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Assessment of *E. coli* and *Salmonella* spp. infection risks associated with different fecal sludge disposal practices in Thailand

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ABSTRACT

The proper management of fecal sludge (FS), to block the transmission pathways of pathogens, is rarely enforced in many parts of the world. Health risks associated with different disposal practices of FS in peri-urban settings of a large metropolis in Thailand were assessed; Tha Klong sub-district with indiscriminate FS dumping, and Klong Luang sub-district which has an FS treatment system. The study showed that indiscriminate FS dumping from along the canal banks and discharge of market waste were likely the major sources of *E. coli* and *Salmonella* spp. in contamination of the canal water. The increased microbial pathogen concentrations near the FS treatment facility also indicated contamination risks from poorly designed treatment facilities. Quantitative microbial risk assessment (QMRA) indicated very high water-related infection risk levels compared to the actual locally recorded disease occurrences. These results indicated that the QMRA model needs to be modified to take account of immunological differences between populations in developed countries, where the model was developed, and developing countries. In addition, further sensitivity factors are needed to reflect different societal behavior patterns, and therefore contact with potentially contaminated water, in different sub-populations of many less developed communities.

Key words | *Escherichia coli*, fecal sludge, health risk assessment, recreational water, *Salmonella* spp

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INTRODUCTION

In many large cities of developing countries, fecal sludge (FS) collection and disposal is becoming a serious environmental issue as accelerating urbanization results in increased generation of fecal matter that is beyond the capacity of FS collection and treatment facilities (Strauss *et al.* 1997). According to Kone & Strauss (2004), assuming an average daily per capita generation 1 L FS in urban areas, a city of 1 million inhabitants will need to collect daily 1,000 m^3 FS for treatment or disposal. However, reported daily collection rates for much larger cities, such as Bangkok in Thailand, Hong Kong in China and Accra in Ghana rarely exceed 300–500 m^3. Accordingly, large amounts of FS have been either unofficially disposed or reused in agriculture.

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FS is a major source of infectious pathogens, containing numerous bacteria, protozoa, viruses and helminthes. Safe handling and treatment of FS are thus essential primary barriers to block transmission pathways of such pathogens and so protect public health. However, limited finance and lack of information often hinder the installation of FS treatment systems in developing countries (Sundaresan *et al.* 1978; Boesch & Schertenleib 1985; Ingallinella *et al.* 2002).

In Thailand, sanitation coverage exceeds 96% in both urban and rural settings due to housing construction guidelines that require installation of on-site sanitation (OSS) systems in residential dwellings (WHO/UNICEF 2004). Typical types of OSS systems include flushing toilets with cesspools, septic tanks or soakage pits. Such OSS systems generate FS that needs to be evacuated for treatment and disposal (Heinss *et al.* 1998). However, the collected FS is often dumped without proper treatment into waterways and/or onto public land, or reused in agriculture. This leads to fecal contamination of the water environment and associated public health risks. While some local authorities in Thailand have installed FS treatment facilities, many of these facilities are not well designed and are managed by poorly-trained personnel. As a consequence, human excreta-transmitted communicable diseases are still one of the leading causes of death of all ages in Thailand (Ministry of Public Health 2001).

Quantitative Microbial Risk Assessment (QMRA) is an approach that calculates the risk a water system poses to public health based on knowledge of the distribution, concentration and infectivity of particular pathogens in the system (Hunter *et al.* 2003). QMRA requires quantitative data on all major aspects of the concerned water environment, and to a certain degree has to rely on assumptions and estimations, particularly when it is applied to developing countries where reliable quantitative data is lacking.

The first aim of this study was to use QMRA to compare how different FS management practices in Thailand affected the degrees of fecal contamination in the water environment and associated health risks posed to local communities in the respective areas. The second aim was to assess the suitability of QMRA in developing countries where health and society factors often differ from those in developed countries where QMRA was originally validated.

MATERIALS AND METHODS

Study area

Klong Luang is a district in the northeast of Pathumthani province, and is situated in a very fertile urban–rural fringe of the central plains in Thailand, 46 km north from the center of Bangkok. Two sub-districts were selected as study areas for this investigation, namely Klong Luang sub-district and Tha Klong sub-district (Figure 1). These two sub-districts have similar environmental, geographical and socioeconomic backgrounds, but have different FS management systems. In the former, FS is collected by municipal trucks and disposed of in the municipal FS treatment facility consisting of an anaerobic digester and an open drying bed without any lining inserted, and is adjacent to an irrigation canal, while in the latter, FS is collected by a private collector and disposed of indiscriminately in canals or open lands. General information about each sub-district is given in Table 1.

Sampling sites

There are several canals running in parallel from Tha Klong sub-district to Klong Luang sub-district, originally constructed for agricultural irrigation in the 1880s (Van Beek 1995). However, since agricultural production failed the canals have been regarded as open sewers. In addition, the canals have been used extensively by local residents for washing clothes and household utensils, bathing, catching fish and collecting recyclables. Figure 2((a) and (b))

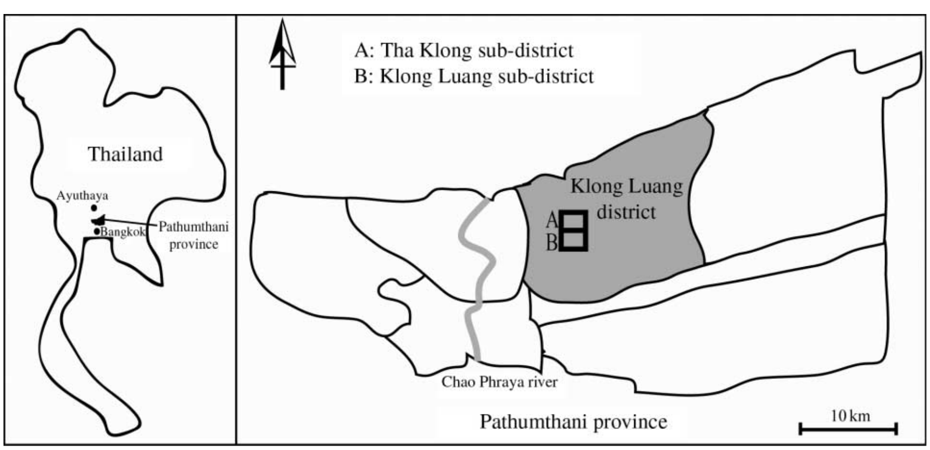

Figure 1 | Map of Thailand (left) and Tha Klong and Klong Luang sub-district in Klong Loang district, Pathumthani province (right).

Table 1 | General information of the two study sub-districts in Pathumthani province

Item	Klong Luang sub-district	Tha Klong sub-district
Population	45,833 (01 Oct, 2004)	35,371 (30 Apr, 2002)
Total area	42,935 km^2	63,000 km^2
No. households	10,565	9,500
Major land use	Residential	Residential
	Industrial	Industrial (1 industrial estate)
	Green area	Green area
	Large-scale food market (Tara Thai)	Institutional (academic & medical)
Major industries	Service industries, food and drink processing, mechanical, vehicle and engine industries, chemical, plastic and metal processing, wood processing, garments, agricultural processing	Service industries, food processing, mechanical vehicle and electronic industries, chemical, plastic and metal processing, garments, furniture, agricultural processing
Types of latrines in use	Circular cesspool and soakage pits (90%)	Cesspool and soakage pits (50%)
	Septic tanks (10%)	Septic tanks (30%)
		Cesspool without soakage pits (20%)
FS collection	Municipality trucks (1 truck = 6,000 L)	Private collectors' trucks sub-contracted by the municipality (1 truck = 6,000 L or 4,000 L)
Monthly FS collection	45,000–66,000 L (Average 66 households per month or 1,500 L per day)	30,000–40,000 L (1,000 L per tank × 30–40 tanks per month)
FS disposal /treatment method	Municipal FS treatment plant (collection pits & FS drying bed; only one in the sub-district)	Sold to fish farms (50%) Discharged to canals (30%) Buried on public land (20%)

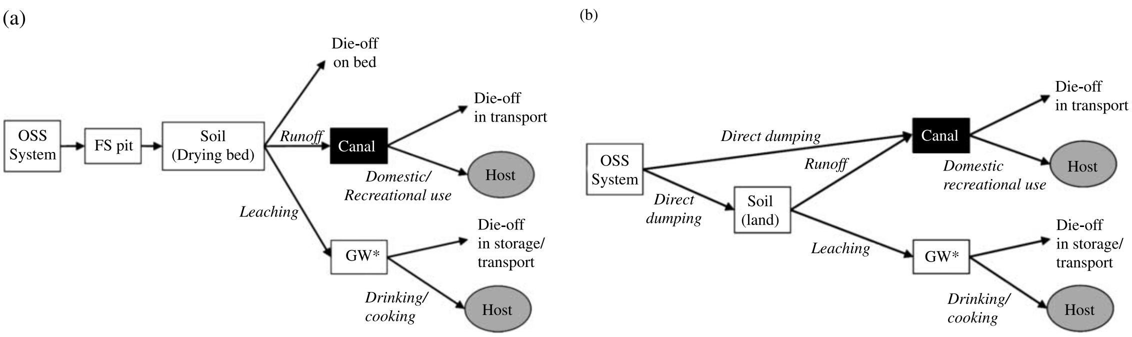

Figure 2 | Event tree for transmission of fecal bacteria in (a) Klong Luang sub-district and (b) Tha Klong sub-district.

presents schematic diagrams of the potential pathogenic pathways originating from the FS disposal sites to the surrounding environment for the two sub-districts. Eight sampling sites, four from each sub-district, were selected to represent overall pathogenic contamination levels in each sub-district, based on these pathways (Figure 3). Domestic waste water is directly discharged into Klong Nung canal (hereinafter referred to as Canal B) from the numerous residential houses along its banks. In contrast, Klong Song canal (hereinafter referred to as Canal A) is a relatively shallow and stagnant ditch with only a few residential huts along the banks. A few meters upstream of Site 6 there is a large-scale food market that seems to discard market waste, including fish and meat debris, into the canal. Site 7 is 10 m from the FS treatment facility of Klong Luang sub-district.

Sample collection

Monthly water sampling was conducted at eight sampling sites between November 2004 and March 2005. Water samples were collected from 30 cm depth at the canal sampling sites, using 500 mL sterilized bottles. FS samples were collected directly from the tank of the FS collection truck of Klong Luang municipality using sterilized bottles. The collected bottle samples were immediately transported to the laboratory and stored at 4°C. The subsequent microbiological analysis was carried out within 8 hours of sample collection.

Laboratory analysis

Escherichia coli was recovered from both water and FS samples using the standard five-tube most probable number (MPN) technique (Oblinger & Koburger 1975). A tenfold dilution series were made from each sample. 1 mL sample of each of ten dilution levels was inoculated in 10 mL lactose broth (E-MA49, Eiken Chemical Co., Ltd., Japan) at 37°C for 24 ∼ 48 hours. 1 loopful of the culture was transferred into 10 mL EC medium (E-MB06, Eiken Chemical Co., Ltd., Japan) and incubated at 44.5°C for 24 ∼ 48 hours. The culture was then streaked in EMB agar (Eiken Chemical

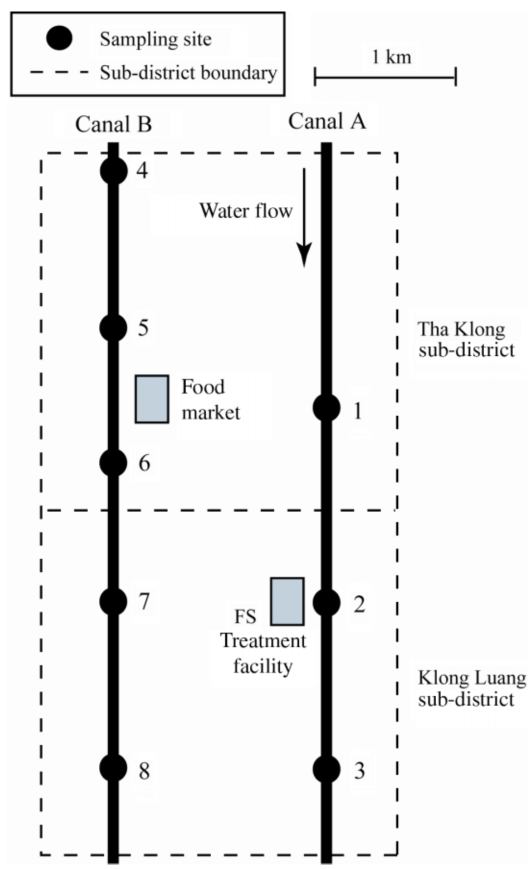

Figure 3 | Sampling sites along Klong Nung and Klong Song canals.

Co., Ltd., Japan) and incubated at 37°C for 24 hours. The positive samples were analyzed with IMVIC tests in accordance with APHA (1998), and those which showed positive results in the Indole and Methyl Red Tests, and negative results in Voges-Proskauer and Simmon's Citrate Tests were confirmed as positive *E. coli* samples.

Salmonella spp. was recovered from both water and FS samples using a modified APHA standard method (Fukushi *et al.* 2003) where a tenfold dilution series were made from each sample and 1 mL sample of each of ten dilution levels was inoculated in 10 mL EEM broth (E-MA23, Eiken Chemical Co., Ltd., Japan) at 37°C for 24 ~ 48 hours and 1 mL of the culture was then transferred to 10 mL Rappaport broth (E-MB25, Eiken Chemical Co., Ltd., Japan) at 37°C for 24 ~ 48 hours. The culture was streaked in SS agar (Eiken Chemical Co., Ltd., Japan) and incubated at 37°C for 24 hours. The results were expressed as most probable numbers per 100 mL (MPN/100 mL).

Statistical analysis

Data were log transformed and analyzed using two-way ANOVA without replication to assess the effects of sampling sites and months on pathogenic contaminations. Significance was determined at the 5% level. The respective contamination levels of *E. coli* and *Salmonella* spp. in Klong Nung and Klong Song were compared by one-way ANOVA. Subsequently, the geometric mean, maximum and minimum concentrations recorded at each site were compared in order to further explore the spatial trends of *E. coli* and *Salmonella* spp. concentrations in each study canal. All statistical analysis was conducted using Microsoft Excel.

Quantitative microbial risk assessment (QMRA)

Risk characterizations were made using established beta-Poisson dose–response models, as given below, to calculate the probability of infection after a single exposure (P_1) to *Salmonella* spp. and *E. coli*, respectively (Haas *et al.* 1999).

$$P_1 = 1 - [1 + d/N_{50}(2^{1/\alpha} - 1)]^{-\alpha}$$

The models have been generalized as the functions of two parameters, namely the median infectious dose (N_{50}) and the slope parameter (α) of the dose–response curve specific to the organism describing the probability (Beta) distribution of N_{50} (Hunter *et al.* 2003). In addition, d is the dose or exposure (i.e. the average number of organisms to be ingested) necessary to initiate infection (Ryu *et al.* 2005). In our study we applied the best fitting parameters for α and N_{50}, according to Haas & Eisenberg (2001). These were respectively 0.3126 and 23,600 for *Salmonella* spp. (multiple non-typhoid pathogenic strains; *S. pullorum* excluded), and 0.1778 and 8.60×10^7 for *E. coli* (multiple non-enterohaemorrhagic strains except for O111).

Four scenarios were developed for the ingestion of canal water based on the authors' observations and local interviews, as shown in Table 2. The typical ingestion dose associated with each scenario was assumed in reference to Covello & Merkhofer (1993) and Steyn *et al.* (2004).

Risk was calculated for exposure to the maximum, geometric mean and minimum concentrations of each microorganism. The annual risk of infection (P_n) was calculated for each scenario using the following equation (Haas *et al.* 1999):

$$P_n = 1 - \prod_{i=1}^{n} (1 - Pi)$$

The results were expressed as the number of cases per 10,000 people, and compared with the acceptable risk of 1 case per 10,000 persons per annum, as defined by the

Table 2 | Involuntary canal water ingestion scenarios and dose assumptions

Scenario	Condition	Ingestion dose of water
1	Bathing/swimming (every day)	100 mL/day 7 times/week 336 times/year
2	Bathing/swimming (3 times per week)	100 mL/day 3 times/week 144 times/year
3	Bathing/swimming, immersed fishing and recyclables collection (once per week)	100 mL/day Once/week 48 times/year
4	Laundry	10 mL/day 6 days/week 288 days/week

USEPA. This acceptable risk was defined using *Giardia* as a reference organism, which is known to be more resistant to disinfection than other microbial pathogens (Macler & Regli 1993). The estimated data were then compared with actual data using recently recorded water-related disease occurrence rates obtained from the Department of Health in Klong Luang sub-district office.

RESULTS

Spatial trend of pathogenic contamination in the two sub-districts

The concentrations of *Salmonella* spp. and *E. coli* in the canal water samples are summarized in Table 3. The influence of water temperature was considered negligible as it remained at 32–34°C during the monitoring period. The two-way ANOVA showed no significant effect of sampling time on *E. coli* or *Salmonella* spp. levels ($P = 0.414$ for *E. coli*, $P = 0.234$ for *Salmonella* spp.) but there was a significant difference between sampling locations ($p < 0.001$ for both *E. coli* and *Salmonella* spp.). The geometric mean concentrations of both *Salmonella* spp. and *E. coli* were higher in Tha Klong sub-district than in Klong Luang sub-district. Table 4 summarizes the levels of *Salmonella* spp. and *E. coli* in each canal by sub-district. There was also a significant difference in pathogen concentrations in the two canals ($P = 0.009$ for *E. coli*, $P < 0.001$ for *Salmonella* spp.), with the numbers of pathogenic microorganisms in Canal B being significantly greater than those in Canal A. In Canal A, the levels of both *Salmonella* spp. and *E. coli* were lowest at

Table 3 | Summary of *Salmonella* spp. and *E. coli* concentrations in canal water

				Concentrations (MPN/100 mL)		
Item	**Sampling point**	**Canal**	**No. of samples**	**Geomean**	**Max**	**Min**
Salmonella spp. (MPN/100 mL)	*Tha Klong sub-district*					
	Sampling site 1	A	10	56	1.1×10^2	30
	Sampling site 4	B	10	2.1×10^3	5.4×10^3	7.0×10^2
	Sampling site 5	B	10	4.0×10^2	2.2×10^3	1.3×10^2
	Sampling site 6	B	10	6.6×10^3	1.7×10^4	2.2×10^3
	Sub-total		40	7.5×10^2	1.7×10^4	30
	Klong Luang sub-district					
	Sampling site 2	A	10	1.1×10^2	2.3×10^3	2
	Sampling site 3	A	10	1.6×10^2	3.4×10^2	50
	Sampling site 7	B	10	5.5×10^2	7.0×10^3	40
	Sampling site 8	B	10	3.7×10^2	2.2×10^3	40
	Sub-total		40	2.5×10^2	7.0×10^3	<2
E. coli (MPN/100 mL)	*Tha Klong sub-district*					
	Sampling site 1	A	10	1.5×10^2	3.4×10^2	80
	Sampling site 4	B	10	8.4×10^4	1.7×10^5	3.5×10^4
	Sampling site 5	B	10	2.8×10^3	1.6×10^4	7.9×10^2
	Sampling site 6	B	10	1.6×10^5	1.6×10^6	9.0×10^3
	Sub-total		40	8.6×10^3	1.6×10^6	80
	Klong Luang sub-district					
	Sampling site 2	A	10	1.1×10^2	1.1×10^3	1.7×10^2
	Sampling site 3	A	10	7.0×10^3	1.7×10^4	1.7×10^3
	Sampling site 7	B	10	1.4×10^4	3.5×10^4	3.3×10^3
	Sampling site 8	B	10	4.3×10^3	9.2×10^4	90
	Sub-total		40	4.6×10^3	9.2×10^4	90

Table 4 | Summary of *Salmonella* spp. and *E. coli* concentrations in Klong Song and Klong Nung

			Concentrations (MPN/100 mL)		
Canal	**Species**	**Sub-district**	**Geomean**	**Max**	**Min**
A	*Salmonella* spp.	Tha Klong	56	1.1×10^2	30
		Klong Luang	1.5×10^2	2.3×10^3	2
	E. coli	Tha Klong	3.3×10^4	1.6×10^6	7.9×10^2
		Klong Luang	7.7×10^3	9.2×10^4	90
B	*Salmonella* spp.	Tha Klong	2.1×10^3	1.7×10^4	1.3×10^2
		Klong Luang	4.5×10^2	7.0×10^3	40
	E. coli	Tha Klong	8.6×10^3	1.6×10^6	80
		Klong Luang	4.6×10^3	9.2×10^4	90

site 1 and highest at site 3. In Canal B, the levels of *Salmonella* spp. at site 4 and site 6, downstream of the large-scale food market, were considerably higher than the other sites. The geometric mean concentrations of *E. coli* showed a similar spatial trend.

Risks of *Salmonella* spp. and *E. coli* infection in the two sub-districts

Figure 4 summarizes the mean risks of *Salmonella* spp. and *E. coli* infection in Klong Luang and Tha Klong sub-districts, respectively, under the four scenarios. The error bars indicate the range between the minimum and maximum risk values. The mean risks of both *Salmonella* spp. and *E. coli* infection were greater in Tha Klong sub-district than in Klong Luang sub-district. However, all estimated risks were much higher than the acceptable risk of 1 case/10,000 persons. Table 5 shows the occurrences of water-related diseases recorded between 2001 and 2004 in Klong Luang by the Department of Health in Klong Luang sub-district office. The occurrence of acute diarrhea in 2004

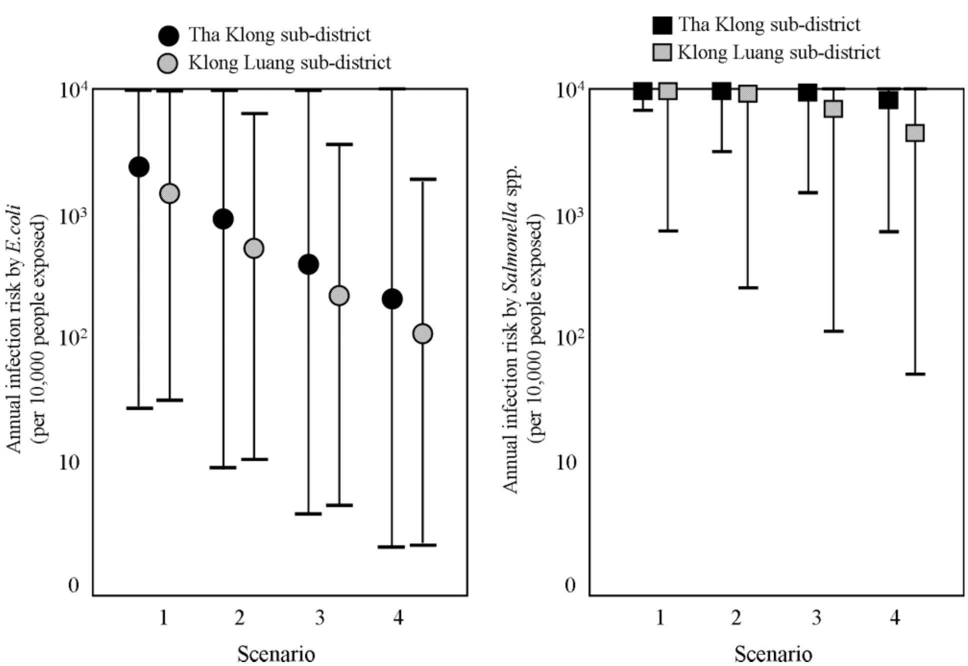

Figure 4 | Estimated yearly mean risks of *Salmonella* spp. (left) and *E. coli* infection (right) in Tha Klong and Klong Luang sub-districts under the following scenarios: (1) daily bathing, (2) bathing 3 times per week, (3) weekly bathing, and (4) daily laundry in the canals. The error bars indicate the range between the minimum and maximum risk values.

Table 5 | Waterborne disease occurrences over 4 years in Klong Luang sub-district

	2004		2003		2002		2001	
Disease	**Total**	**Death**	**Total**	**Death**	**Total**	**Death**	**Total**	**Death**
Acute diarrhea	6,500	0	11,296	0	10,971	1	9,578	0
Dysentery	27	0	58	0	132	0	159	0
Enteric Fever	2	0	12	0	19	0	12	0

Source: Klong Luang sub-district office (2004).

was equivalent to 1,418 cases/10,000 persons, which was much lower than the estimation by QMRA using the data from samples collected during the same period. No epidemiological data had been recorded for Tha Klong sub-district.

Correlation of fecal indicator organism and actual pathogenic organism

There was a strong correlation between the concentrations of *Salmonella* spp. levels (Figure 5). *E. coli* concentrations exceeded those of *Salmonella* spp. at all sampling sites (Table 3, Figure 5) but the infection risks of *Salmonella* spp. estimated from these same microbial concentrations were considerably higher than those of *E. coli*. In fact, the infection risks of *Salmonella* spp reached 100% at many sampling sites, preventing a clear demonstration of the correlation between *Salmonella* spp and *E. coli* infection risks ($r = 0.49$). Geometric mean concentrations of *E. coli* and *Salmonella* spp. in FS samples were 7.5×10^6 and 3.5×10^5 MPN/100 mL, respectively.

DISCUSSION

Health risks associated with different disposal practices of fecal sludge were assessed in peri-urban settings of a large metropolis in Thailand. The water quality results in Klong Luang and Tha Klong sub-districts showed considerable spatial variation, indicating that land use along the canal banks affected the contamination of the canal water. The levels of both *E. coli* and *Salmonella* spp. were high adjacent to the FS treatment facility in Klong Luang sub-district and the food market in Tha Klong sub-district. In Klong Luang sub-district, the FS drying bed is likely to have been the main source of excreta-oriented pathogens because it had no lining to prevent leachate from entering the canal. However, the pathogen levels were higher in Tha Klong sub-district. The concentrations of *E. coli* and *Salmonella* spp. in FS samples were 1 log different. Those in canal water samples also showed 1 log difference except for 2 log difference in site 6 and 7. This indicates that *E. coli* and *Salmonella* spp. in canal water samples are likely to be originated from indiscriminate FS dumping in the canals

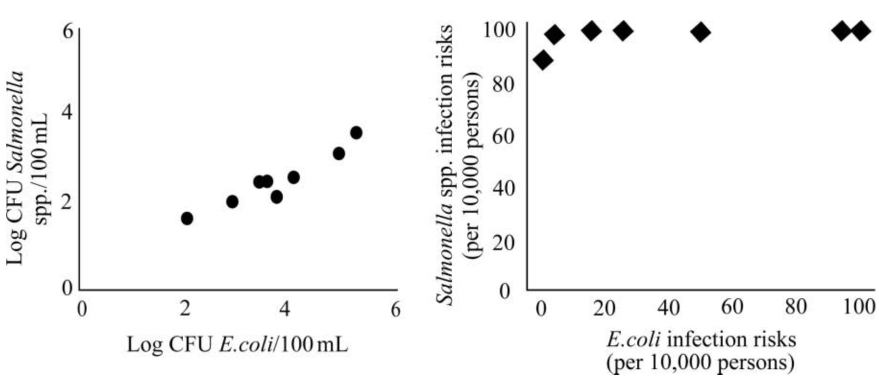

Figure 5 | Correlation between *Salmonella* spp. and *E. coli* concentrations (left) and correlation between yearly risks of *Salmonella* spp. and *E. coli* infection from daily bathing (right).

except for site 6 and 7 where additional input of these species occurred presumably in the form of effluent discharge from the food market. It is thus implied that the installation of an appropriately designed FS treatment system alone will not be sufficient, and that stringent restrictions on canal water use for local communities is required to protect the public's health.

Based on the QMRA, the estimated risks of *E. coli* and *Salmonella* spp. infection were greater in Tha Klong sub-district, where FS was indiscriminately discarded, than in Klong Luang sub-district where FS is collected in the FS treatment facility. General assumption is that higher concentrations of *E. coli* lead to higher estimation of infection risks. However, the results of our study was contradictory.

The higher estimated infection risks from *Salmonella* spp. than from *E. coli*, despite the lower *Salmonella* concentrations, could be due to the very low infection dose of *Salmonella* spp., that produce infection at very low concentrations. Another possibility is that correlation between concentrations of *E. coli* and those of *Salmonella* spp. in freshwater and soil in developed countries where the dose–response models were originally validated is different from that in tropical environment in less developed countries. As Winfield & Groisman (2003) argues, the natural populations and survival rates of *E. coli* and *Salmonella* spp. varies by several factors in the specific locations such as temperature, moisture, sunlight, nutritional levels and presence of predators. Therefore the application of the existing dose–response model in QMRA to estimate *E. coli* is not likely to accurately estimate infection risks from the other fecal bacteria in less developed countries.

In our study the estimated risks of both *E. coli* and *Salmonella* spp. infections were extremely high compared to the acceptable risk of 1 case/10,000 persons. This indicated that almost the entire population along the canals would suffer annual infection from these two pathogens. However, cross-comparison with recorded occurrences of water-related disease in the sub-district showed that the QMRA greatly overestimated the infection risk. As mentioned earlier, QMRA requires quantitative data on all major aspects of the related water environment, and therefore has to rely on assumptions and estimates when recorded data is unavailable. This is particularly true when QMRA is applied to less developed countries where there is often a particular shortage of recorded information. Typically the QMRA model has been developed using data from healthy adults in developed countries who have been exposed to various doses of microorganisms (Haas & Eisenberg 2001). Therefore the lack of agreement between the QMRA estimated risk and recorded occurrence of disease in our study indicates that the QMRA model needs to be modified for accurate use in less developed countries. In particular research is needed to produce adjusted factors to incorporate the immunological differences between healthy adults in developed and less developed countries.

In addition there is often a wide range of social groups that have different degrees of immunity, contact with the water environment and societal behavior, and so this makes it impractical to integrate the varied health risks to produce an overall community-level risk. Therefore, in our study we produced a scenario-based risk estimate rather than a community-level risk estimate by targeting the population with a high probability of contact with canal water; namely residents along the canal who are typically middle to low income class and use the canal water daily for laundry and bathing. However, the possible development of immunity from repeated exposure to the pathogens in daily life was not taken into consideration. Based on the results of our study, it is therefore clear that in addition to modifying factors for less developed countries, we also need to determine different factors to take account of different sub-populations whose societal behavior results in different degrees of contact with potentially contaminated water.

CONCLUSIONS

The following conclusions were drawn from this study:

1. Indiscriminate FS dumping and discharge of market waste were identified as possible sources of fecal contamination in the canals in the peri-urban region of Thailand, highlighting the need for the stringent restriction of public use of canal water, as well as for the installation of an appropriately designed FS treatment system.
2. While *E. coli* can be effectively used as an indicator organism of concentrations of *Salmonella* spp., estimated

risks of *E. coli* infection do not accurately indicate the risk of infection from *Salmonella* spp.

3. The application of QMRA as a tool to accurately assess infection risks in less developed countries requires the determination of sensitivity factors, based on systematic collection of comprehensive epidemiological data in the region, in order to reflect immunological differences between different sub-populations.

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