extensive movement of strains among Southeast Asian countries could be expected, which would explain the similarity of PFGE patterns of Indonesian strains and those from other countries.[10] Although these data demonstrate that Indonesia-specific *S. typhi* strains might circulate, none of the studies so far has correlated genetic profiles or specific protein bands with severity of illness.[127] Interestingly, the j-flagellar antigen appears to be more prevalent in Indonesian strains and may be associated with a milder course of disease.[5] In agreement with the hypothesis of cross-border travel, significant genetic homogeneity among *S. paratyphi A* isolates from Pakistan and Indonesia has been observed.[115]

Two risk factor studies have been carried out in Indonesia: in Ujung Pandang (Sulawesi) and Semarang (Java).[105,107] The latter study compared 75 blood culture-confirmed typhoid fever cases with 75 neighbourhood controls and identified poor housing and inadequate food and personal hygiene as risk factors, such as the lack of connection to the water mains, living in houses with open sewers and rarely washing hands before eating. The study in Ujung Pandang was a hospital-based study, used other inclusion criteria for cases (i.e., clinical presentation and Widal test confirmation) and identified poor hand-washing hygiene as a risk factor and also street food consumption.

These studies demonstrated that all distinctive routes of transmission of typhoid fever could play a role in Indonesia, i.e., person-to-person spread within households by poor personal hygiene, and spread at community-level by inadequate drinking water supply and sanitation, and purchase of contaminated street foods. Evaluation of these factors in every endemic situation is essential for the public health agencies and municipal authorities to target the predominant routes of transmission in order to control the spread of disease.

Outline of the thesis

- Introduction on typhoid and paratyphoid fever
In the introduction the bacterial cause of typhoid fever is discussed: bacterial aspects, pathogenesis, diagnosis, treatment, epidemiology and prevention are reviewed to enhance understanding of the subjects raised in the articles. Similarly, paratyphoid fever is discussed.
- Indonesia and (para)typhoid fever
In this chapter the available data on typhoid and paratyphoid fever from Indonesia are briefly reviewed.
- Chapter I: Identification of typhoid fever and paratyphoid fever cases at presentation in outpatient clinics in Jakarta, Indonesia
The first article is the description of the surveillance study in East Jakarta in which typhoid and paratyphoid fever patients were identified. Specific patient characteristics are evaluated and compared with that of non-enteric fever patients to develop an index-of-suspicion for local physicians, which could help them to target empiric treatment to suspected enteric fever patients.
- Chapter II: Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia
The second article deals with the risk factors of personal hygiene, water supply and quality, and eating habits for typhoid and paratyphoid fever in the study area, because the identification and determination of the contribution of risk factors are essential for the development of effective control strategies.
- Chapter III: Risk factors for transmission of food borne illness in restaurants and street vendors in Jakarta, Indonesia
This chapter describes the identification of the determinants in the transmission of food borne diseases, such as (para)typhoid fever, in commercial food handling in restaurants, food stalls and pushcarts.
- Chapter IV: A survey of the supply and bacteriologic quality of drinking water and sanitation in Jakarta, Indonesia
In this chapter different drinking water sources are compared, and sanitary conditions evaluated to identify transmission routes for waterborne diarrheal diseases in Jakarta.
- Chapter V: Helicobacter pylori infection and typhoid fever in Jakarta, Indonesia
The final article determines the association of enteric fever and *Helicobacter pylori* infection of the stomach as a possible host-dependent predisposing factor.
- General discussion
In this section the chapters will be evaluated and summarized.

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I

Identification of typhoid and paratyphoid fever cases at presentation in outpatient clinics in Jakarta, Indonesia

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Abstract

Objective: In Jakarta, Indonesia, over eighty percent of patients with typhoid fever or paratyphoid fever are treated in outpatient setting. We evaluated the clinical presentation of (para)typhoid fever to develop a clinical prediction rule that may help focus empiric antibiotic treatment to cases with suspected (para)typhoid fever rather than all febrile patients, or refer patients for additional diagnostic tests.
Methods: Standardized interviews were obtained from 59 blood culture-confirmed typhoid, 23 paratyphoid fever and 259 non-enteric fever outpatients, who were identified in a community-based prospective passive surveillance study.
Results: Decisions on empiric antibiotic treatment and advice on hygiene measures in patients with suspected (para)typhoid fever should take into account: duration of fever, absence of cough, and chills in the first week of fever, and, in the second week of illness delirium. This prediction rule will increase the likelihood of (para)typhoid fever from 1 : 10 in the first week to at most 2 : 3 in the second week of a febrile illness. However, the clinical prediction rule cannot be used as absolute screening method, because of the low sensitivity of presenting symptoms in (para)typhoid. A lack of these symptoms may suggest absence of (para)typhoid fever in a febrile outpatient, but is less useful in identifying (para)typhoid cases. Furthermore, paratyphoid fever could not be distinguished clinically from typhoid fever.
Conclusion: Clinical symptoms alone cannot provide certainty whether a febrile patient suffers from (para)typhoid fever or another febrile illness, and a robust clinical prediction rule to help focus empiric antibiotic therapy and replace the more definite blood culture method could not be proposed.

Introduction

Typhoid fever constitutes a serious public health problem in developing countries with approximately 16 million cases and 600 000 deaths per year worldwide (Pang et al., 1998). Also paratyphoid fever is an endemic disease in developing countries, but its incidence fever is lower than that of typhoid fever (ratio 1 : 5-20)(Arya and Sharma, 1995).
Diagnosis of typhoid and paratyphoid fever requires culture of blood, bone marrow, stools or urine to confirm growth of *Salmonella typhi* or *S. paratyphi A, B* or *C*. However, in developing countries culture facilities are expensive and mostly confined to hospitals, and because most typhoid patients are diagnosed and treated in outpatient setting, the insensitive Widal test or a diagnosis based on clinical presentation are predominantly applied in the diagnostic process.
A correct diagnosis followed by directed antibiotic treatment are required to shorten duration of illness, to prevent complications and to monitor the spread of disease at community-level. A unique feature in the transmission chain of typhoid fever is the continued excretion of bacteria in stools in a small proportion of patients (i.e., about 4%) during years after the acute infection, i.e., the chronic carriers (Parry et al., 2002; Christie, 1987). Typhoid fever will therefore remain endemic as long as hygiene, water and sanitation are inadequate and carrier detection and treatment are not effectively carried out (Cvjetanovic et al., 1971).
Typhoid fever is difficult to differentiate clinically from other causes of fever, because its clinical presentation consists of non-specific symptoms such as fever, chills, headache, malaise, anorexia, nausea, abdominal discomfort, a dry cough or myalgia (Parry et al., 2002). Only in the later phase of illness, more specific physical signs such as rose spots and splenomegaly may be observed. Comparative data on the clinical presentation of (para)typhoid fever and non-enteric fever in outpatient setting are scarce because most data is derived from hospitalized patients (Yew et al., 1991; Ross and Abraham 1987; Butler et al., 1991). In developing countries 60-90% of typhoid fever patients are treated as outpatients (Parry et al., 2002).
When all patients with a prolonged fever were treated as (para)typhoid fever patients, without use of blood culture for confirmation of (para)typhoid fever, the empiric treatment would inevitably include many febrile patients without *S. (para)typhi* infection. At the level of the individual patient this may imply unnecessary exposure to antibiotic agents in case of a viral cause of febrile illness (e.g., dengue). In addition, the isolation of bacteria is essential for determination of antibiotic susceptibility of bacteria to target adequate treatment and monitor spread of increasingly common multi-drug resistant strains (Rowe et al., 1997). Also at the community-level a correct diagnosis is required to monitor the transmission chain of typhoid fever and to determine clusters of patients and

local transmission routes. Detection of sources of infection related to, for instance, recent typhoid fever in household contacts (Luxemburger et al., 2001), commercial food handlers (Reeve and Dwyer, 1995) or contaminated drinking water sources (Mermin et al., 1999; Swaddiwudhipong and Kanlayanaphotporn, 2001) is essential to design effective preventive measures for the containment of disease. Any successful disease surveillance starts with adequate diagnostic methods.

Considering the costs of cultures and predominant outpatient treatment of typhoid fever, a clinical prediction rule would be useful to limit the use of cultures to those febrile outpatients with a high index-of-suspicion for (para)typhoid fever, that would still allow correct identification of (para)typhoid patients and adequate public health monitoring. We initiated a community-based prospective passive-surveillance study in Jakarta, in which 1019 consecutive patients with fever for 3 or more days were enrolled, as described (Vollaard et al., 2004). We compared the clinical presentation of (para)typhoid fever outpatients with that of outpatients with other causes of fever in order to predict or exclude (para)typhoid fever. The latter would efficiently target blood culture confirmation of diagnosis to those outpatients with a high index-of-suspicion for (para)typhoid fever or target effective empiric treatment in case culture facilities are absent.

Materials and methods

Febrile illnesses surveillance: An eastern district of 10.6 km^2 in Jakarta, Jatinegara, with 262 699 registered inhabitants was selected as study area, because of its varied socio-economic conditions and good access to public community health centers (*puskesmas*). A passive surveillance study was established involving all 4 hospitals in (immediate vicinity of) the area, all 12 *puskesmas*, and 8 of the 13 small local private outpatients clinics, as was described elsewhere (Vollaard et al., 2004). The *puskesmas* provide medical care for low-income residents in Indonesia. In the fee of $ 0.35 three days of treatment is included, but cultures or Widal tests are not part of the usual diagnostic practice.

This study was ethically approved by the Indonesian National Institute of Health Research and Development (*Litbangkes*) and the provincial authorities.

Patients: 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. Dependent on the age, 5-10 mL of blood was collected into culture bottles (aerobic) containing antibiotic absorbing resins (Bactec, Becton Dickinson, USA) that were provided to the centres free of charge. Cases were defined as eligible patients with blood culture-confirmed *S. typhi* or *S. paratyphi* infection. Non-enteric fever patients fulfilled the same criteria, except that the blood cultures showed no growth or other bacteria as cause of fever. Malaria could be excluded in the differential diagnosis of prolonged

fever, because it is not transmitted in Jakarta. The drug of choice for the treatment of suspected typhoid fever and other causes of febrile illness was left to the discretion of the referring physician.

Household visits: The households of cases and every second non-matched non-enteric fever patient (i.e., fever control) were visited within 1 month after the febrile episode that led to blood culture. This selection of non-enteric fever patients was introduced to obtain cases and controls in a ratio of about 1 : 4, to maximize statistical power while comparing cases and controls. Only the first reported eligible patient from a household was interviewed. Cases and controls were interviewed by trained medical graduates using standardized questionnaires. The presence of symptoms of disease was verified with household members. Delirium was defined as impaired consciousness, diarrhoea as three or more loose stools per day, constipation as absence of defecation during 3 or more days and cough as either productive or dry cough in the course of disease. When cases or controls were less than 13 years of age, the mother or guardian was interviewed. At the household visit a single stool sample was collected by the subjects in a vial with Cary Blair transport medium that was transported and processed within 24 hours after collection.
From all participants or from parents or guardians a written informed consent was obtained at the household visit.

Laboratory methods: Blood culture vials from outpatient facilities were transported on the day of collection to the central reference lab of Mitra Internasional, one of the participating private hospitals. Blood and stool culture methods are described elsewhere (Vollaard et al., 2004).

Statistical methods: Data from questionnaires was entered twice using Epi6 software (CDC, Atlanta, USA), validated and imported in SPSS version 11. Unpaired t-tests (normally distributed variables) or Mann Whitney U tests (not-normally distributed variables) were used to test numerical variables. Significance levels were p-values < 0.05. Measures of association were expressed as odds ratios with their respective confidence limits (OR [95% CI]) when categorical exposures were explored. To control for confounding a multivariate analysis was performed by the use of logistic regression with a variable selection using a forward likelihood ratio test with the significantly associated variables from the univariate analysis. The sensitivity (i.e., the probability that a patient with enteric fever presents with a certain symptom), specificity (i.e., the probability that a non-enteric fever patient would not present with that symptom), positive predictive value (PPV, the probability that a fever patient presenting with that symptom has enteric fever) and negative predictive value (NPV, the probability that a patient without that symptom would have another disease than enteric fever) were calculated for presenting symptoms of cases and fever controls. Combinations of symptoms in individual patients were separately compared and predictive values calculated. The PPV of symptoms can be calculated taking into

account the prevalence of (para)typhoid fever in the patient-population being investigated (p), by use of the formula: PPV = {sensitivity x p} / {(sensitivity x p) + [(1-specificity)(1-p)]}. Negative predictive value = {specificity x (1-p)} / {[specificity x (1-p)] + [(1-sensitivity) x p]}. For the calculation of p at a duration of fever of x days: p = {cases with fever for x days} / {(cases with fever for x days) + 2 x (febrile controls with fever for x days)}. The factor 2 for the febrile controls was introduced because of the 1 : 2 selection from the non-enteric fever patients for interviews.

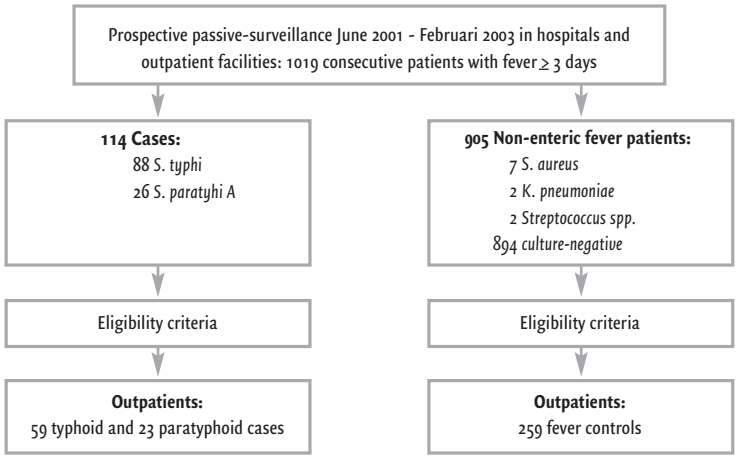
Results

Febrile illness surveillance: Blood cultures were obtained from 1019 consecutive patients with fever for 3 or more days from June 11th 2001 - February 4th 2003, yielding 88 *S. typhi* (8.6%), 26 *S. paratyphi A* (2.6%), 11 (1%) other bacterial pathogens (7 *S. aureus,* and *K. pneumoniae* and *Streptococcus* spp. in 2 patients each) and 894 negative cultures **(Figure 1)**. Three (3%) of *S. typhi* and none of the *S. paratyphi A* strains were resistant to chloramphenicol, ampicillin and cotrimoxazole. Only 113 (11%) patients were included in the hospitals, the other fever outpatients were included in the *puskesmas*: 717 (70%), and private practices: 189 (19%). The relative number of (para)typhoid fever among all febrile patients was identical in the patients enrolled by the various health care providers (p = 0.81). None of the (para)typhoid fever patients died.

Interviews in household visits: The eligible 114 cases and 450 fever controls (i.e., every second non-enteric fever patient) were interviewed in a household visit, if they had not died or moved, if they were the first patient in their household, their address could be traced, and did not refuse, as was described elsewhere **(Figure 1)**. Subjects were interviewed median 24 days (IQR 21-29) after taking of the blood culture. At the household visit stool cultures were obtained from 95% of the cases and 94% of the fever controls. Two fever controls had a positive stool culture (1 *S. typhi* and 1 *S. paratyphi A*) and were therefore excluded from further analysis. In stools from 5 fever controls (2%) non-typhoidal *Salmonellae* were cultured. Three (5%) of the 65 sampled typhoid fever patients and 1 (4%) of the 23 sampled paratyphoid fever patients were still excreting *Salmonella* bacteria in their stools 4 weeks after blood culture.

Outpatient characteristics: The characteristics of the 59 typhoid and 23 paratyphoid fever outpatients and 259 non-enteric fever controls that had been included in outpatient facilities were compared **(Table 1)**. The median age (IQR) of the (para)typhoid fever cases was 19 (11-26) and of controls 20 (13-30) years. No significant differences in age or sex-ratio were observed **(Table 1)**. The median time of residence (IQR) in the study area was 6 (1-13) and 11 (3-21) years for cases and fever controls, respectively. Inclusion of (para)typhoid fever and non-enteric fever outpatients had been in the *puskesmas* (63 cases

Figure 1. Inclusion of (para)typhoid fever cases and fever controls in Jatinegara, Jakarta



Eligibility criteria: living in study area (Jatinegara), identified address, alive, first patient in household, non-refusal, fever controls: every second non-enteric fever patient and stool culture negative for *S. (para)typhi*, (para)typhoid fever and non-enteric fever outpatients only.

and 208 controls) and private practices (19 cases and 51 controls). Patients who were included by private practitioners had a higher family income than patients who consulted the *puskesmas* ($p = 0.001$, t-test).

Fever controls reported to be diagnosed and treated at the moment of blood culture or during consecutive visits to health centres for the following diagnoses: suspected typhoid fever 126 (49%), dengue fever 5 (2%), respiratory tract infection 9, tuberculosis 3, influenza 3, and gastroenteritis 1. The remaining 112 outpatients (43%) were treated without being informed on the working diagnosis. Enteric fever resulted in longer absence from work or school than other causes of fever: median 7 days (IQR 3-14) for enteric fever versus 5 days (IQR 1-7) in controls ($p < 0.001$).

Duration of fever: Most patients were subject to blood culture in the first week of onset of fever: after 4 days of fever (median; IQR 3-7). Enteric fever cases had a significantly longer duration of fever before blood culture ($p = 0.001$, Mann Whitney U test); median 5.5 (IQR 3-7) compared to 4 (IQR 3-7) days in the controls **(Table 1).**

The use of prior antibiotics before culture was similar in both groups (32 vs. 37%, respectively) ($p = 0.47$). When subjects had taken prior antibiotics, cases reported mean 9.2

Table 1. Comparison and univariate analysis of characteristics of interviewed (para)typhoid fever and non-enteric fever outpatients

Characteristic	Cases	Fever controls	OR (95% CI)	p
n	82 [a]	259		
Age:				
- Range	4-59	1-75		0.13
- Median years (IQR)	19 (11-26)	20 (13-30)		
Sex: male	40 (49%)	148 (57%)		0.19
Days of preceding fever, median (IQR)	5.5 (3-7)	4 (3-7)		0.001
Duration of fever, 7 or more days	38 (46%)	73 (28%)	2.19 (1.31-3.65)	0.002
Prior antibiotics [b]	17 (32%)	61 (37%)	0.78 (0.41-1.51)	0.47
Antibiotic treatment of index event [c]				
- Chloramphenicol	45 (74%)	102 (52%)		
- Cotrimoxazole	8 (13%)	59 (30%)		
- Beta-lactams	5 (8%)	22 (11%)		
- Other	3 (5%)	12 (6%)		
Absence of work/school, median days (IQR)	7 (3-14)	5 (1-7)		< 0.001
Symptoms:				
- Chills	57 (70%)	134 (52%)	2.13 (1.25-3.61)	0.005
- Headache	63 (77%)	214 (83%)	0.70 (0.38-1.28)	0.24
- Delirium	11 (13%)	15 (6%)	2.52 (1.11-5.73)	0.02
- Nausea	59 (72%)	157 (61%)	1.67 (0.97-2.87)	0.06
- Abdominal pain	48 (59%)	115 (44%)	1.77 (1.07-2.92)	0.03
- Diarrhea	28 (34%)	68 (26%)	1.46 (0.85-2.48)	0.17
- Constipation	28 (34%)	76 (29%)	1.25 (0.74-2.12)	0.41
- Absence of cough	58 (62%)	125 (43%)	2.20 (1.36-3.55)	0.001

a: 59 typhoid and 23 paratyphoid fever patients

b: Data provided by referring physicians for 54 cases and 165 fever controls

c: Data provided by referring physicians for 61 cases and 195 fever controls

days of fever and fever controls 7.0 days before blood culture (p = 0.09, Mann Whitney U test), whereas cases and fever controls without prior antibiotic treatment were included earlier in the course of illness: mean 6.0 days and 4.2 days, respectively (p = 0.006).

Treatment: Data was obtained from the referring physicians on prescribed treatment in 61 cases and 195 controls **(Table 1)**. Most cases (45; 74%) had been treated with chloramphenicol versus 102 (52%) in the fever control group.

Typhoid versus paratyphoid fever: The age of typhoid fever outpatients did not differ significantly from that of paratyphoid fever outpatients: median 19 years (IQR 11-25) and 22 years (IQR 14-33), respectively (p = 0.26 (Mann Whitney U test)), nor did the duration of fever before blood culture: median 6 days (IQR 4-7) and 5 days (IQR 3-10), respectively (p = 0.70). None of the symptoms of disease was reported significantly more often in one of the two groups: chills were reported by 73% of typhoid and 61% of paratyphoid fever

outpatients, respectively (p = 0.29), delirium by 14% and 13% (p = 0.95), abdominal pain by 56% and 65% (p = 0.44), diarrhoea by 32% and 39% (p = 0.55) and constipation by 31% and 44% (p = 0.27), respectively. The period of inactivity due to disease did not differ between the 2 groups (p = 0.72, Mann Whitney U-test). Since typhoid and paratyphoid presentation was not significantly different, data of enteric fever outpatients was pooled for further comparison of clinical presentation with that in the group of non-enteric fever outpatients.

The comparison of clinical symptoms in outpatients: Duration of fever (≥ 7 days), chills, abdominal pain, delirium and absence of cough were significantly associated with (para)typhoid fever in univariate analysis **(Table 1)**. Diarrhoea (in 34% of cases and 26% of controls) and constipation (in 34% of cases and 29% of controls) were non-discriminative symptoms. In a multiple logistic regression analysis duration of fever (> 1 week) (OR 2.03 (1.20-3.43)), presence of chills (OR 1.91 (1.11-3.30)) and absence of cough (OR 2.20 (1.36-3.55)) were independently associated with (para)typhoid fever.

Predictive values of symptoms in outpatients: The two independent characteristic symptoms of (para)typhoid fever, chills and absence of cough, in combination with additional symptoms as reported by individual patients were used to calculate the predictive values of reported symptom-combinations for (para)typhoid fever in febrile patients for two categories of duration of fever at presentation **(Table 2, 3)**.

First week of illness: The starting points were 1) the a-priori chance of (para)typhoid fever of 11% in patients presenting with less than 7 days of fever, and 2) the presence of chills or absence of a cough with sensitivity of 68% and 73% and specificity of 52% and 63%, respectively **(Table 2)**. These two symptoms were neither very sensitive (i.e., not all cases suffered from these symptoms) nor specific (i.e., also a considerable proportion of the controls suffered from chills or did not cough), and consequently the PPVs were not much higher than the a-priori chance (14% and 19%, respectively). The use of more restrictive disease-specific criteria (i.e., presence of other symptoms in addition to chills or absence of cough) to increase the specificity (fewer false-positives) resulted in a lower number of cases with these specific symptom-combinations, i.e., a reduction of the sensitivity. However, although the number of cases presenting with these specific symptom-combinations was low, the PPV reached values of twice the a-priori chance and these combined symptoms should therefore raise the index-of-suspicion. As expected, the presence of diarrhoea or constipation in cases did not markedly improve either sensitivity or PPV.

Second week of illness: The starting points in patients with fever for 7 or more days were 1) an a-priori chance of (para)typhoid fever of 21%, and 2) the presence of chills or the absence of a cough with a sensitivity 71% and 50%, and specificity of 38% and 49%, respectively **(Table 3)**. Again, the PPVs of these rather non-specific symptoms equaled the

Table 2. Sensitivity, specificity and predictive values (%) of clinical symptoms for (para)typhoid fever in outpatients with fever < 7 days

Symptoms	Sensitivity	Specificity	PPV	NPV
A-priori chance			11	
Chills	68	52	14	93
Chills, absence of cough	50	78	21	93
Chills, absence of cough, abdominal pain	30	91	28	92
Chills, absence of cough, abdominal pain, diarrhoea or constipation	23	93	28	91
Chills, absence of cough, delirium	5	98	25	90
Chills, absence of cough, abdominal pain, delirium [a]	0	100	-	89
Chills, abdominal pain	36	77	16	91
Chills, abdominal pain, diarrhoea or constipation	27	84	17	91
Chills, abdominal pain, delirium [b]	2	100	100	90
Absence of cough	73	63	19	95
Absence of cough, abdominal pain	34	84	20	92
Absence of cough, abdominal pain, diarrhoea or constipation	25	91	26	91
Absence of cough, delirium	7	97	23	90
Absence of cough, abdominal pain, delirium [c]	0	99	0	89

A-priori chance of (para)typhoid fever in febrile patients = 11% (44 cases and 2 x 185 fever controls)

a: Combination reported by none of the cases and controls

b: Combination reported by none of the controls

c: Combination reported by none of the cases and by 2 controls

Table 3. Sensitivity, specificity and predictive values (%) of clinical symptoms for (para)typhoid fever in outpatients with fever ≥ 7 days

Symptoms	Sensitivity	Specificity	PPV	NPV
A-priori chance			21	
Chills	71	38	23	84
Chills, absence of cough	34	74	25	81
Chills, absence of cough, abdominal pain	29	86	35	82
Chills, absence of cough, abdominal pain, diarrhoea or constipation	18	92	37	81
Chills, absence of cough, delirium	8	99	60	80
Chills, absence of cough, abdominal pain, delirium [a]	8	100	100	81
Chills, abdominal pain	55	66	30	85
Chills, abdominal pain, diarrhoea or constipation	34	77	28	82
Chills, abdominal pain, delirium	11	96	40	81
Absence of cough	50	49	20	79
Absence of cough, abdominal pain	39	78	32	83
Absence of cough, abdominal pain, diarrhoea or constipation	29	85	33	82
Absence of cough, delirium	11	96	40	81
Absence of cough, abdominal pain, delirium	11	99	67	81

A-priori chance = 21% (38 cases and 2 x 73 fever controls)

a: Combination reported by none of the controls

a-priori chance. Also, the presence of additional symptoms reduced both the number of cases and controls presenting with these symptom-combinations with similar trends for the specificity (i.e., up), sensitivity (i.e., down) and PPV (i.e., up). Delirium in patients with fever ≥ 7 days in addition to other symptoms would increase the likelihood of (para)typhoid fever to at least 2 times the a-priori chance (up to 67% and in 1 combination to 100%).

Use of clinical symptoms for screening and further diagnostic tests: When culture facilities are available in (immediate vicinity of) a health care facility, suspected typhoid fever patients should be subject to blood culture. Whether the clinical symptoms of febrile patients can be used as a screening test to decide on further diagnostic steps (i.e., blood culture or not) depends on a high sensitivity of the symptom-combinations in individual patients. No true enteric fever patient should be missed and therefore wide inclusion criteria should guarantee a high sensitivity and subsequent entrance to further diagnostic steps, even if that meant that the blood of a proportion of non-enteric fever patients, with similar symptoms as cases, would be cultured as well. The highest sensitivity was found in the first week of illness: absence of a cough (73%) **(Table 2).**

However, application of this presentation as a criterion for further diagnostic testing would mean that 27% of the cases would be missed and also that 37% of the non-enteric fever patients would be cultured (specificity 63%).

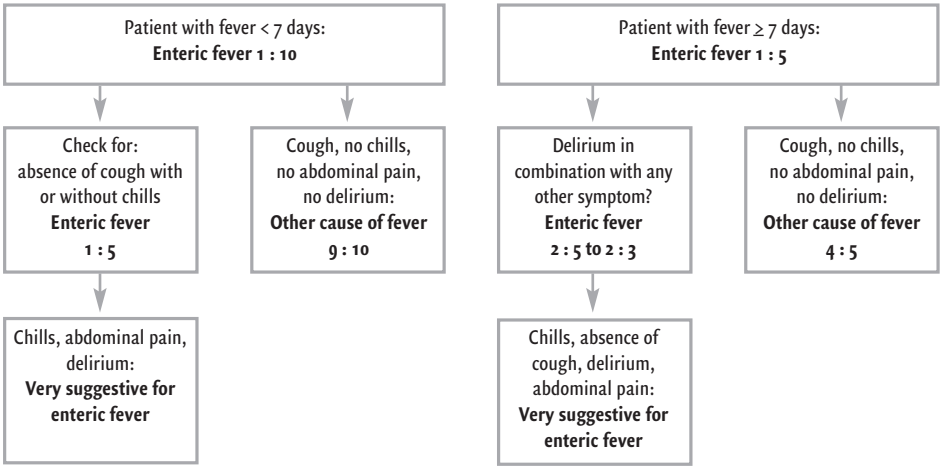
None of symptom(-combination)s yielded a sufficiently high detection rate of true (para)typhoid cases due to the limited sensitivity. Therefore, the clinical presentation cannot be used as a screening instrument for (para)typhoid fever to decide whether an individual patient should additionally be subject to blood culture for the confirmation of (para)typhoid fever or not.

Use of clinical symptoms for empiric treatment of (para)typhoid fever: When culture facilities are not available, empiric treatment should be started in patients with suspected typhoid fever to avoid the serious complications of (para)typhoid fever (i.e., hemorrhage and perforation) and advice on hygiene should be given to patients to reduce the risk of further spread. For that purpose the predictive values of symptom-combinations **(Table 2, 3)** are useful, because physicians could check for symptoms in febrile patients to increase the index-of-suspicion for (para)typhoid fever (**Figure 2**, expressed as ratios).

A first clue should be the duration of fever, because the a-priori chance increased twofold in patients with fever ≥ 7 days (from 11% to 21%). In the first week of fever the absence of cough with or without chills would increase the probability of (para)typhoid fever in a febrile patient twofold as well (from 11% to 19-21%). The presence of other symptoms in addition to absence of cough and/or chills did not increase the PPV any further in the first week, and are therefore not very helpful to increase the index-of-suspicion. For patients with 7 or more days of fever delirium in combination with other symptoms increased the

a-priori chance to a maximum of 67% for the combination of absence of cough, abdominal pain and delirium, although this combination was not very common in cases (sensitivity 11%). Also, two symptom-combinations were very suggestive for (para)typhoid fever and patients should be treated as such; in the first week of fever the combination of chills, abdominal pain and delirium was a strong predictor of (para)typhoid fever (PPV 100%), as well as the combination of chills, absence of cough, abdominal pain and delirium after one week of fever (PPV 100%).
Although in presence of other symptom-combinations than the this above-mentioned,the suggestion of (para)typhoid fever in febrile patients remains rather low (PPV 11-67%), in absence of these symptoms the evidence against (para)typhoid fever is strong (NPV 79-95%), especially in the first week **(Figure 2).**

Figure 2. Checklist for symptoms to raise index-of-suspicion for enteric fever in febrile patients in outpatient setting



Discussion

In our recent surveillance study in the Jatinegara district, Jakarta, we observed that 88% of (para)typhoid fever patients was treated in outpatient setting in Jakarta, making an evaluation of (para)typhoid fever outpatients relevant. Paratyphoid fever could not be distinguished clinically from typhoid fever. Our study confirmed quantitatively that in outpatients (para)typhoid fever presentation is relatively similar to other febrile illnesses. In consequence, due to the limited sensitivity of symptom-combinations the clinical presentation cannot be used as a screening method for further diagnostic tests and therefore all febrile patients should be subject to blood culture (or serological tests) for the confirmation of (para)typhoid fever. When culture facilities are absent decisions on empiric treatment and advice on hygiene measures should be influenced by the duration of fever of patients, absence of cough with or without chills in the first week of fever, and delirium in the second week, to increase the likelihood of (para)typhoid fever in a febrile outpatient from 1 : 10 in the first week to at most 2 : 3 for the latter combination, although numbers of cases presenting as such are low.

Some issues regarding the inclusion of patients need consideration. In our surveillance study Bactec blood cultures were used for confirmation of (para)typhoid fever. The effective neutralization of antibiotics by resins in Bactec bottles (Koontz et al., 1991) reduced the interference of prior antibiotics, and accordingly the relative number of patients using antibiotics before blood culture was equal in both groups. Because 75% of the patients in our study was included in the first week of illness, the lower sensitivity of the Widal test compared to blood culture would have been reduced even more by the short duration of illness (Parry et al., 1999). Furthermore, to minimize misclassification of controls, since the sensitivity of blood culture for confirmation of (para)typhoid fever never reaches 100 percent, faeces was cultured in all subjects at the household visit and two stool culture-positive fever controls were excluded from the analysis. With respect to the use of a questionnaire, although recall-bias cannot be ruled out because of the interval of 3 weeks between blood culture and interview, it would have affected both groups equally. Moreover, most of the fever controls thought they had been treated for typhoid fever or were not informed on the final diagnosis, consequently reducing recall-bias. Finally, the inclusion criterion of three or more days of fever might have affected the inclusion of children, who may have an atypical presentation of typhoid fever (Ferreccio et al., 1984). Consequently, in contrast to reports from India and Bangladesh (Sinha et al., 1999; Saha et al., 2001) few cases were young children and the presented clinical guideline is therefore predominantly applicable in adolescent and adult fever patients.

Most of the controls had been treated empirically without final diagnosis. This treatment strategy is an accurate reflection of the conditions of physicians in outpatient health centres

in many developing countries. In absence of laboratory facilities likelihood estimates are applied that are based on local endemicity levels of typhoid and other febrile illnesses, and the clinical presentation of febrile patients. The latter was explored extensively in our study, but the former might differ in other settings and consequently influence the general applicability of our findings, because we showed that the interpretation of clinical presentation to predict (para)typhoid fever in a febrile patient also depends on the local endemicity levels of the prevailing febrile illnesses, i.e., the a-priori chance of finding (para)typhoid fever as a cause of fever. When the a-priori chance, i.e., the relative number of enteric fever patients in a patient-population, increases, also the PPV of symptoms will increase. Predictive values are therefore more useful for decisions on empiric treatment than the sensitivity of symptoms or odds ratios for symptoms in the total group of patients, because the latter are unaffected by the a-priori chance. The prior chance of having typhoid fever in our study was 8.6% among all patients with fever for three or more days, comparable to rates from other surveillance studies for typhoid fever using the same inclusion criteria: 4.6-8.5% (Lin et al., 2000; Saha et al., 2001; Sinha et al., 1999; Simanjuntak et al., 1991; Bodhidatta et al., 1987), but was shown to increase in patients with fever for at least one week. Our study demonstrated that in daily medical practice in outpatient setting the index-of-suspicion for (para)typhoid fever should be raised in adolescent patients who report prolonged fever, chills, absence of cough and especially delirium after one week of fever, but that these symptoms cannot be used for the screening of patients for further diagnostic testing due to the low sensitivity of symptoms.

The treatment of (para)typhoid fever in Jakarta with relatively cheap, standard antibiotics such as chloramphenicol, ampicillin and cotrimoxazole, still is a valid option, since only 3% of the *S. typhi* and none of the *S. paratyphi A* strains were multi-drug resistant, in line with other reports from Indonesia (Isbandrio et al., 1994; Tjaniadi et al., 2003; Oyofo et al., 2002). The accessibility of the low-priced *puskesmas* and early and adequate treatment in these outpatient centres was likely the cause of the absence of deaths in the case-group and the low rate of hospitalization.

Non-treatment of (para)typhoid cases would increase complication rates of haemorrhage and perforation and also lead to infection of other household contacts if hygiene is poor (Luxemburger et al., 2001). As was shown in stool cultures after 3-4 weeks in treated (para)typhoid case subjects, 5% of the cases continued to excrete bacteria, enabling latter transmission route. Adequate treatment and public health measures in case of suspected enteric fever are therefore essential. The evaluated symptoms allow an increase in the correct identification of (para)typhoid fever patients from an a-priori chance of 11% in febrile patients to 67% at most, when we disregard the few cases with the very rare combination of all chills, abdominal pain and delirium with or without absence of cough. However, in settings without culture facilities the empiric treatment of (para)typhoid fever will inevi-

tably include a substantial number of patients with other causes of fever, because a conclusive clinical picture could be not distilled from our data that may help physicians to restrict the prescription of antibiotics to those patients with a high probability of (para)typhoid fever. Whereas most symptom-combinations were not very useful for the identification of enteric fever patients, in absence of these symptoms other causes for fever in a febrile outpatient are probable in at least 4 out of 5 patients.
We focussed on the clinical presentation of outpatients and did not include laboratory parameters such as white blood cell or thrombocyte count or Widal test in the prediction model (Ross and Abraham, 1987). The additional information that can be obtained using lab parameters in patients with a high index-of-suspicion as identified in our study needs further validation in outpatient centres with laboratory equipment.
In conclusion, as long as culture facilities are limited (and costs inhibitive), and sensitive serological tests are not available, many febrile patients without *S. (para)typhi* infection will receive empiric treatment for suspected (para)typhoid fever. Although chills, delirium, abdominal pain, and/or absence of a cough in adolescent patients with prolonged fever should raise the index-of-suspicion of (para)typhoid fever, no conclusive clinical picture could be presented to differentiate (para)typhoid fever from other febrile illnesses. Culture or serological tests are therefore essential in the diagnosis and in health surveillance. A reduction of the number of cultures in febrile patients could not be proposed, because of the limited sensitivity of the symptoms in (para)typhoid fever compared to other causes of fever. A follow-up visit after 3-5 days of antibiotic treatment of suspected (para)typhoid fever is recommended to decide on prolongation of treatment for patients with delayed fever-clearance, because after administration of standard antibiotics fever due to typhoid fever should reside after 3-5 days of treatment (Parry et al., 2002). Also instruction on adequate hygiene in the first weeks after treatment should be emphasised.

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2

Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia

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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 paratyphoid 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]

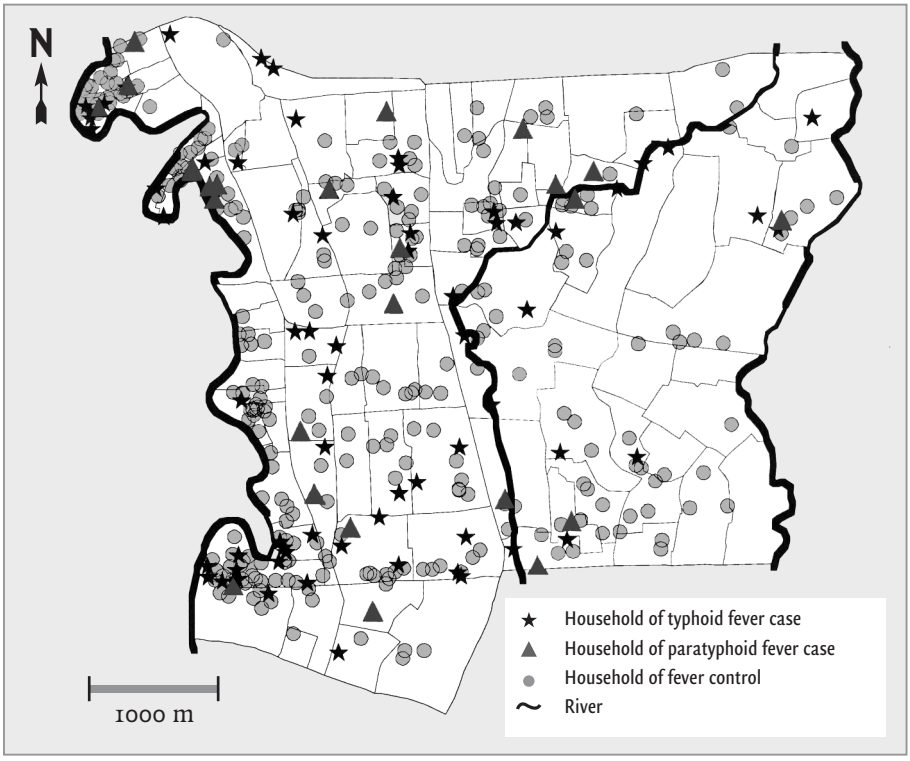
In this community-based case-control study in an endemic area in East Jakarta, Indonesia, we compared case patients having paratyphoid and typhoid fever with random community controls to identify hygienic practices, eating habits, and environmental and household characteristics that could elucidate prevailing 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]

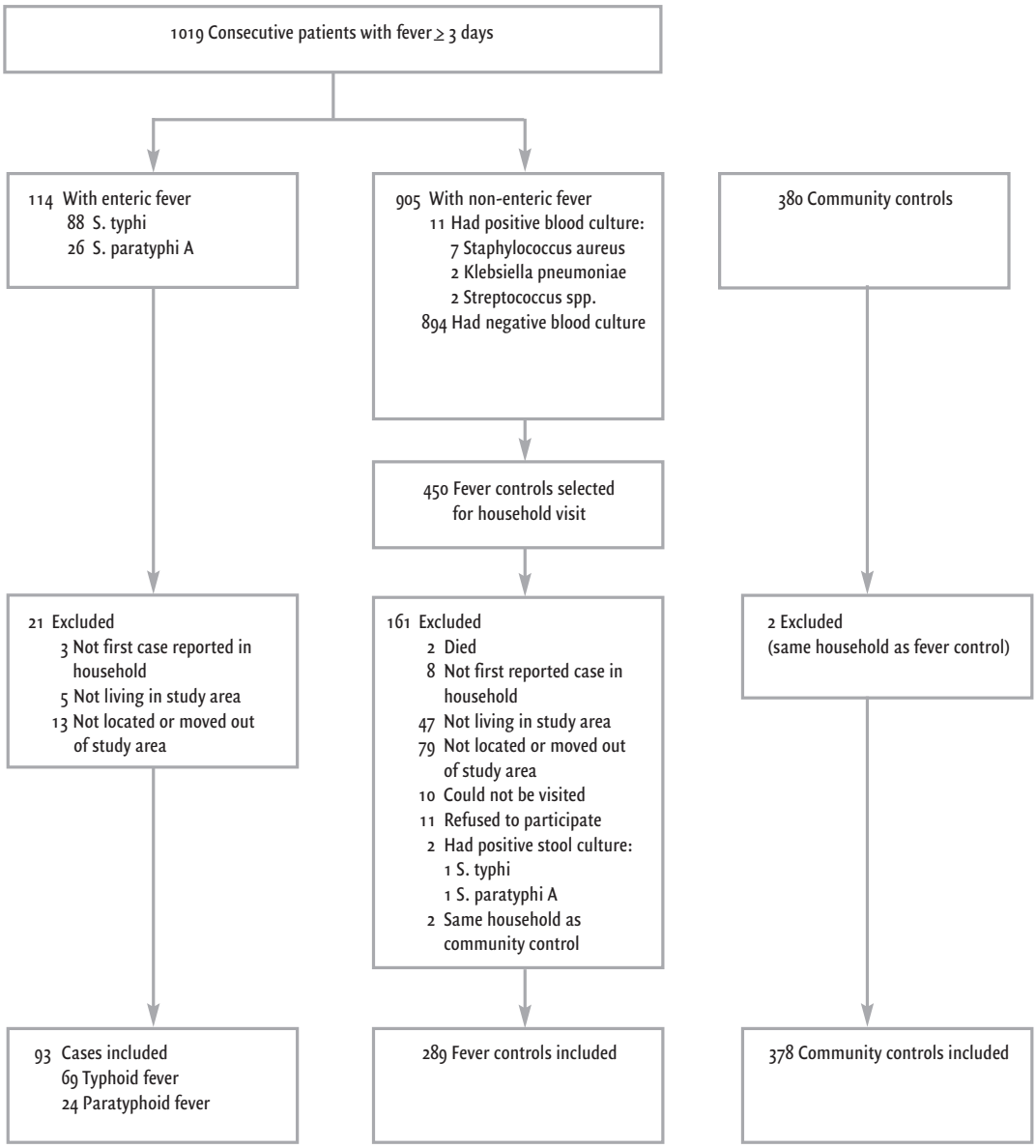
Results

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

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



cases and fever controls, respectively). Due to manpower constraints, 10 fever controls (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

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)

groups, fever controls were more often male, from a lower income group, observed 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 intra-household 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.

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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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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 above-mentioned 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 here is not the exact rate of faecal carriage per se but the finding that the 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 separated. 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.

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4

A survey of the supply and bacteriologic quality of drinking water and sanitation in Jakarta, Indonesia

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Abstract

Background: We assessed water supply, water quality and human waste disposal and their association with diarrhoeal illness in East-Jakarta, where part of the study area has been involved in the *Kampung* Improvement Program (KIP).
Methods: 378 households, randomly selected in the study area, were visited and questioned about water source, sanitation and diarrhoeal illness in the past 3 months. Microbiological quality of drinking water was assessed.
Results: The water sources were boreholes (243; 64%), the water mains (77; 20%), bottled water (45; 12%), or vendors or dug wells (243; 4%). Faecal coliforms were isolated in 56% of the samples (median 23 (IQR 6-240)/100 mL in the contaminated samples). Only 2 (3%) of the water mains' samples contained > 100 faecal coliforms/100 mL, compared to 57 (24%) groundwater samples. Most residents used private toilets with drainage in on-site septic tanks, yet in over one quarter of households human excreta were disposed into rivers or gutters. KIP-areas lagged behind in environmental hygiene. Diarrhoeal episodes were reported in one third of households; these were associated with water contaminated with > 100 faecal coliforms/100 mL (OR 2.4 [95% CI: 1.4-4.2]), but there was no significant association with either water source or environmental contamination. Significantly, all individuals reported to boil water before consumption.
Conclusion: In East Jakarta supply and quality of drinking water –though frequently contaminated with low bacterial numbers– is satisfactory, even in slum areas, but human excreta disposal is inadequate, especially in areas subject to the *Kampung* Improvement Program. This health hazard, as assessed by the frequency of diarrhoeal illness, apparently is countered effectively by the generally endorsed practice of boiling drinking water before use. The expansion and rehabilitation of the watermains should be encouraged to supply high quality drinking water to households.

Introduction

The second greatest loss of Disability-Adjusted Life Years worldwide is attributable to the combination of poor water supply, sanitation and personal hygiene. [1] A substantial proportion of the population in developing countries lacks access to high quality drinking water. [2] Health benefits that can be derived from improved water supplies not only depend on improvements in water quality, but should be accompanied by improvements in sanitation. [3,4] For instance, improvements in water quality alone were shown to reduce the morbidity rates of diarrhoeal illness with 16%, whereas improvements in both water quality and availability resulted in a reduction of 37%. [5] Inadequate water and sanitation adversely affected the nutritional status of children, but better water source alone did not accomplish full health benefits in Peru. [6] Next to improvements in the 'hardware' components of water supply and sanitation (i.e., the physical infrastructure), 'software' interventions are essential to improve health outcomes. [7] The latter refers to the responsibilities of the health sector in water and sanitation interventions: the transfer of knowledge and the initiatives to induce changes in behaviour (i.e., hygiene education, social marketing, surveillance and monitoring).

The interaction of water supply, water quality and sanitation has not been evaluated in Jakarta, Indonesia, where waterborne diseases are endemic. [8,9] We compared the bacteriologic quality of drinking water from different water sources and evaluated sanitary conditions in an urban environment in which some slum-areas had been subject to *Kampung* (slum) improvement projects. Our data may be helpful to determine health risks for citizens in Jakarta and to determine which hardware and software interventions are required to reduce these health risks.

Methods

Study area and population: Jakarta, Indonesia, has an officially registered population of more than 9 million inhabitants of which 40-50% are slum dwellers. The city is located at the Java Sea shore and 40% of its surface is below sea-level. The rainy season lasts from December to April and dry season from May to November. In 1998 the municipal water supply in Jakarta became a joint-venture of the city-owned utility PAM Jaya and two foreign partners, Thames and ONDEO, each providing water to about 300 000 households in North-East and West-Central Jakarta, respectively. At the initiation of the cooperation 43% of Jakarta's population had access to the municipal water network that distributes treated and chlorinated surface water. Both companies apply a cross-subsidy regime in multi-tiered tariff grid to guarantee provision of piped water to the urban poor.

The study area, Jatinegara, is a district in East-Jakarta with an official population of

262 699 inhabitants living in an area of 10.6 km² (March 2002), hence the population density is 24 783/km². No sewage system exists in the study area. The municipal water supply in the area originates from a single distribution station. Three rivers cross the area, making the adjacent sub-districts (*kelurahan*) particularly flood-prone. Three slum-areas have been subject to the *Kampung* Improvement Program (KIP) that has been implemented in several rounds in Jakarta since 1969 by the City Authorities with support from the World Bank (*kampung* = slum). Its objective was to improve the environmental health and conditions of *kampung* dwellers by provision of hand-powered pumps, public toilets, roads, and wastewater drainage.

From March 2002 - February 2003 within a typhoid fever risk factor study [10] we had randomly selected 378 households in every third *rukun tetangga* (RT) of a total of 1140 RTs; RT being the smallest administrative unit comprising 40-60 households. The primary food handlers in the households, i.e., housewives, were approached for an interview. Questions were asked about demographic and socio-economic characteristics, drinking water sources, human waste and wastewater disposal of the households and diarrhoeal episodes of household members in the 3 months preceding the interview. Diarrhoea was defined as three or more liquid stools per day. Informed consent was obtained from all respondents. This study was approved by the Indonesian National Institute of Health Research and Development (*Litbangkes*) and provincial authorities.

Water sample collection: During the study period of 12 months 341 samples from running drinking water sources were collected within each of the 8 sub-districts in at least 6 distinct months. In 45 households purchased mineral water was used for consumption, from these 9 were examined. A 150 mL sample was collected in sterilized bottles according to WHO-guidelines. [11] Faucets of piped water connections and pumps were sterilized and water kept running for one minute before samples were collected. Piped water samples were collected in bottles containing 0.1 mL of 10% sodium-thiosulphate to neutralize the bactericidal effect of chlorine during transport. Water samples were stored in cool boxes with ice and processed within 6 hours after collection in the central water examination laboratory. Samples were examined for total and faecal coliform counts by use of Most Probable Number method: serially diluted water samples were incubated in Endolactose broth and Brilliant Green to detect specific colour changes and gas formation. Faecal contamination was defined as the presence of faecal coliforms (≥ 1 MPN Index/100 mL). [11] The upper detection limit was 1600/100 mL. End-point free chlorine residuals (ppm) had been determined in the study area by Thames PAM Jaya using colorimetry as part of their standard control measurements. Rainfall measurements were obtained from Halim Meteorology Station in East Jakarta.

Statistical analysis: Data from the questionnaires were entered twice using EpiInfo 6.04b software (CDC, Atlanta, USA) and after validation imported in SPSS version 11.5

(SPSS Inc, Chicago, Ill) for statistical analysis. For numerical values the median and interquartile range (IQR) are given. Mann Whitney U-tests were used for comparison of not-normally distributed numerical data from two groups and Kruskal Wallis tests for data from more than two groups. For the comparison of proportions between groups Chi square tests (χ^2) were used. Measures for association were expressed as odds ratios with their confidence limits (OR [95%CI]) for categorical variables. Correlation between variables was tested by a univariate procedure in a regression analysis using a general linear model.

To control for confounding a multivariate analysis was performed by the use of logistic regression with a variable selection using a forward likelihood ratio test with the significantly associated variables from the univariate analysis. Significance levels were $p < 0.05$.

Results

1. Demographic data: The characteristics of the 378 households that were visited are given in **Table 1.** In total 123 households were included in KIP-area and 255 in non-KIP-area, the latter having a significantly lower population density ($p = 0.05$) (**Table 2**). Between the areas no significant differences in demographic variables such as income and median number of household members were found.

Figure 1. Faecal contamination of drinking water sources in Jatinegara, Jakarta

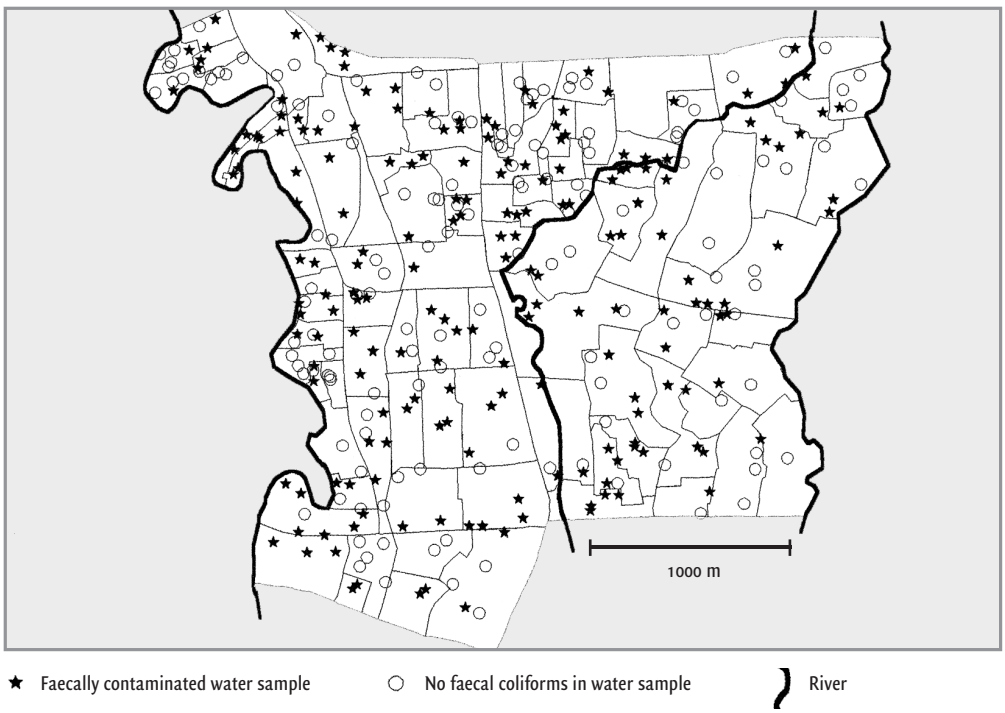


Table 1. Population characteristics, Jatinegara, Jakarta

Variable	Households
n	378
Educational level of family head [a]	
- Max. primary school (%)	140 (37%)
- Higher education (%)	234 (62%)
Profession of family head	
- Self employed / private sector	209 (55%)
- Blue collar worker	56 (15%)
- Housewife	37 (10%)
- Civil service	32 (8%)
- Unemployed	24 (6%)
- Retired	16 (4%)
- Student	4 (1%)
Median number of household members (IQR)	6 (4-7)
Family income [b]	
- Median	900 000 Rp
Ownership of the house	
- Self-owned	326 (86%)
- Rented	52 (14%)

a. Data missing for 4 respondents, family head = breadwinner and/or most senior household member
b. Exchange rate Indonesian Rupiah: 1$ = 9400 Rp. (June 2004)

Table 2. Comparison of districts subject to Kampung Improvement Project (KIP) and other districts in Jatinegara, Jakarta

Characteristic	KIP-area	Non-KIP area	OR (95% CI)	p
Interviewed households (n)	123	255		
Population density (km^{-2})	36 670	20 412		0.005
Years of residence (median, IQR)	25 (8-36)	20 (7-31)		0.19
Number of household members (mean, range)	5.7 (2-14)	6.4 (1-50)		0.11
Low income [a]	58 (47%)	124 (49%)	0.94 (0.61-1.45)	0.79
Water source [b]				
- Water mains	31 (25%)	46 (18%)	1.53 (0.91-2.57)	0.11
- Pumped groundwater	69 (56%)	173 (68%)	0.61 (0.39-0.94) [d]	0.03
Sanitation [b]				
- Use of private toilet	91 (74%)	235 (92%)	0.24 (0.13-0.45)	< 0.001
- Use of public toilet	21 (17%)	16 (6%)	3.08 (1.54-6.13)	0.001
Toilet disposal				
- Septic tank	69 (66%)	214 (89%)	0.23 (0.13-0.41) [d]	< 0.001
Garbage disposal [b]				
- In river/gutter	38 (31%)	21 (8%)	4.98 (2.77-8.97) [d]	< 0.001
Drinking water quality [c]				
- Presence of faecal coliforms	54 (51%)	137 (60%)	0.70 (0.44-1.11)	0.13
- Median faecal coliforms (IQR)	23 (4-300)	23 (6-220)		0.77

a: Below median income of all households (< Rupiah 900 000)
b: Respective category was compared to the combined other categories
c: Bottled water was excluded from comparison; comparison of numbers: Mann Whitney U-test
d: Independently associated in multivariate analysis

2. Water source: Most residents (64%) used groundwater extracted by electrically or hand-powered pumps from boreholes (**Table 3**). The reported depth ranged from 3 to 35 meter (mean 16 meter). Only 5 households used public pumps (2%), the others had access to privately constructed boreholes in their houses. Twenty percent of the households used the water mains as the source of drinking water and 12% only drank bottled mineral water (**Table 3**). The latter households also had connections to the water mains (30%) or boreholes (71%) from which water was used for personal hygiene and/or food preparation. Only very small proportions used water from unprotected dug wells (2%) or water from vendors (2%) as a source of drinking water.
The high-income group had more often private connections to the water mains than the low-income group: 55 vs. 44%, but this difference was not significant (p = 0.09). Consumers of bottled water were left out from this comparison. This group had a significantly higher income than the users of all other sources: 84% vs. 47% were from the high-income group, respectively (p < 0.001).
Fewer pumps were present in households in KIP-area compared to non-KIP area (56% vs. 68%, p = 0.03), but the expansion of the water mains starts to compensate for this deficit in water access, because more residents in KIP-area had private connections to the water mains than in non-KIP area (25% vs. 18%, respectively, p = 0.11) (**Table 2**).

3. Bacteriologic quality of drinking water: In 33 water samples only total coliforms but no faecal coliforms were detected (**Table 4**). Piped water was less often faecally contaminated than the combined other sources (OR 0.10 [0.05-0.19]). In contrast to 57 (24%) of the pumped groundwater samples only 2 (3%) of the piped water samples contained more than 100 faecal coliforms/100 mL (p < 0.001), a level of contamination classified as high risk. [11] However, the comparison of the median numbers of faecal coliforms in contaminated piped water and those from pumped groundwater: 4 (IQR 2-50) versus 23 (IQR 6-280) faecal coliforms/100 mL, showed that the difference with groundwater was non-significant (p = 0.07, Mann Whitney U-test).
Three mineral water bottles contained total coliforms including one bottle with faecal coliforms. These bottles had been refilled in local refill outlets after prior use.
Water from dug wells and water vendors had particularly high rates and levels of contamination (**Table 4**).

4. Sanitation: Most residents (86%) used private toilets in their houses and smaller proportions used public toilets (10%) or the riverbanks (4%) (**Table 5**). In 86% of the private toilets excreta were collected in on-site septic tanks. Human excreta and wastewater were directly disposed into rivers or gutters by 98 households (26%) including the 46 households with a private toilet but without an on-site septic tank.

Table 3. Water provision

Variable	Households
Drinking water source:	
- Boreholes (pumped groundwater)	243 (64%)
- Connection to water mains	77 (20%)
- Bottled water	45 (12%)
- Water from vendors	7 (2%)
- Dug well	6 (2%)
Water used for washing foods and dishes[a]:	
- Groundwater from boreholes	286 (74%)
- Piped water from water mains	88 (23%)
- Other sources	13 (3%)

a. Non-boiled water. Answer given by 387 food handlers in the households.

Table 4. Bacterial contamination of drinking water sources

Water source (number of samples)	≥ 1 total coliforms/ 100 mL	≥ 1 faecal coliforms/ 100 mL	Total coliforms / 100 mL[a]	Faecal coliforms / 100 mL[a]
Water pump (243)	195 (80%)	167 (69%)	30 (2-900)	23 (6-280)
Piped water (76)	15 (20%)	13 (17%)	0 (0-0)	4 (2-50)
Bottled water (9)	3 (33%)	1 (11%)	36	-
Water from vendors (7)	6 (86%)	5 (71%)	140 (2-1600)	170 (12-535)
Dug wells (6)	6 (100%)	6 (100%)	590 (88-1600)	102 (7-1600)
Total (341)	225 (66%)	192 (56%)	220 (28-1600)	23 (6-240)

a. Median (IQR) MPN Index per 100 mL in contaminated samples only.

Table 5. Sanitation and garbage disposal

Variable	Households
Use of toilet:	
- Private toilet	326 (86%)
- Public toilet (with direct disposal onto river)	37 (10%)
- River banks	15 (4%)
Human excreta disposal of private toilets:	
- Septic tank	280 (86%)
- River or gutter	46 (14%)
Garbage disposal:	
- Collection by garbage collector	169 (45%)
- Brought to landfill or container	140 (37%)
- Thrown in the river or gutter	59 (16%)
- Burned	10 (3%)

We calculated the hypothetical distance between all septic tanks in Jatinegara study area using the data from our study group. Assuming a median number of household members of 6 and that 86% of the households had a private toilet of which 86% had a septic tank, we found a radius of 10.2 m around each septic tank in the total study area of 10.6 km^2 with 263 000 inhabitants.

Next to human excreta also garbage was directly disposed into rivers or gutters by 16% of the households.

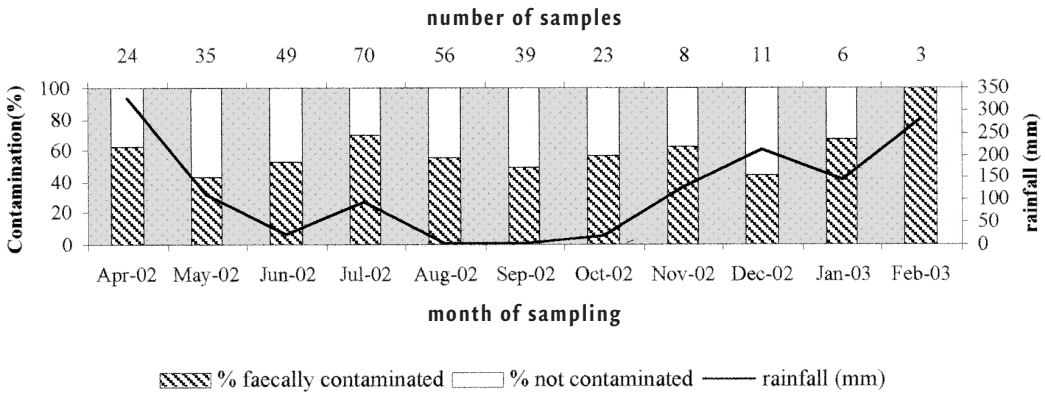
In KIP-area sanitation was still lagging behind, because significantly fewer households used private toilets (74 vs. 92% in non-KIP-area) and consequently more often public toilets were used (17 vs. 6%, respectively) (**Table 2**). The disposal of the private toilets in KIP-area was less frequently collected into septic tanks than in non-KIP area (66 vs. 89%, respectively, $p < 0.001$). Garbage was more frequently thrown into gutters or rivers in KIP-area than in the other area (31 vs. 8%, $p < 0.001$).

5. Factors influencing the contamination rate and level

Distance to the river: Faecal contamination was not confined to areas immediately bordering the rivers (**Figure 1**) nor to one of the 8 sub-districts ($p = 0.38$, χ^2), also not if pumped groundwater samples were analysed separately ($p = 0.65$). The number of piped water samples in some sub-districts was too small to allow statistical comparison of contamination rates and levels in piped water between the distinct sub-districts. The number of faecal coliform in all samples was not significantly different for the 8 distinct sub-districts ($p = 0.40$, Kruskal Wallis test), also not when analyzed for pumped groundwater separately ($p = 0.68$).

Seasonality: The faecal contamination rate of all water samples did not vary significantly per month ($p = 0.13$, χ^2) (**Figure 2**), also not when piped water was analysed separately ($p = 0.63$) or for the pump water samples only ($p = 0.64$). Also, no significant differences in the numbers of faecal coliform per month were demonstrated for piped water samples ($p = 0.46$, Kruskal Wallis test) or pumped water ($p = 0.53$).

Fig. 2. Proportional contamination of samples from groundwater and water mains (n=324) and rainfall per month



KIP-area: Contamination rates (51% vs. 60%, p = 0.13) and levels (p = 0.77) in the KIP and non-KIP-area were not significantly different (**Table 2**).
Depth of boreholes: From 168 pumps both the depth of the borehole and contamination level was known. In a linear regression model for depth of the borehole and numbers of faecal coliforms significantly lower numbers were found in deeper boreholes (p=0.017, R = 0.2), but high bacterial counts were found over the full range of depths. Thirteen outliers were not confined to one sub-district in the study area and were mostly sampled in dry season (11 from 13). The mean depth of boreholes containing less than 100 faecal coliforms/100 mL was 17.0 m compared to 14.2 m in those with > 100 bacteria/100 mL(p = 0.02).
Chlorination: Thames PAM Jaya monthly measured end-point free chlorine residuals (ppm) at 17 locations in the study area during 2002, during the study period. An end-point level of 0.3 ppm free chlorine residual is the minimum bactericidal level the water company applies which was met in 74% of the measurements. In regression analysis month and location were both independently (p = 0.03 and p < 0.001, respectively) of influence on the chlorine level, but rainfall was not independently associated (p = 0.53).

6. Diarrhoeal illness
In 34% of the households diarrhoeal episodes were reported for at least one of the household members in the 3 months preceding the interview. The occurrence of diarrhoea was not associated with the use of other sources than piped water for consumption (OR 1.03 [0.61-1.75]) or for washing food (OR 1.21 [0.74-1.97]). Also non-associated with diarrhoea were flooding (OR 1.40 [0.83-2.33]), the use of private toilets (OR 0.95 [0.52-1.77]), residence in a KIP-area (OR 1.11 [0.70-1.75]) or faecal contamination of the drinking water source (OR 1.09 [0.70-1.72]). However, in households with water contamination > 100 faecal coliforms/100 mL diarrhoea was reported more frequently (51 vs. 30%) (OR 2.40 [1.38-4.16]).
All respondents reported to boil their drinking water before consumption with the exception of the 45 consumers of bottled water and 2 subjects who drank non-boiled water from the water mains. Non-boiled groundwater from boreholes was also used in 74% for food preparation.

7. *Kampung* Improvement Program
The significant differences in water supply and sanitation in the areas that had been subject to KIP compared to non-KIP area from univariate analysis include: less access to pumped water, less use of private toilets and, consequently, more use of public toilets, fewer septic tanks and inadequate garbage disposal (**table 2**). In a multivariate analysis by logistic regression independent characteristics of the KIP-area were: less access of households to groundwater from pumps as a source of drinking water (OR 0.54 [0.33-0.89]);

more frequent garbage disposal in rivers and gutters (OR 2.89 [1.33-6.27]); and less presence of septic tanks for collection of human excreta (OR 0.33 [0.17-0.65]).

Discussion

The main finding of the present study in East-Jakarta is that although the supply and quality of drinking water – though frequently faecally contaminated with low bacterial numbers – are satisfactory, human waste disposal and environmental hygiene are poor. Areas subject to the prior *Kampung* Improvement Program especially were still lagging behind with respect to provisions of sanitation and environmental hygiene. However, the health hazard resulting from the latter situation, as assessed by frequency of diarrhoeal illness, apparently is countered effectively by the generally endorsed practice of boiling drinking water before consumption.

With respect to the study design the following should be considered. We examined the drinking water quality at the source and not in containers after storage or boiling. We choose this method to compare the drinking water quality from different water sources, since comparative data are scarce in Jakarta. The only samples that had been stored prior to sampling were those from the water vendors and high numbers of coliforms were demonstrated in these samples. Contamination was most likely caused by unhygienic handling or infrequent cleaning of the water containers. Similarly, if boiled water is stored in unhygienic conditions, the initial gains of microbiologically safe water supply would be nullified. Second, we only examined the microbiological quality of drinking water and therefore nothing can be said about the chemical pollution of groundwater. However, considering the lack of wastewater-treatment in Jakarta and uncontrolled garbage disposal this problem must be considerable. Finally, the occurrence of diarrhoea in household members as reported by the food handlers in the households might be an under-estimation of the true incidence.

The access to water sources in the study area was adequate, because most households had private pumps or connections to the water mains. Freely available groundwater extracted by pumps was the predominant source of drinking water and water used for food preparation. Although low income could prevent residents from buying bottled water or acquiring a direct connection to the water mains, the projected future scarcity of groundwater in Jakarta may eventually limit its availability. The industrial and domestic over-exploitation of groundwater caused a drop in the groundwater table and consequent salination by intrusion of seawater (Indonesia Water Resources and Irrigation Reform Implementation Project, Strategic Management Plan Ciliwung-Cisadane, 2003). In the first 4 years of operation (1998-2002) the number of connections to the water system network of Thames PAM Jaya increased with 30%. Further expansion and continuation of the existing

cross-subsidy system are therefore necessary to compensate for the expected increase in demand for piped water.

Chlorinated piped water was least frequently contaminated. Although no significant difference in the number of faecal coliforms in the contaminated samples from piped water and groundwater was found, groundwater contained more often contamination levels of more than 100 faecal coliforms per 100 mL. That contamination level was associated with the occurrence of diarrhoea in households making water treatment at home (i.e., boiling or addition of hypochlorite) and hygienic storage crucial for prevention of diarrhoeal illness. Below this threshold level most likely other transmission routes of diarrhoeal disease (e.g., food and personal hygiene) contribute equally or more to transmission of diarrhoeal disease, similar to findings of Moe et al. [12] The association may be explained by the frequent use of non-boiled groundwater for food preparation, but other factors, such as unhygienic storage of boiled drinking water, might have contributed as well.

We assume that the inadequate sanitation and human waste disposal in our study area and the subsequent intrusion of wastewater into water pipes and boreholes are responsible for the contamination of piped water and groundwater, respectively. Four observations supported this hypothesis.

First, in our study area with a high population density the septic tanks are located in close proximity to each other and the minimum recommended distance of 10 m between septic tanks and water sources is therefore infrequently met. [10,12] The septic tanks require adequate outflow of the liquid effluent and repeated emptying of faecal sludge. Both requirements are hard to enforce, since Jakarta lacks good sewage systems to dispose the liquid effluent and the impaired infrastructure in overcrowded slum-areas makes emptying difficult or impossible.

Second, contamination of groundwater samples was evenly spread over the study area. Other factors than proximity to the river (i.e., nearby septic tanks and open gutters) must therefore be associated with faecal contamination as well. Also the fact that high numbers of faecal coliforms were found in shallow and even in some of the deep boreholes demonstrated that groundwater is likely contaminated by intrusion of wastewater from superficial ground layers into boreholes (and evidently also into dug wells). Also poor construction or maintenance of boreholes might have contributed in this respect.

Third, uncontrolled garbage disposal in open sewers is complicating the drainage of wastewater. Overflow or leakage from clogged gutters could result in intrusion of faecal bacteria into adjacent boreholes or water pipes. The *Kampung* Improvement Project as initiated by the city authorities did provide for concrete gutters, but participation of residents in maintenance is essential. Garbage collection in Jakarta is a logistic challenge but necessary to prevent the obstruction of water flows by direct disposal of garbage

into gutters and rivers.
Fourth, fluctuating levels of chlorination were demonstrated in samples from the water mains. Intrusion of wastewater into the water mains decreases the chlorination level and consequently bacterial numbers rise. Intrusion is certainly possible, because administrative and technical leakage of the water mains is calculated to cause a loss of 50% of the initial water supply in Jakarta (Case studies from Dhaka, Jakarta and Manila, Malou Mangahas, Philippine Center for Investigative Journalism, www.adb.org, 2002). As a consequence, water pressure in the water mains is low and supply is not continuous. Residents store water at home to bridge intervals in supply. This could give rise to contamination of stored water by immersion of soiled hands or utensils into water reservoirs or by the use of dirty storage vessels. [14-16] Negative pressures during interruptions in supply and back-siphonage can facilitate intrusion of surrounding wastewater into leaking water pipes resulting in post-treatment contamination of the water mains. This mechanism could cause outbreaks of disease as was illustrated by a massive typhoid fever epidemic in Tajikistan in 1997 that was associated with the use of municipal water. [17] Rehabilitation of the water network, as was already initiated by the water company, is consequently a priority in water management to guarantee the distribution of high quality water and to anticipate the increasing demand for piped water.
In conclusion, the adequate availability of water in most households, the low contamination levels in most-used drinking water sources and the entrenched habit of boiling piped and groundwater before consumption reduce the risk of diarrhoeal illness in East Jakarta. However, the inadequate human excreta disposal is a threat to the quality of both piped water and groundwater. With respect to hardware interventions in water supply and sanitation the rehabilitation and expansion of the existing water network combined with a 24-hour water supply (i.e., permanent pressure in the water mains) are needed to prevent intrusion of wastewater into pipes and to allow supply of high quality water from the water network. The separation of human excreta and water provisions is essential to prevent that intrusion. Future *Kampung* Improvement Programs should specifically focus on human waste disposal, since water supply in slum-areas is increasingly covered by the expansion of the water network in recent years. Specific software interventions by the health sector in Jakarta should address the following issues in public health campaigns: the continuation of boiling groundwater and piped water before consumption; the use of piped water or bottled water for food preparation; the warning for the high contamination rates and levels in water from dug wells and water vendors; the construction of boreholes of sufficient depth; the monitoring of mineral water refill outlets; the promotion of cheap access to the water mains for the urban poor; adequate garbage disposal; and the construction in safe distance from water sources and frequent emptying of septic tanks.

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Helicobacter pylori infection and typhoid fever in Jakarta, Indonesia

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Abstract

We evaluated the association between typhoid fever and *Helicobacter pylori* infection, as the latter microorganism may influence gastric acid secretion and consequently increase susceptibility to *Salmonella typhi* infection. Anti-*H. pylori* IgG and IgA antibody titres (ELISA) and gastrin concentration (RIA) were determined in plasma of 87 blood culture-confirmed typhoid fever cases (collected after clinical recovery) and 232 random healthy controls without a history of typhoid fever, in the Jatinegara district, Jakarta. Patients with typhoid fever more often than controls were seropositive for *H. pylori* IgG (67 vs 50%, p<0.008), when antibody titres were dichotomized around median titres observed in controls. *H. pylori* IgA-seropositivity was not associated with typhoid fever. Plasma gastrin concentrations indicative of hypochlorhydria (i.e., gastrin ≥ 25 or ≥ 100 ng/L) were not significantly elevated in typhoid fever cases as compared to controls (p=0.54 and p=0.27, respectively). In a multivariate analysis, typhoid fever was independently associated with young age (< 27 years, median age of all subjects): Odds Ratio (OR) 4.53 [95%-confidence interval (CI): 2.53-8.10], and *H. pylori* IgG-seropositivity: OR 1.78 [95%-CI: 1.02-3.09]. Typhoid fever was independently associated with *H. pylori* IgG-seropositivity, but not with elevated gastrin concentration. Therefore, the association suggests a common risk of environmental exposure to both bacteria, e.g., poor hygiene, rather than a causal relationship via a reduced gastric acid production.

Introduction

Typhoid fever is a serious systemic illness that each year affects over 20 million people, predominantly in developing countries. [1] Infection with *Salmonella typhi* is transmitted by faecal-oral route and in several epidemiological studies risk factors were identified that suggested either waterborne transmission [2-6] or foodborne transmission. [2,7-9] The determination of the relative contribution of distinct environmental risk factors for transmission of disease is essential to focus local control strategies. Also host-related risk factors for infection have been examined, identifying both genetic factors [10,11] as well as concurrent *Helicobacter pylori* infection, that was interpreted as a cause of a reduced gastric-acid barrier. [8]

A high incidence of salmonellosis has been observed in individuals with surgically induced or other types of achlorhydria (pernicious anemia and chronic atrophic gastritis). [12-14] Also *H. pylori* infection may exert an effect on the secretion of gastric acid. Approximately 50 percent of the world population is infected with *H. pylori* [15], and even higher prevalences have been reported in developing countries [16], where acquisition occurs at a younger age than in the developed world. [17,18] Active infection with *H. pylori* is associated with a transient hypochlorhydria that may be present for several months. [19] Furthermore, *H. pylori*-induced chronic gastritis of the body of the stomach reduces acid secretion and the persistent hypochlorhydria constitutes a risk for the development of gastric cancer. [20,21]

In absence of the acid-mediated inhibition of gastric gastrin release, the serum gastrin concentration increases. In contrast, antral-predominant, body-sparing gastritis due to *H. pylori* increases gastric acid secretion, resulting in duodenal ulcer disease. [21-23] The localization of gastritis depends on the pre-morbid acid secretory status of a subject, dietary, genetic and bacterial factors [22,24], and the age of acquisition of *H. pylori* infection. [18] Consequently, the association between *H. pylori* infection as an indicator of hypochlorhydria and the susceptibility to other gastro-intestinal infections is ambiguous. An increased susceptibility for enteric infections in *H. pylori*-infected individuals, as measured by anti-*H. pylori* IgG-response, was documented for cholera [25,26] and typhoid fever. [8] However, the evidence for the association of *H. pylori* infection and diarrhea is conflicting [27-32] and even a protective effect of *H. pylori* infection was demonstrated. [33,34]

To examine the association of *H. pylori* infection and typhoid fever, we determined the anti-*H. pylori* antibody titres and plasma gastrin in patients who had just recovered from typhoid fever and healthy controls in a case-control study in Jakarta, Indonesia.

Material and Methods

Study design: From June 2001-October 2003 we identified patients with typhoid fever (*Salmonella typhi* infections) in a passive community-based surveillance study among individuals with 3 or more days of fever in the Jatinegara district, Jakarta, Indonesia, as described. [35] In addition, 6 patients with typhoid fever were enrolled in the Medistra Hospital in Central Jakarta. Patients were interviewed using a standardized questionnaire and blood samples were collected during a household visit, conducted within one month after making the diagnosis by blood culture, at a time when the patient had recovered from typhoid fever.

During the study period healthy controls were randomly selected in a random household in every third *rukun tetangga* (RT) of a total of 1140 RT's in Jatinegara; RT being the smallest administrative unit of 40-60 households. Controls were enrolled in a ratio of cases to controls of 1 : 4 to maximize statistical power of the analysis. The selection of controls was non-matched for age or sex to prevent over-matching. When a control reported any type of fever in the 30 days preceding the interview or refused participation, the house on alternating sides of the initially selected household was approached. From 378 healthy controls 291 (77%) allowed collection of a blood sample. Fifty-nine (20%) of these 291 controls were excluded from the analysis because they reported a history of typhoid fever (defined as an episode of 3 or more days of fever, requiring antibiotics prescribed by a physician who had made a diagnosis of typhoid fever). If cases or controls were less than 13 years of age, the mother or guardian was interviewed.

This study was approved by the Indonesian National Institute of Health Research and Development (*Litbangkes*) and provincial authorities. From all participants a written informed consent was obtained at the household visit or during hospitalization.

Laboratory diagnosis of typhoid fever: The diagnosis of typhoid fever required confirmation by blood culture. To this end, blood was collected of the febrile patients (dependent on the age, 5-10 mL), inoculated in aerobic bottles (Bactec, Becton Dickinson, Franklin Lakes, NJ) and incubated. The identification of cultured micro-organisms was done by biochemical tests and agglutination, as described. [35]

Samples in patients and controls: In the household visits blood samples were collected from non-fasting cases and controls in EDTA-tubes. Following centrifugation, plasma was separated and frozen at –70 °C.

Measurement of anti-*H. pylori* IgA and IgG antibodies by ELISA: The ELISA technique for determination of IgG and IgA antibodies against *H. pylori* as well as information on the intra- and interassay variability of the results obtained by this ELISA have been described in detail. [36,37] The antigen is a mixture of sonicates of six different *H. pylori* strains; these had been obtained by sonicating whole bacteria for 6 minutes on a Branson

sonifier (stage 4; 30 000 cycles/sec), and adjusting the suspension to a protein concentration of 3 mg/mL. Optimum concentrations of reagents was determined by checker board titration as described. [36] ELISA results are expressed as absorbance index (AI):
AI = [Patient's OD – OD of blank reading] / [Reference OD – OD of blank reading],
where OD is the optical density. Samples were assayed in blinded fashion in one run.
Measurements of gastrin concentrations: Plasma gastrin concentration was measured using a previously validated radioimmune assay (RIA) as described. [38] Samples were assayed in blinded fashion in one run.
Statistical methods: Data from the questionnaires were entered twice using EpiInfo 6.04b software (CDC, Atlanta, USA), validated and imported in SPSS version 11.5 (SPSS Inc, Chicago, Ill.) for further analysis. Unpaired t-tests for normally distributed variables or Mann Whitney U-tests for not-normally distributed variables were used to test numerical variables. Correlation between numerical variables was tested by linear regression and the Pearson correlation coefficient (R) was provided. For the comparison of proportions the Chi-square test or Fisher's Exact test was used. To correct for age strata Mantel-Haenszel statistic was applied. Measures of association were expressed as odds ratios with their respective confidence limits (OR [95%-CI]) when categorical exposures were evaluated. To control for confounding a multivariate analysis was performed by the use of logistic regression.

Results

Demographic characteristics: Plasma samples were available of 87 typhoid fever cases, and were collected at home following clinical recovery of the patient 22 days [median, IQR: 20-27] after the confirmatory blood culture. Thirteen patients had been hospitalized and 74 patients were included in local health outpatient facilities. During the same period blood samples were obtained from 232 healthy controls. The median age of all subjects was 27 years [IQR: 17-45]. Patients with typhoid fever had a median age of 20 year [IQR: 13-27], and were significantly younger than the controls (median age 33 [IQR: 19-50] years; $p<0.001$ by Mann Whitney U-test). The sex ratio did not differ between the typhoid fever group and controls, i.e., 53 and 59 percent were female individuals, respectively ($p = 0.29$, χ^2).
Concentration of IgG and IgA antibodies against *H.pylori*: Taking all subjects together, the median titre of anti-*H. pylori* IgG amounted to AI 0.126 (IQR: 0.084-0.186) and for IgA to AI 0.094 (IQR: 0.057-0.170). In a linear regression model IgG and IgA were significantly correlated, though not strongly considering the correlation coefficient ($p < 0.001$, $R = 0.23$).

Table 1: H. pylori-serology of typhoid fever cases and controls

Age	Cases	Controls	Cases IgG (AI) [a]	Controls IgG (AI) [a]	IgG ≥ 0.119 (AI) [b]		p [c]
					Cases	Controls	
0-14	28	23	0.132 (0.068-0.179)	0.114 (0.069-0.155)	17 (61%)	10 (43%)	0.22
15-29	43	82	0.151 (0.097-0.190)	0.118 (0.084-0.162)	30 (70%)	39 (48%)	0.02
30-44	12	48	0.143 (0.098-0.192)	0.128 (0.093-0.206)	8 (67%)	26 (54%)	0.43
≥ 45	4	79	0.236 (0.090-0.261)	0.128 (0.080-0.230)	3 (75%)	41 (52%)	0.62
Total:	87	232	0.146 (0.092-0.190)	0.119 (0.082-0.185)	58 (67%)	116 (50%)	0.008
			IgA (AI) [a]	**IgA (AI) [a]**	**IgA ≥ 0.092 (AI) [b]**		**p [c]**
0-14	28	23	0.073 (0.046-0.118)	0.038 (0.016-0.102)	12 (43%)	6 (26%)	0.21
15-29	43	82	0.100 (0.060-0.164)	0.069 (0.042-0.126)	25 (58%)	29 (35%)	0.02
30-44	12	48	0.105 (0.072-0.231)	0.119 (0.073-0.221)	7 (58%)	30 (63%)	0.79
≥ 45	4	79	0.105 (0.074-0.226)	0.134 (0.078-0.296)	2 (50%)	51 (65%)	0.62
Total:	87	232	0.098 (0.060-0.156)	0.092 (0.054-0.178)	46 (53%)	116 (50%)	0.65

a: Median absorbance index (25-75% range)

b: Median antibody AI in total number of healthy controls

c: Chi square test (Fisher's Exact test if cells contained less than 5 subjects)

Table 2: Univariate analysis: associations with typhoid fever

Risk factor	Cases (n=87)	Controls (n=232)	OR (95% CI)	p
Age [a]				
- < median (i.e., 27 years)	64 (74%)	94 (41%)	4.09 (2.37-7.04)	< 0.001
Sex				
- Male	41 (47%)	94 (41%)	1.31 (0.80-2.15)	0.29
Anti-H.*pylori* antibodies				
- IgG positive (AI ≥ 0.119) [b]	58 (67%)	116 (50%)	2.00 (1.20-3.35)	0.008
- IgA positive (AI ≥ 0.092)	46 (53%)	116 (50%)	1.12 (0.69-1.84)	0.65
Plasma gastrin				
- ≥ 25 ng/L	80 (92%)	208 (90%)	1.32 (0.55-3.18)	0.54
- ≥ 100 ng/L	18 (21%)	36 (16%)	1.42 (0.76-2.66)	0.27

a. Independently associated in multivariate analysis: OR 4.53 (95% CI: 2.53-8.10), p < 0.001

b. Independently associated in multivariate analysis: OR 1.78 (95% CI: 1.02-3.09), p = 0.04

In individuals who had suffered from typhoid fever in the preceding month the anti-*H. pylori* IgG-titres (median 0.146 [IQR: 0.092-0.190]) were higher than those in controls (median 0.119 [IQR: 0.082-0.185]), but the difference did not reach a level of significance ($p = 0.24$, by Mann Whitney U-test). Also the IgA titres did not differ significantly ($p > 0.50$) between typhoid fever cases (median 0.098 [IQR: 0.060-0.156]) and controls (median 0.092 [IQR: 0.054-0.178]).

In typhoid fever patients and controls alike, IgG and IgA against *H. pylori* increased with age (**Table 1**), and in a linear regression model IgG titres and age were significantly correlated ($p = 0.005$, $R = 0.16$) as were IgA AI and age ($p < 0.001$, $R = 0.30$). In all age cohorts higher rates of IgG-seropositivity were observed in the group of individuals with typhoid fever, and in the age cohort of 15-29 years this difference reached a level of significance ($p = 0.02$).

Next, the median AI of IgG and IgA in the healthy controls were used to dichotomize the data to obtain a cutoff-level of *H. pylori* seropositivity (IgG ≥ 0.119 and IgA ≥ 0.092, respectively) comparable to study procedures of Bhan et al. [8] The percentage of individuals with typhoid fever who had an IgG titre above this level ($n = 58$; 67%) was significantly ($p = 0.008$) higher than that in the controls ($n = 116$; 50%) (**Table 1**). No significant difference of IgA-seropositivity between individuals with typhoid fever and controls was observed: i.e, 46 (53%) versus 116 (50%), respectively ($p = 0.65$). In a stratified analysis for all individuals correcting for age group by use of Mantel-Haenszel statistic, IgG-seropositivity remained significantly associated with typhoid fever ($p = 0.007$), whereas IgA-seropositivity reached borderline significance ($p = 0.05$).

Gastrin concentration: Taken all subjects together, plasma gastrin concentration (ng/L) ranged from 6 to 1253 ng/L, with a median concentration of 54 (IQR 36-85) ng/L. On average, gastrin concentrations did not change with age ($p > 0.50$, $R = 0.004$). The gastrin concentration in individuals with typhoid fever (median 53 ng/L [IQR: 37-85]) did not differ significantly from that in controls (median 54 ng/L [IQR: 36-85]) ($p = 0.74$, Mann Whitney U-test).

Gastrin concentrations of ≥ 25 ng/L and ≥ 100 ng/L have been evaluated as markers of hypochlorhydria as a condition of chronic atrophic gastritis. [39] In the analysis of the total group, i.e., both cases and controls, anti-*H. pylori* antibody indices were higher in the group of individuals with gastrin ≥ 25 ng/L than in those with a lower plasma gastrin, reaching significance for IgA AI ($p = 0.02$, Mann Whitney U test) and borderline significance for IgG AI ($p = 0.06$). The IgG and IgA AI of individuals with gastrin < 100 ng/L did not differ significantly from those with gastrin ≥ 100 ng/L ($p = 0.44$ and $p > 0.50$, respectively). However, the proportions of typhoid fever cases and controls that had gastrin ≥ 25 ng/L did not differ significantly ($p = 0.54$) (**Table 2**). Similarly, in the group of typhoid fever patients, 18 (21%) individuals had a gastrin concentration ≥ 100 ng/L, as

compared to 36 (16%) in the control group (p = 0.27).

Univariate and multivariate analysis

H. pylori IgG- and IgA-seropositivity and plasma gastrin concentrations in cases and controls were evaluated by univariate analysis (**Table 2**). Significantly associated with typhoid fever were: young age (dichotomized at 27 years, the median age of all subjects): OR 4.09 (95%-CI: 2.37-7.04), and *H. pylori* IgG-seropositivity: OR 2.00 (95%-CI: 1.20-3.35). *H. pylori* IgA-seropositivity nor elevated plasma gastrin concentrations were significantly associated with typhoid fever.

In a multivariate analysis using multiple logistic regression to control for confounding the occurrence of typhoid fever was independently associated with *H. pylori* IgG-seropositivity: OR 1.78 (95%-CI: 1.02-3.09), and young age (< 27 years): OR 4.53 (95%-CI: 2.53-8.10).

Discussion

The main finding of this case-control study in Jakarta is that the prevalence of *H. pylori* IgG-seropositivity is higher in individuals who recently had recovered from blood culture-confirmed typhoid fever than in healthy controls without a history of typhoid fever. However, we did not find an association between typhoid fever and an elevated plasma gastrin concentration indicative of hypo- or achlorhydria. Furthermore, typhoid fever was independently associated with young age, which suggests that either exposure to *S. typhi* or the proportion of those exposed that develop symptomatic infection (e.g., due to absence of a prior acquired immune response) is greater among young people.

A reduced secretion of bactericidal gastric acid due to acute or chronic *H. pylori* infection [22] may increase susceptibility to infection with enteric pathogens. This hypothesis was supported by case-control studies that examined anti-*H. pylori* IgG antibody concentrations in patients with typhoid fever and cholera, and observed a higher prevalence of *H. pylori* seropositivity among the cases than controls. [8,25,26] To this end, seropositivity was defined as an antibody concentration above the median antibody level of the controls [8], a method we applied as well to be able to compare our results and also to confirm the demonstrated association of typhoid fever and *H. pylori* infection.

Although the anti-*H. pylori* IgG-response reliably indicates prior infection with *H. pylori*, *H. pylori* colonization in the mostly adult typhoid fever cases and controls most likely occurred at a much younger age, i.e., in early childhood [17], than the acquisition of typhoid fever in our study. Moreover, the infection itself may exert different effects on the gastric acid secretion and is therefore not one-dimensionally associated with hypochlorhydria as a predisposing condition for enteric infections. [21] In addition, the evidence for a correlation between anti-*H. pylori* IgG-antibody levels and the severity of gastritis or

H. pylori density remains conflicting [40-43], thereby diminishing the likelihood of a more severe gastritis in typhoid fever cases compared to controls. Also, anti-*H. pylori* antibodies may be a sensitive marker of chronic atrophic gastritis as a cause of hypochlorhydria, although not being a very specific marker [39], which can explain the higher antibody titres in the individuals with gastrin ≥ 25 ng/L in our study. However, we did not find evidence for a higher prevalence of chronic atrophic gastritis, as determined by elevated concentrations of plasma gastrin, among cases of typhoid fever compared with healthy controls.

A more likely explanation of the association of increased anti-*H. pylori* IgG antibodies and typhoid fever might be a higher susceptibility to colonization by and re-exposure to *H. pylori* as a consequence of low hygienic standards and shared risk factors for both infections, than a supposedly decreased gastric acid secretion in typhoid fever cases. Since re-infection with *H. pylori* after eradication is common in developing countries [44] signifying continuous exposure, the association of typhoid fever and *H. pylori* IgG-seropositivity may be confounded by poor hygiene.

Some issues related to the outcome of our study need consideration. We did not find an association between *H. pylori* IgA-seropositivity and typhoid fever, most likely due to the fact that the anti-*H. pylori* IgA serological response is a less sensitive marker for *H. pylori* infection than anti-*H. pylori* IgG. [45-47] We assume that *H. pylori* infection as determined by anti-*H. pylori* antibody response preceded typhoid fever infection, because it takes 1-3 months after *H. pylori* infection to develop anti-*H. pylori* IgG antibodies and typhoid fever cases were visited within 1 month after blood culture-confirmation of *S. typhi* infection.[48] Also, in some cases the antibiotic treatment of typhoid fever might have interfered with the quantitative anti-*H. pylori* IgA antibody response, because in *H. pylori* eradication studies IgA-titres decreased faster than IgG six weeks after antibiotic treatment, but even then IgA and IgG titres will remain elevated during 6-12 months after eradication. [37,44,47,49] Furthermore, samples for determination of plasma gastrin were obtained from non-fasting subjects, but the gastrin concentrations in our study showed little elevation compared to concentrations in a population sample in Mexico, where samples were taking in a fasting state and median serum gastrin amounted to 35 ng/L [IQR 26-52]. [39] Finally, we assume that the association of *H. pylori* infection as defined by anti-*H. pylori* IgG-response and typhoid fever may be the result from shared risk factors for exposure to both bacteria. Infection with *H. pylori* occurs in early childhood within families. [17,20] Several risk factors for *H. pylori* infection have been identified without determination of the exact source of infection, but most are associated with poor hygiene and sanitation within households, and could coincide with the intra-household risk factors for typhoid fever in Jakarta. [17,35] The improvement of hygiene within households might therefore reduce the risk of exposure to both bacteria. Continuous exposure may also explain the correlation of age

and the anti-*H. pylori* antibody absorbance index in our study, similar to findings of Chen et al. [40]

In conclusion, our findings demonstrated that typhoid fever is associated with *H. pylori* IgG seropositivity, whereas the gastrin concentrations were not significantly higher in typhoid fever cases compared with healthy controls. The findings do not support the hypothesis that the association points to *H. pylori*-induced hypochorhydria as risk factor for typhoid fever, but suggest that the epidemiological association is likely confounded by shared risk factors for infection with both bacteria.

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General discussion

This thesis describes the epidemiology and clinical presentation of, and risk factors for *Salmonella typhi* and *S. paratyphi* infection, two endemic diseases in Jakarta. Since both bacteria are strictly human pathogens, eradication of (para)typhoid fever is possible if human exposure would end. Next to improvements in water supply, human excreta disposal and general hygienic measures, control of disease requires the development of a system for disease surveillance through public health agencies and the effective use of antibiotic agents in patients and chronic carriers.
The above-mentioned perspectives on the control of typhoid and paratyphoid fever in Jakarta were kept in mind in the present thesis that provided empirical evidence on the risk factors of disease in Jakarta. The presented articles also suggested initiatives for the control of disease at the level of residents, health care providers, food vendors, public health planners and municipal authorities.

Health care providers

At the level of health care providers suggestions were provided for the identification of (para)typhoid fever cases among patients with fever, in order to target antibiotic treatment and to give advice on specific measures for the prevention of further spread of the disease in households or professions of patients (**chapter 1**). In a passive surveillance study during 20 months involving 4 hospitals and 20 outpatient centres, 88 blood culture-confirmed typhoid and 26 paratyphoid fever patients were identified among 1019 consecutive fever patients. A conservative estimate of the incidence of enteric fever using our surveillance data was 0.03% in the study area, lower than the previously reported rate in another part in Indonesia (1%). Some factors might have influenced the inclusion rate of patients in the surveillance study: self-treatment through the use of over-the-counter antibiotics, the consultation of other private health care providers than the participating health centres, and a possible under-representation in our study of young children who may present in an atypical way. However, the demonstrated incidence rate was much lower than anticipated, which may be due to an improved water supply and consequent boiling of drinking water in Jakarta (**chapter 4**).
Also was shown that paratyphoid fever was the cause of disease in one quarter of the enteric fever patients. This finding contrasted with other surveillance studies that showed percentages of five to twenty percent of paratyphoid fever in enteric fever. One reason could be, apart from a relative decrease of the incidence of typhoid fever, that the expanding urban population in post-Asian crisis Jakarta has become increasingly dependent on street food for nutritional needs. As was shown, hygiene in food stalls and pushcarts is poorer than in restaurants (**chapter 3**). Consequently, the hands of recent paratyphoid fever patients among street vendors might contaminate street foods in which paratyphoid

bacteria can multiply to reach a sufficiently high dose for disease in customers (**chapter 3**). Health care providers in outpatient clinics, being the physicians dealing most frequently with (para)typhoid fever patients in Jakarta, should bear in mind that the clinical presentation of (para)typhoid fever is relatively similar to other febrile illnesses (**chapter 1**). Paratyphoid fever could not be distinguished clinically from typhoid fever and should therefore be considered in the differential diagnosis of any febrile illness as well. When culture facilities are absent, suggestions were provided to increase the index-of-suspicion for (para)typhoid fever in patients presenting with fever. Decisions on empiric treatment and advice on hygiene measures should be influenced by the duration of fever of patients, absence of cough with or without chills in the first week of fever, and delirium in the second week, to increase the likelihood of (para)typhoid fever in a febrile outpatient from 1 : 10 in the first week to at most 2 : 3 for the latter combination. However, no conclusive picture could be distilled from our data that could help physicians in the outpatient clinic to help differentiate enteric fever from other causes of fever. Therefore, blood cultures are necessary for the diagnosis, for the determination of antibiotic susceptibility of *Salmonella* strains, and for disease surveillance and monitoring of public health interventions. Fortunately, in Jakarta multi-drug resistance of *S. typhi* is rare, and expensive fluoroquinolones or hospitalization to administer intravenous antibiotics are currently not required for the treatment of both typhoid and paratyphoid fever. Ironically, the empiric treatment strategy in many outpatient clinics to treat any patient with prolonged fever as a suspected typhoid fever patient might have helped to limit the spread of (para)typhoid fever. Notwithstanding the short-term benefits of this rigorous strategy for the treatment of ten percent of the fever patients with the actual disease, long-term consequences might be the appearance of multi-drug resistant bacteria. Also unwanted and serious side-effects, e.g., aplastic anemia due to chloramphenicol, are the price that non-enteric fever patients pay for the overexposure to antibiotic agents. However, considering the lack of culture facilities in most centres, the absence of sensitive serological tests, and the high costs of cultures for most residents, disease surveillance and identification of chronic carriers by use of cultures will currently remain infeasible instruments in public health practice in Jakarta.

Health care providers should emphasize adequate hand washing hygiene of (suspected) typhoid fever patients, because typhoid fever appeared to be spread predominantly within households by cases. In our study (para)typhoid fever patients continued to excrete bacteria in their stools for several weeks after the acute infection. The combination of bacterial excretion and both inadequate hand washing hygiene and sharing food with other household members, could facilitate intra-household transmission (**chapter 2**). In the chapter describing risk factors for transmission of food borne illness, hand washing hygiene in commercial food handling was poor, especially in the food stalls and pushcarts, which

could explain the association of paratyphoid fever and consumption of street food (**chapter 3**). Apart from the use of soap for hand washing, also boiling of drinking water appeared to be an effective preventive measure to counteract the spread of disease at household-level, and this practice should be stimulated explicitly in health campaigns (**chapter 2, 4**).

In conclusion, in Jakarta (para)typhoid fever is an illness predominantly treated in out-patient setting. Health care providers in Jakarta should consider (para)typhoid fever in about 10% of the presenting fever patients, especially in those with prolonged fever and delirium. The differentiation of (para)typhoid fever from other febrile illnesses is difficult on clinical grounds only, as is the differentiation of paratyphoid from typhoid fever. Standard first-line antibiotics suffice for the treatment of (para)typhoid fever, and treatment should be accompanied with advice on adequate hand-washing hygiene in the weeks during and following treatment .

Public health agencies

The initiation of mass immunization campaigns does not seem to be a cost-effective public health intervention in Jakarta. The relatively low-endemicity level of typhoid fever, the high proportion of paratyphoid fever in enteric fever (that is not covered in current typhoid vaccines), the cheap and adequate antibiotic treatment options, and the more effective intervention method of the propagation of the use of soap (which would also help reduce the overall incidence of febrile illnesses) provide little substantial support for the initiation of costly mass typhoid fever immunization programmes in Jakarta. Scarce health resources might better be allocated to the development of culture facilities, the distribution of soap to suspected typhoid fever patients and to educational programs in the *puskesmas* on personal hygiene, safe human waste disposal and domestic treatment of drinking water.

Empirical evidence on the risk of transmission of waterborne disease was provided in **chapter 4**. The assessment was based on the examination of water supply, water quality and sanitation in 378 households in Jakarta. Piped water was much less often contaminated and, when contaminated, contained less frequently high numbers of faecal coliforms that were associated with diarrheal illness in households. These two factors and the expected reduction of the availability of groundwater due to its overexploitation in Jakarta, should encourage city administrators and water companies to expand the water network to supply the remaining 80% of the households in Jatinegara that not are connected to the water mains.

Also was shown that expansion alone, without the rehabilitation of the existing water network to allow permanent pressure in water pipes, could limit the health gains of the

provision of good quality drinking water due to the poor environmental hygiene and sanitation in especially the slum-areas that have been subject to *Kampung* Improvement Programs. Future improvement programs should specifically focus on human excreta disposal in slums, because the lack of a sewage system in Jakarta resulted in frequent contamination of drinking water by intrusion of wastewater into the water mains and boreholes of pumps. This potential health hazard appeared to be countered effectively by the entrenched habit of boiling drinking water, but failure to do so could result in outbreaks of typhoid fever if transient or chronic carriers contaminate central sources.
In conclusion, drinking water supply and quality are not the major contributing factors in the transmission of typhoid fever, but potential outbreaks of disease should be prevented by the provision of drinking water of good quality, i.e., water from the water mains, and adequate human waste disposal. Scarce health care resources might better be allocated to educational programs on hand-washing hygiene and domestic treatment of drinking water, than to mass vaccination campaigns.

Food vendors

Because of the association of paratyphoid fever and street food, a cross-sectional study was carried out in the same study area that compared hygiene practices, water quality and faecal carrier rates of *Salmonella* bacteria of 128 street vendors with that of 74 vendors in restaurants (**chapter 3**). The main finding was that one in every twenty-five vendors excreted *Salmonella* bacteria, including one case of *S. typhi* in an asymptomatic individual among the food vendors. In addition, street vendors observed poorer hand washing hygiene compared to the other vendors and in food stalls and pushcarts direct hand contact with foods occurred more frequently. Consequently, since faecal carrier rates of enteric pathogens did not differ between the two groups of commercial food handlers, hygienic behavioral components in the street food trade appeared to be contributing most to the transmission of food borne illness in Jakarta.
Dishwashing standards were inadequate in the street food trade, with high rates of faecal contamination of dishwater. Samples of drinking water and ice cubes in all food units were frequently faecally contaminated and might additionally contribute to the transmission of waterborne diseases such as typhoid fever. The lower hygiene standards of street vendors compared to those of food handlers in restaurants could therefore explain the association of (para)typhoid fever and street food, because of the higher probability of contamination of street food through hands, dishes and water. In the warm climate of Jakarta bacteria can multiply rapidly in foods and reach sufficiently high doses for disease in customers.
In conclusion, relatively simple interventions could reduce the role of food vendors in

the transmission of food borne illness in Jakarta: instruction on hand washing hygiene and food handling hygiene, frequent renewal of dishwater and the use of soap in dishwater. To reduce the risk of transmission of waterborne illness in outlets of commercial food handlers, attention should be given to the quality of drinking water and ice cubes.

Host factors

A reduced gastric acid barrier could predispose to enteric infections. Therefore, the contribution of concurrent *Helicobacter pylori* infection - that can cause hypochlorhydria and chronic atrophic gastritis - was determined in typhoid fever patients (**chapter 5**). *H. pylori* infection exerts diverse effects on the gastric acid secretion, depending on the pattern of gastritis, bacterial factors, nutritional status, pre-morbid acid status and genetic factors of the human host and the age of acquisition. An independent association of increased anti-*H. pylori* IgG-titres and typhoid fever was demonstrated in the comparison of typhoid fever cases and healthy controls. However, since an association with increased gastrin and typhoid was not found in the typhoid fever cases, the association might as well be confounded by poor hygiene that predisposes subjects to both infections.
In conclusion, *H. pylori* infection and typhoid fever share intra-household risk factors for disease, and associations between both diseases more likely reflect the shared environmental exposure to both pathogens than causative relations within the host.

Methodological aspects

The risk factor study in **chapter 2** demonstrated how the outcome of a case-control study may be influenced by the selection of the control-group used for comparison. In this study typhoid and paratyphoid fever patients were compared with two groups of controls. The selection of random controls from the community was essential to obtain the prevalence of risk factors in the population.
Compared to the community controls typhoid cases observed poorer hygiene, and therefore a predominant intra-household transmission of typhoid fever in Jakarta was postulated. Several factors consistently hinted at this likely transmission route: non-use of soap, other household contacts with recent typhoid fever, and sharing of food. However, other risk factors for typhoid fever (use of ice cubes and female sex) were demonstrated in the comparison with the fever control-group, because the hygienic standards of the fever controls were relatively similar to those of typhoid cases. Considering the importance of personal hygiene for the reduction of the transmission of many endemic febrile illnesses in Jakarta, e.g., diarrhoeal illness and viral respiratory tract infections, this outcome from the comparison of typhoid cases and fever controls is not surprising.

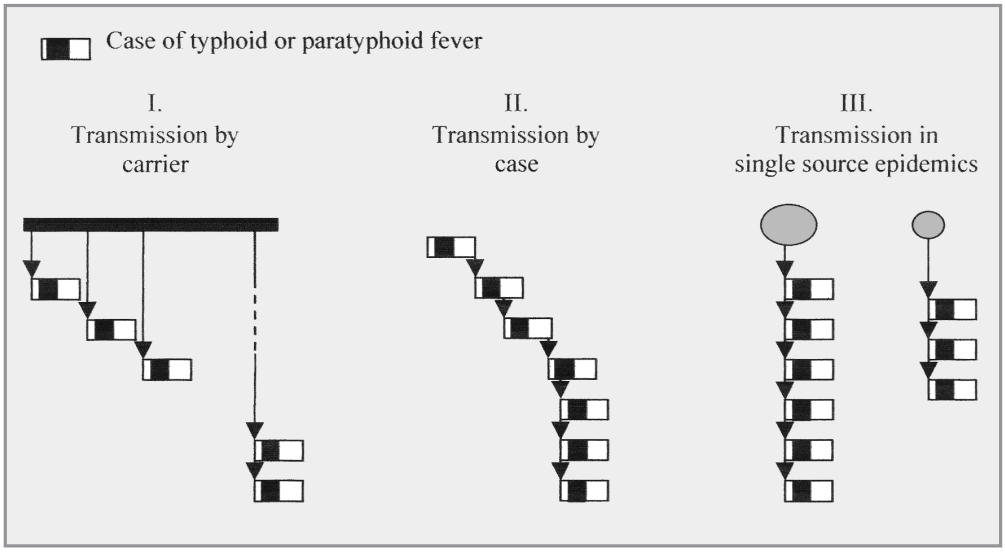
The latter illustrates that matching of cases and controls for disease presentation (i.e., fever for 3 or more days), age, health care seeking behaviour, neighbourhood, or other factors will generate similarities between the two groups that will inevitably influence the outcome of the statistical comparison. A non-matched study design to avoid pre-inclusion selection bias would therefore be preferable. After data collection from all subjects, the independent contribution of a risk factor for disease can be evaluated in a multivariate analysis to control for confounding.

Conclusion

Typhoid and paratyphoid fever are two clinically comparable diseases that cause about ten percent of fever episodes in Jakarta. The two diseases can be transmitted through water and food in conditions of inadequate hygiene. In Jakarta paratyphoid and typhoid fever are associated with distinct routes of transmission, with the risk factors for disease either mainly outside (paratyphoid) or within the household (typhoid).

Three epidemiologic models for the transmission of typhoid and paratyphoid fever can be distinguished, as depicted in the figure below. The first model focuses on the role of the chronic carrier, who can intermittently infect other individuals during a prolonged period of time, e.g., while preparing food in a household or in a restaurant and not observing proper hand washing hygiene. The second model depicts person-to-person spread by cases leading to subsequent or simultaneously occurring secondary cases. The latter model describes outbreaks of disease, due to drinking water sources or shared meals that have been contaminated by a carrier or case.

Epidemiologic models for the transmission of typhoid and paratyphoid fever



These simplified transmission models can overlap and intermingle in endemic regions, but they constitute a useful take-off point for the identification of the weakest link in the transmission chain. In consequence, the number of reported cases within a household or a community and the timeframe of occurrence of cases could help to determine the most likely source or vehicle of transmission of disease. Our surveillance study did not detect any outbreaks of disease (i.e., model 3) nor intra-household carriers among the primary food handlers (i.e., model 1).

The first finding can be explained by the entrenched habit of boiling drinking water before consumption in the study area, and by the fact that most households had private access to drinking water sources, which decreases the likelihood of waterborne transmission on a large scale in the community. However, drinking water was frequently faecally contaminated due to inadequate human waste disposal, which potentially could result in outbreaks of disease, if domestic treatment of drinking water were neglected. Also paratyphoid fever patients among food vendors could initiate micro-epidemics among customers, if inoculation size or the number of replicated bacteria on their foodstuff exceeds the minimal infective dose.

The second finding increases the likelihood of transmission of disease by (convalescent) cases instead of chronic carriers (model 2). This was particularly true for typhoid transmission within households, where recent typhoid cases observed poor personal hygiene. We identified shared food as a likely vehicle for transmission of typhoid fever. The most likely explanation of the finding that transmission of paratyphoid fever within households was not likely to occur, is that the relatively higher infective dose for paratyphoid fever compared to that for typhoid fever, is only infrequently met in food that is shared with household contacts. Food can be implicated as a vehicle in the transmission of paratyphoid fever as well, but our study showed that food purchased from street vendors was a more likely vehicle than shared food items within households. This finding could be explained by the presence of chronic carriers or paratyphoid cases among street vendors (model 1 or 2), who observed inadequate hand washing hygiene and touched food items with bare hands frequently. The contamination of foods with paratyphoid (or typhoid) bacteria and favorable conditions for bacterial growth during storage could result in sufficiently high doses for illness in customers.

In Jakarta containment strategies should focus on the prevention of intra-household spread of typhoid fever by instruction on adequate hygiene of (recent) cases and, to a lesser extent, immunization of household contacts, especially those without a history of typhoid fever. The control of paratyphoid fever requires monitoring of food vendors (e.g., by repeated stool samples) and the detection of convalescent cases and carriers among vendors operating in a district where recent paratyphoid cases have been reported. Simple interventions such as hand washing using soap at home and in commercial food

handling could reduce the food borne transmission of typhoid and paratyphoid fever. The growing global urbanization has important consequences for the epidemiology of infectious diseases, such as typhoid fever. In the first half of the 20th century the incidence of typhoid fever in developed countries rapidly declined due to the improved water supply in urban areas and chlorination of drinking water. However, other members of the *Salmonella* family continue to circulate and infect people, because of their host-specificity (i.e., not confined to humans alone) and because they effectively infiltrated in the industrialized food-processing in these countries. Although water supply and sanitation are far from ideal in many developing countries, improvements are underway, such as in the *Kampung* Improvement Program in Jakarta and through the expansion of the water network. A future prediction would therefore be a reduced global importance of typhoid fever as we already signaled in our study, and a predominantly food borne transmission of *S. typhi*. Consequently, an improved food processing hygiene and personal hygiene would be detrimental for the survival of all *Salmonella* family members, including its relatively fragile (i.e., most host-specific) members *S. typhi* and *S. paratyphi*.

Nederlandse samenvatting

In dit proefschrift is geëvalueerd hoe buiktyfus zich presenteert in Jakarta en welke risicofactoren een rol spelen bij de transmissie van deze infectie. Buiktyfus wordt veroorzaakt door een infectie met de *Salmonella typhi* bacterie, welke alleen de mens treft. Infectie met *Salmonella paratyphi*, vaak met de term paratyfus aangeduid, geeft een vergelijkbaar ziektebeeld, maar komt ongeveer tien keer minder vaak voor dan buiktyfus.

Buiktyfus en paratyfus

Buiktyfus is een verwarrende naam, want de ziekte geeft –behalve in ernstige gevallen– in het algemeen weinig klachten van de buik. De naam is terug te voeren op de tijd dat koortsende ziekten niet nader konden worden gedetermineerd, en buiktyfus zich alleen van die bij tyfus leek te onderscheiden door het optreden van zweren in de darm. Inmiddels weten we dat tyfus een koortsende ziekte is die wordt overgedragen door beten door luizen, vlooien of mijten en veroorzaakt wordt door infectie met *Rickettsiae*. Deze infectieziekte heeft met een *Salmonella* infectie dus niks te maken.

Buiktyfus was een gevreesde ziekte die onbehandeld tien tot twintig procent van de patiënten deed overlijden. In Europa en de VS kwamen vroeger massale epidemieën voor, maar daar is sinds de aanleg van waterleidingen en chlorering van het drinkwater een drastische daling in opgetreden. Tegenwoordig zijn de meeste buiktyfus-patiënten in Nederland toeristen die uit het buitenland terugkomen. In ontwikkelingslanden komen buiktyfus en ook paratyfus nog frequent voor.

S. typhi en *S. paratyphi* hebben geen dierlijk reservoir en veroorzaken uitsluitend ziekte bij mensen. De overdracht van infectie is faeco-oraal, dat wil zeggen via ontlasting dat water of voedsel heeft verontreinigd. Er zijn waarschijnlijk minimaal 100 tot 1000 bacteriën nodig om ziek te worden van buiktyfus. In één gram ontlasting van een patiënt met buiktyfus kunnen vele miljoenen *Salmonella* bacteriën worden aangetroffen, en deze veroorzaken door verontreiniging van drinkwater of voedsel overdracht van de infectie. In voedsel, zoals melk, roomijs, kokosmelk en vleesprodukten, kan de *Salmonella* bacterie zich vermenigvuldigen - de bacterie deelt dan elk half uur - waardoor het aantal microorganismen in het voedsel toeneemt en de kans op overdracht van ziekte groter wordt. Paratyfus zou met name via voedsel worden overgedragen, omdat er meer bacteriën nodig zijn voor een infectie dan voor buiktyfus.

De lichamelijke gevolgen van het inslikken van een voldoende dosis bacteriën manifesteren zich pas na ruim één week. Er ontstaat oplopende lichaamstemperatuur, soms met rillingen en ijlen, en daarbij: misselijkheid, apathie, gebrek aan eetlust, vage buikpijn, soms diarree of juist obstipatie en soms hoest. Met antibiotica is de infectie goed te

behandelen en na drie tot vijf dagen zakt dan de koorts, waarna de patiënt verder kan herstellen. Voordat antibiotica geïntroduceerd werden, kreeg tien procent van de patiënten bloedingen uit de darmwand door erosie van het laatste stuk van de dunne darm. Soms traden daarbij perforaties van ernstig ontstoken darmwandgedeelten op. Die perforaties behoeven altijd operatief ingrijpen om het gat in de darm af te dichten, want zonder behandeling leidt dit tot een fataal verlopende buikvliesontsteking.
Behalve dat patiënten tijdens een acute infectie bacteriën uitscheiden in de ontlasting gedurende drie tot vier weken, blijft een kleine groep patiënten (4%) gedurende jaren na de infectie grote aantallen bacteriën uitscheiden, overigens zonder zelf klachten te hebben. Deze bacterie-strooiers worden chronische dragers ('carriers') genoemd. Zo kan de bacterie ondanks zijn kieskeurigheid voor de menselijke gastheer zich toch langdurig handhaven in een populatie, mits de hygiëne, sanitaire voorzieningen en drinkwatervoorziening de verspreiding van bacteriën uit ontlasting van acute patiënten of chronische dragers via drinkwater of voedsel veilig stellen. En daarmee blijft de mens opgezadeld met deze infectieziekte, tenzij ze erin slaagt verbeteringen door te voeren in de drie bovengenoemde risicofactoren voor buiktyfus en paratyfus.

Onderzoek in Jakarta

In dit proefschrift wordt beschreven hoe buiktyfus- en paratyfus-patiënten werden geïdentificeerd in Jakarta. In Jatinegara, Oost-Jakarta, werkten 4 ziekenhuizen, 12 gezondheidscentra van de overheid (*puskesmas*) en 8 privé-kliniekjes mee gedurende 20 maanden aan een surveillance studie. Bij alle patiënten met drie of meer dagen koorts werd 5-10 mL bloed afgenomen en in een speciale kweekfles geïnjecteerd. Die kweekflesjes waren door de studiegroep zonder kosten voor patiënten en dokters gedistribueerd en werden in een laboratorium op kweek gezet. In de onderzoeksperiode voldeden er 1019 patiënten aan de onderzoekscriteria, waarvan de meeste (89%) buiten de ziekenhuizen werden geïncludeerd. In het bloed van 88 patiënten werd een *S. typhi* en bij 26 een *S. paratyphi* A bacterie geïsoleerd in het centrale laboratorium van een privé-ziekenhuis. De buiktyfus- en paratyfus-patiënten werden thuis bezocht, evenals de helft van de mensen met koorts bij wie in het bloed geen *Salmonella* bacteriën waren geïsoleerd. In totaal werden 69 mensen met buiktyfus geïnterviewd, 24 paratyfus-patiënten en 289 patiënten met een koortsende ziekte van andere origine (de zogenaamde koorts-controles). Daarnaast werden nog 378 huishoudens in het studiegebied bezocht, die volstrekt willekeurig van een lijst werden gekozen. Dit was de gemeenschaps-controlegroep, die een afspiegeling gaf hoe het er in de bevolking aan toeging.
In al deze huishoudens werd er drinkwater afgetapt dat in het laboratorium op bacteriën werd onderzocht, dat wil zeggen op bacteriën die normaal in de ontlasting voorkomen (en dus niet speciaal *S. typhi* of *S. paratyphi*). Hierdoor kan nagegaan worden of besmet-

ting van de waterleiding met fecaliën is opgetreden (en daarmee in potentie ook de mogelijkheid om *S. typhi* over te dragen). Bovendien werd er bij alle bezochte mensen en van de personen in het huishouden die het eten klaarmaakten, ontlasting onderzocht om na te gaan of er carriers in het huishouden voorkwamen. Naast het interview, het monster van het drinkwater en ontlasting, werd er ook van iedereen bloed afgenomen voor onderzoek naar gastheer-factoren.
Tenslotte werden in het onderzoeksgebied 202 voedselverkopers geïnterviewd, met als doel inzicht te krijgen in de hygiëne in restaurants, en in stalletjes en karren. Daarbij werd ook ontlasting van deze verkopers onderzocht op pathogene bacteriën en parasieten en werd drinkwater en afwaswater onderzocht op fecale verontreiniging.

Hoofdstuk 1: Ziektepresentatie

Om voor artsen in de ambulante gezondheidszorg een richtlijn te ontwerpen die zou kunnen helpen buiktyfus en paratyfus in een vroeg stadium te kunnen herkennen, werden de symptomen van 59 buiktyfus-, 23 paratyfus- en 259 andere koorts-patiënten vergeleken. De meeste patiënten waren tieners of jong-volwassenen. Er was geen significant verschil tussen symptomen bij buiktyfus en die bij paratyfus. Wel waren er verschillen met de andere koortsende patiënten: langere duur van koorts voor er een bloedkweek werd afgenomen, vaker last van koude rillingen en ijlen, vaker buikpijn en minder vaak klachten van hoest. Met de symptomen zoals die door de patiënten waren gerapporteerd in de interviews, kon er een richtlijn gegeven worden aan de artsen. Bij patiënten met minder dan 7 dagen koorts is er een kans van 10% dat een patiënt buiktyfus of paratyfus heeft. Als de patiënt niet hoest of daarnaast ook nog rillingen heeft, wordt die kans twee keer zo groot. Voor patiënten met een week koorts of meer, is de aanwezigheid van ijlen ('delier') belangrijk in de diagnostiek, want daardoor stijgt de kans op buiktyfus of paratyfus voor een patiënt van 20% naar maximaal 67%. Maar ondanks het feit dat sommige symptomen suggestief zijn voor buiktyfus of paratyfus, is de klinische presentatie van beide ziekten in hoge mate overeenkomstig met die van andere infectieziekten in Jakarta, zoals dengue of darminfecties. Bloedkweken of serologische testen blijven daarom noodzakelijk voor het definitief vaststellen van een infectie met *S. typhi* of *S. paratyphi*, omdat de klinische presentatie alleen te weinig onderscheidend vermogen biedt. Wel bleek dat de behandelingsopties in Jakarta goed zijn, want de *S. typhi* bacteriën waren maar in een gering percentage (3%) ongevoelig voor de meest gebruikte, goedkope antibiotica.

Hoofdstuk 2: Risicofactoren

Om te onderzoeken welke risicofactoren een rol spelen in de overdracht van buiktyfus en paratyfus in Jakarta, werden beide patiëntengroepen vergeleken met de beide controlegroepen. Paratyfus blijkt met name buiten het huishouden opgelopen te worden, vooral

door het eten van straatvoedsel. Ook bleek overstroming van het huishouden een risicofactor voor ziekte te zijn. Deze beide risicofactoren kwamen naar voren in vergelijking met beide controlegroepen. Voor buiktyfus lag dat anders. In de vergelijking met de gemeenschapsgroep waren het vooral factoren die binnen het huishouden lagen: een eerdere buiktyfus episode van een huisgenoot in het laatste jaar, het niet wassen van handen met zeep, delen van voedsel van hetzelfde bord en de afwezigheid van een toilet. Ook jonge leeftijd was een risicofactor voor buiktyfus. De factoren die met onvoldoende hygiëne te maken hadden, speelden niet mee toen buiktyfus-patiënten werden vergeleken met de andere patiënten-controlegroep. Alleen het gebruik van ijsklontjes was gerelateerd aan buiktyfus en bovendien waren buiktyfus-patiënten vaker van het vrouwelijk geslacht dan de andere patiënten. Het was wel te verklaren dat in de vergelijking met deze controlegroep de factoren die wezen op een slechtere hygiëne van deze patiënten niet naar voren kwamen, omdat onvoldoende hygiëne de overdracht van andere ziekten die gepaard gaan met koorts, zoals sommige vormen van diarree of virale luchtweginfecties, eveneens bevordert.

Hoofdstuk 3: Voedselverkopers

In hetzelfde gebied werden verkopers van etenswaren geïnterviewd: 11 uit restaurants, 63 uit kleine restaurantjes (*warung*), 110 uit stalletjes en 18 mobiele verkopers. Omdat in het risicofactoronderzoek gebleken was dat het eten bij de laatste twee groepen van straatverkopers een risico inhield op het oplopen van paratyfus, werden deze twee groepen gezamenlijk vergeleken met de eerste twee groepen. Naast de interviews, werden ontlasting, drinkwater en afwaswater onderzocht. Bij één op de vijfentwintig verkopers werden *Salmonella* bacteriën gekweekt in de ontlasting, waarvan bij één (circa 0.6%) *S. typhi* werd geïsoleerd. De hygiëne van de straat-verkopers was minder dan die van de verkopers in restaurants. Niet alleen werd er minder vaak handen gewassen, maar ook werd minder vaak zeep gebruikt en vaker etenswaren met blote handen aangeraakt. Afwaswater bleek in het algemeen besmet met fecale coliforme bacteriën, waarbij de hoogste aantallen bacteriën werden aangetroffen bij straatverkopers, die hun water zelden verversten. Bovendien was drinkwater vaak verontreinigd, zonder dat er verschillen waren tussen beide groepen van verkopers. In 23 monsters van ijsklontjes bleken alle monsters verontreinigd. Op basis van deze gegevens kan worden begrepen dat straatvoedsel een risicofactor is voor de overdracht van paratyfus en buiktyfus. Slechte handwashygiëne na ontlasting en voor voedselbereiding, en direct contact van handen met voedsel in de stalletjes en karren leidt tot contaminatie van de etenswaren, waarna de bacteriën verder kunnen groeien, zodat in de etenswaren het noodzakelijke aantal bacteriën voor ontwikkeling van ziekte wordt bereikt.

Hoofdstuk 4: Drinkwater en sanitaire voorzieningen

De gegevens over drinkwatervoorziening en –kwaliteit en over sanitaire voorzieningen van de willekeurig gekozen controlegroep uit het onderzoeksgebied werden afzonderlijk nader uitgediept. Van de 378 huishouden had 20% een aansluiting op de waterleiding, 64% gebruikte grondwater uit pompen en het restant gebruikte of mineraalwater, of water van waterventers of uit putten. Het water van de waterleiding was in een gering percentage besmet met faecale bacteriën (17%) en bovendien waren de aantallen bacteriën laag. Ondanks het feit dat grondwater vaker verontreinigd was (69%), lag het aantal bacteriën in het grondwater van pompen daar niet significant boven, maar wel werden vaker hogere aantallen micro-organismen aangetroffen. In ruim een kwart van de huishoudens loost het toilet rechtstreeks in een open goot of de rivier. De bron van drinkwater of het bezitten van een privé toilet had geen invloed op het vóórkomen van diarree. Wel werd er vaak diarree gerapporteerd in huishoudens met drinkwater dat zwaar verontreinigd was (> 100 fecale coliforme bacteriën per 100 mL). Van belang is verder te melden dat alle inwoners vertelden dat ze hun drinkwater langdurig kookten voor gebruik, waarmee ze het risico op een infectieuze diarree natuurlijk verminderen, ondanks de gebrekkige sanitaire voorzieningen. Behalve verdere uitbreiding van het waterleidingnetwerk is ook vervanging van lekkende pijpen, waarin verontreinigd omgevingswater kan binnendringen, van belang om hoge kwaliteit drinkwater aan de inwoners van Jakarta te kunnen leveren.

Hoofdstuk 5: Helicobacter pylori infectie en buiktyfus

De maaginfectie met *Helicobacter pylori* komt geregeld voor over de hele wereld, vooral in ontwikkelingslanden (tot bij 80% van de volwassenen). Afhankelijk van de plek in de maag waar de infectie een ontstekingsreactie genereert, kan er een afname van maagzuurproduktie optreden. Als gevolg van verminderde zuurproductie zou de kans om bij geringe aantallen *S. typhi* in het voedsel toch buiktyfus op te lopen toenemen. Om dit na te gaan werd bij patiënten met buiktyfus en controle-personen de concentratie van antilichamen tegen *H. pylori* bepaald in het bloed (zowel IgG als IgA). Ook werd de gastrine concentratie in het bloed bepaald, als maat voor een lage maagzuurproduktie, zoals die bijvoorbeeld wordt gemeten voor de bepaling van atrofie (slijmvliesverlies) van de maag. Er werd een associatie van optreden van buiktyfus en anti-*Helicobacter* IgG-spiegels vastgesteld, maar de gastrine concentraties waren niet verschillend tussen patiënten met buiktyfus en controles. Het lijkt er dus op dat de associatie tussen *Helicobacter* infecties (afgemeten aan IgG) en buiktyfus niet op een causaal verband berust. Deze associatie werd in een ander onderzoek gedacht te berusten op een verlaagde zuurproductie bij mensen met een gelijktijdige *Helicobacter* infectie. Maar het is eerder waarschijnlijk dat de gebeurtenissen die leiden tot een *Helicobacter* infectie en/of buiktyfus geassocieerd zijn, bijvoorbeeld door een gebrek aan (voedsel-)hygiëne in het huishouden.

Conclusie

Buiktyfus en paratyfus zijn twee endemische ziekten in Jakarta, die klinisch niet van elkaar te onderscheiden zijn en gezamenlijk circa 10% van de episodes met 3 of meer dagen koorts veroorzaken. Bloedkweken of gevoelige serologische bepalingen zijn noodzakelijk om de infectie met zekerheid vast te stellen, want de klinische presentatie van beide ziekten biedt te weinig houvast voor artsen om een eenduidig onderscheid te maken met andere koortsende ziekten. Zowel buiktyfus als paratyfus worden overgedragen via drinkwater of voedsel, als de toilet- en voedselhygiëne onvoldoende in acht genomen wordt door patiënten die de bacteriën in hun ontlasting uitscheiden. Uit ons onderzoek in Jakarta werd duidelijk dat paratyfus met name buiten het huishouden overgedragen wordt (via straatvoedsel), maar dat de meeste risicofactoren voor buiktyfus binnen het huishouden lagen (personen die eerder een buiktyfus opgelopen hadden).

Weinig bewijs werd gevonden voor overdracht van beide ziekten via drinkwater, omdat de mensen in Jakarta hun water consequent koken voorafgaande aan consumptie, en ook omdat in de laatste jaren de drinkwatervoorziening verbeterd is door uitbreiding van de centrale waterleiding. Toch bevatte het drinkwater vaak fecale coliforme bacteriën, een besmetting die erop wijst dat er contact is van menselijke ontlasting met de drinkwatervoorziening. Verbetering van sanitaire voorzieningen zou dus moeten nagestreefd worden, door het aanleggen van goede riolering en 'septic' opvangtanks. Immers, door slechte omgevingshygiëne zouden er epidemieën kunnen ontstaan als een buiktyfus patiënt een centrale waterleiding besmet met *S. typhi*, en er tegelijkertijd minder zorgvuldig met het drinkwater wordt omgegaan (als bijvoorbeeld het koken van drinkwater wordt veronachtzaamd).

Het was duidelijk dat beide ziektes overgedragen worden via voedsel. Voor buiktyfus: gedeeld voedsel binnen het huishouden, en voor paratyfus: straatvoedsel. Voor beide ziekten bleek een belangrijke rol weggelegd voor voedselhygiëne, of beter gezegd een gebrek daaraan: bij het handenwassen wordt niet consequent zeep gebruikt en daarmee wordt in veel gevallen voorbijgegaan aan een eenvoudige doch efficiënte manier om overdracht van buiktyfus en paratyfus te voorkómen.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