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Original Contribution

Quantification of Diarrhea Risk Related to Wastewater Contact in Thailand

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Abstract: Wastewater reuse contributes to closing the nutrient recycling loop as a sustainable way of managing water resources. Bangkok has over a thousand man-made drainage and irrigation canals for such purposes. Its use for agricultural and recreational purposes has a long tradition in rural and peri-urban areas. However, the continuation of these practices is increasingly questioned since potential health risks are an issue if such practices are not appropriately managed. The microbial and chemical quality of canal water has considerably deteriorated over the last decade, mainly because of discharged, untreated domestic and industrial wastewater. It is important to understand the health risks of wastewater reuse and identify risky behaviors from the most highly exposed actors promote the safe use of wastewater. This study assessed diarrhea infection risks caused by the use of and contact with wastewater in Klong Luang municipality, a peri-urban setting in Northern Bangkok, using quantitative microbial risk assessment. Wastewater samples were collected from canals, sewers at household level, and vegetables grown in the canals for consumption. Samples were also collected from irrigation water from the agricultural fields. Two protozoa, *Giardia lamblia* and *Entamoeba histolytica,* were quantified and analyzed by real-time PCR, exposure assessment was conducted, and finally, the risk of infection due to contact with wastewater in different scenarios was calculated. The results showed that canal water and vegetables were heavily contaminated with *G. lamblia* and *E. histolytica*. Infection risk was high in tested scenarios and largely exceeded the acceptable risk given by WHO guidelines.

Keywords: *Entamoeba histolytica*, *Giardia lamblia*, peri-urban, QMRA, wastewater, reuse

INTRODUCTION

Water shortage and limited access to clean drinking water have been major problems in many parts of the world. Today, this issue is exacerbated by climate change and world population growth. The need for clean water can be partially supplied by reusing wastewater if combined with planned, sustainable sanitation and water management (Conradin et al. 2010). Use for irrigation, fertilizer for agriculture and aquaculture, reduced costs and energy, and income generation for the poor are all added values of wastewater reuse (Zhang et al. 2009). However, wastewater reuse without appropriate treatment may lead to public

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health problems such as diarrhea, helminth infection, skin diseases (Campos 2008; Dietrich et al. 2009). A question has therefore been how to mitigate the health risks associated with the practice of wastewater reuse without compromising livelihoods (Seidu et al. 2008).

Thailand has a dense man-made canal network. In particular, Bangkok has 1,165 canals that span 2,280 km, more than two times the road network (1,014 km). This canal network contains 45.6 millions m^3 of wastewater. Bangkok has 490 sub-canals carrying 2.7 millions m^3 of surface wastewater (Diallo et al. 2008). During the last 20 years, Thailand has experienced rapid urbanization without careful consideration of future impact (Pradhan and Perara 2006). The uncontrolled urbanization has been concentrated around man-made canal networks because of access to water and around areas with good road connections such as Klong Luang—our study area in a district north of Bangkok. In Klong Luang, wastewater from households, industry, and markets are discharged into the canal systems, mostly untreated, which causes environmental and health impact by chemical pollution and microbial contamination for water bodies in the setting (Anceno et al. 2007b; Diallo et al. 2008; Surinkul and Koottatep 2009; Yajima and Koottatep 2010).

Globally, microbial contamination of water represents one of the most significant risks to human health (Lemarchand et al. 2004). Human feces from infected persons contain a large range of pathogens like *Salmonella*, *Giardia*, *Cryptosporidium*, *Entamoeba*, and *rotavirus*. They cause mainly gastroenteritis or milder respiratory infections, but others can cause more severe diseases like hemolytic ureic syndrome (Westrell et al. 2004). Improperly treated wastewater is a favorable environment for these pathogens in their stable form where they can move fast and find new hosts. Therefore, when wastewater is used for food production, these diseases are easily be transmitted when people are fishing, bathing, cleaning, or consuming vegetables grown in wastewater.

This article is part of a larger study on combined health and environmental assessment of environmental sanitation in developing countries (Nguyen-Viet et al. 2009), aimed at assessing the health risks related to the pathogen transmission of wastewater in Klong Luang municipality, Pathumthani Province, Thailand using the method of quantitative microbial risk assessment (QMRA). This method is used to estimate the probability of health risks following the ingestion of pathogens and depends on the availability of numerical data and consumption pathways. The quality of a QMRA depends largely on the quality of the data, the assumptions taken, and the validity of the model (Boone et al. 2009). The contribution of this article is to provide evidences on the health risk on different potential infection points surrounding and in the wastewater canal systems by assessing health risk related to the exposure to wastewater. This will help understand more the environmental system in Klong Luang for further intervention for environmental and health improvement.

Materials and Methods

Study Site

Klong Luang is one of seven districts of Pathumthani Province, which is located 20 km north of Bangkok (Fig. 1). The municipality of Klong Luang is a peri-urban community with 18 villages, 49,296 inhabitants on 43 km^2, and a total of 11,949 households (Pradhan and Perara 2006). Around the year 1902, a canal irrigation system was developed due to the high agricultural activity. In the last 20 years, Klong Luang became an attractive place for the settlement of new industries. Since then the population has increased, notoriously bringing people from other parts of Thailand who were not used to living in urban settings (Hung and Yasuoka 2008). Among several canals in Klong Luang, canal 1 received wastewater mainly from the market and households and qualified as a wastewater canal. Canal 2 received more water discharged from households (Fig. 1).

Quantitative Microbial Risk Assessment (QMRA)

The methodology used for this study was based on the QMRA framework described by Haas et al. (1999). The QMRA analysis characterizes infection risk with four steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization.

Hazard Identification

Diarrhea is the fourth cause of death in adults in developing countries and is responsible for 11% compared to 17% by HIV/AIDS but also by lower respiratory infections, and 16% by heart diseases (Atlas 2002). In a previous study, Surinkul and Koottatep (2009) described, in the same area, health risk caused by *Escherichia coli* within an environmental sanitation and agricultural system. However, their study focused much more on the transmission pathways of

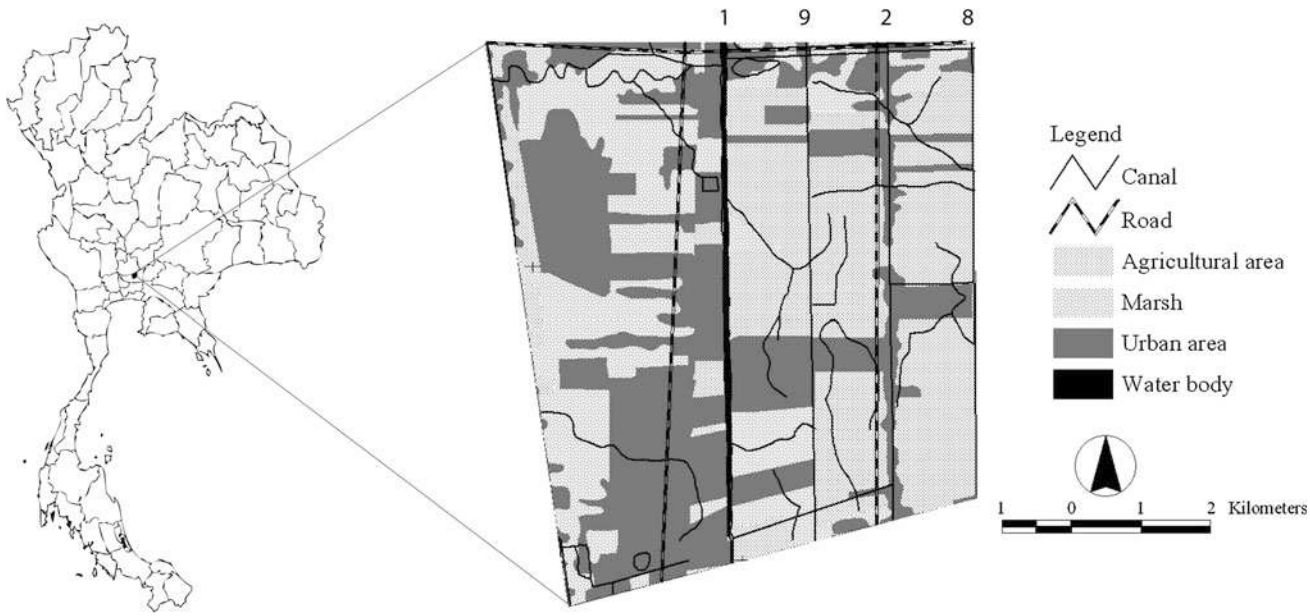

Figure 1. *Left* study site at Klong Luang Municipality, Thailand. *Right* vertical canals are labeled (1, 9, 2, and 8) at the *top* of the detailed area.

E. coli in these systems and did not examine in details exposure assessment as well as pathogenic microorganisms. Our study focused on two pathogenic protozoa, *Entamoeba histolytica* and *Giardia lamblia*. Both cause diarrhea and are free-living, therefore, very resistant to adverse environmental conditions such as desiccation, starvation, high temperatures, and disinfectants (Haas et al. 1999).

Exposure Assessment

This step consists of identifying the pathways of a protozoan to reach a person and cause infection. In order to assess the exposure to wastewater, one needs the microorganism concentration in the water. The protozoa concentration was measured by real-time polymerase chain reaction (qPCR) as described by Haque et al. (2007) as well as the amount of water consumed by the population. Further methodology of qPCR can be found in the microbial analysis section below. Subsequently, the frequency of exposure was determined by interview surveys. Among many potential wastewater exposure routes, this article focused on five main scenarios: (1) contact with water while bathing, (2) while fishing, (3) while swimming, (4) while collecting vegetables grown in the canals, and (5) via consumption of these vegetables. The survey also quantified the number of people exposed to each risk scenarios and their frequency of exposure. First, two clusters (villages) were randomly selected in Klong Luang municipality then 160 households from these two villages were selected with a systemic sampling approach using simple street systems. To select randomly the households for interviews, the interviewer walked all the streets from the selected village and interviewed each fifth household. In case, one household was absent then went to the following household and so on until one interview could be conducted. One interview was conducted per household with the head of the household by a Thai research assistant. After conducting an interview successfully, one could skip the next 5 coming households. 80 questionnaires were conducted in each village. Only one interview had to be rejected due to missing data, giving a total of 159 questionnaires completed. Finally, the estimated volume of wastewater involuntarily ingested in scenarios was taken from the literature (Covello and Merkhofer 1993; Haas et al. 1999; Steyn et al. 2004; Surinkul and Koottatep 2009).

Dose–Response Assessment

Dose–response assessment examines the incidence of diarrhea infection or diseases as a health outcome in an exposed population. It represents the relationship between a dose (number of pathogens entering in the body to cause infection) and the response of the organism, which is the infection caused by pathogens. Two dose–response models are widely used from literature, exponential and beta-Poisson models, as they fit well to several microorganisms (Haas et al. 1999).

The exponential model was used for *Giardia lamblia* (Haas and Eisenberg 2001):

$$P_{\text{inf}=}1 - \exp(-rd) \quad (1)$$

where P_{inf} is probability of infection, r is the constant showing the probability that a single organism can reach to the target organ to cause an infection, and d is the ingested dose. In some cases, a value k is used as an alternate parameter calculated from r as $k = 1/r$. For *G. lamblia*, $r = 0.0199$, $k = 50.23$.

The parameter is not available in the literature but Haas and Eisenberg (2001) describes the best-fit dose response parameter for *Entamoeba coli*. For this study, we assumed that the similarity between the two species was sufficient so that the parameters related to human infection from both species should be comparable. This was confirmed through a discussion of this assumption with QMRA specialists Prof. Charles N. Haas (Drexel University, Philadelphia, USA) and Prof. Peter Teunis (RIVM, Bithoven, The Netherland), a Beta-Poission dose–response model was used for *E. histolytica* (Furumoto and Mickey 1967a, b; Haas 1983):

$$P_{\text{inf}} = 1 - \left[1 + d/N_{50}\left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \quad (2)$$

where P_{inf} is the probability of infection, d is dose, N_{50} is the median infectious dose, and α is the slope parameter. For *E. histolytica*, $N_{50} = 341$, $\alpha = 0.1008$ (Rendtorff 1954).

The P_{inf} in both cases above is probability of infection by a single exposure. We also calculated the yearly risk as follows:

$$P_{\text{inf(y)}} = 1 - (1 - P_{\text{inf}})^n \quad (3)$$

where $P_{\text{inf (y)}}$ is probability of yearly infection, n is the number of exposures per year.

Risk Characterization

Risk characterization integrates the information from the three previous steps into a single mathematical model, to calculate risk as a probability of infection, illness, or death. In our study, we calculated infection risk. Risk was calculated using a stochastic approach, with inputs as probability density functions (PDFs) of each parameter if data were available and plausible for fitting a PDF and Monte Carlo simulation. Calculation showed that concentrations of pathogens in different replicates fit the best to a negative binomial distribution. Monte Carlo simulation gives a range of possible risks, including average and worst case scenarios. The output of the model was run with the Monte Carlo simulation with 10,000 permutations. Results were expressed as the risk of infection, per person, per single exposure, as well as by yearly risk with multiple exposures. In the latter, the risk was presented by mean of 10,000 simulation values of risk.

Sampling Strategy

Water and vegetable samples were collected at four locations along the wastewater canal of the study site: (1) household sewer, (2) canal surface water, (3) irrigation water from agricultural field, and (4) vegetables from the canal. The idea of having these four sampling locations is to better understand the pathogen flows within the environmental sanitation system in the study site and to see how these pathogens infect populations through wastewater. A total of 12 sampling points from these 4 locations were selected: 3 points from household sewer, 6 from canal surface water in two canals 1 and 2 (3 points per canal), 1 from agricultural field, and 2 for vegetable—the morning glory was collected directly in canals 1 and 2. As mentioned above, canal 1 received wastewater mainly from the market and households and qualified as a wastewater canal, whereas canal 2 received more water discharged from households (Fig. 1). For canal 1, sampling point 1 is at the upstream; point 2 is in the middle canal and close to the big market, Thalad Thai, and wastewater treatment plant; and point 3 is at the downstream of the canal. For canal 2, sampling points are also located at the upstream, middle, and downstream. At each sampling point, either wastewater or vegetables were collected from three to five times with 3 day intervals. Thus, a total of 42 samples were collected for the period from the 13th of May to the 5th of June 2008.

For each wastewater sample, 2 L of wastewater were collected. All water samples from the canals were collected at 20 cm depth from the surface, whereas at the household sewer level, they were collected directly from the sewer in front of the houses. The samples were immediately measured in the field for pH and temperature by a pH meter Knick, model 911 pH then transported on ice to the laboratory within 2 h, in clean wide-mouth screw-capped 1 L bottles.

Morning glory (*Ipomoea aquatica,* "Pak poong" in Thai language) was collected for the study. This species grows in the wastewater canals and is eaten raw (as salad) or cooked in not only Thailand but also throughout South-East Asia. At each sampling point, vegetables were sampled

at five different points, 1 m from each other, in the canal and mixed to have one final composite sample.

To extract protozoa from the vegetables, first, after the vegetables were harvested, they were left 1 h to remove the canal water and then weighed for the fresh mass. Then, they were rinsed twice with a physiological solution (0.95% NaCl). This allowed us to have the final data in number of cysts of *G. lamblia* and *E. histolytica* per gram of fresh vegetable. The first rinse was with 2 L of physiological solution and the second time with 1 L, both times with vigorous shaking to free the protozoa that could be attached to the vegetable surface.

Microbiology Analysis

qPCR allows the quantification of an exact number of cysts in a small volume of water. Samples were filtered through a 800-µm porosity filter to avoid sediment and then centrifuged in 500 mL tubes during 10 min at 5,000 rpm. The pellet was divided into five parts. The DNA was extracted from one and the four remaining parts were kept as a reservoir at 4°C. DNA was extracted using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Three replicates of each DNA extraction were used to run qPCR. The primers and *Taq*man probes were as described by (Haque et al. 2007). Primers were designed on small subunit ribosomal RNA gene and the amplified targets were 134 and 62 bp for *E. histolytica* and *G. lamblia*, respectively. All primers and *Taq*man probes used in this study were provided from Eurogentec (Seraing, Belgium). Amplification was performed following the details found in Haque et al. (2007). The reactions were done in a volume of 12.5 µL with Bio-Rad iQ SYBR Green Supermix containing: 100 mM KCl, 40 mM Tris–HCl, pH 8.4, 0.4 mM each dNTP, 50 U/mL i*Taq* DNA polymerase, 6 mM $MgCl_2$, and stabilizers specifically optimized for qPCR using SYBR Green I. Depending on the protozoa studied, 0.4 µmol/L of each correspondent primer (Eh-f and Eh-r or Gd-80F and Gd-127R) and 1.75 µL extracted DNA were used in each reaction. Differing from Haque et al. (2007), to minimize the impact of PCR inhibitors present in DNA environmental samples, 500 ng/µL bovine serum albumin at final concentration was added to the RNase-free water.

SYBR Green with fluorescence at 490 nm was used for this experiment and was not binding specifically. Therefore, it was necessary to do a melting curve at the end of the amplification to see if the amplified product was actually the DNA section of interest. Amplification consisted of 1 cycle of 3 min at 95°C, 45 cycles of 30 s at 95°C/30 s at 55°C (Real-time photo)/30 s at 72°C, 1 cycle of 30 s at 95°C, 1 cycle of 30 s at 55°C, 80 cycle of 10 s at 55°C (Melt curve). Amplification, detection, and data analysis were performed with the i-Cycler iQ™ Real-Time PCR detection system (model 170-8740, BioRad, USA). Fluorescence was measured during the annealing step of each cycle. The ramping of the machine was 3.3°C/s in every step. The FAM/SYBR filter that was used measures by excitation at 490/20 nm and emission at 518/30 nm.

Statistical Analyses

All parameters are presented as mean and standard deviation (SD). The statistical analysis and calculations from the questionnaire data were done using STATA 10 statistics and data analysis software (StataCorp, Lakeway Drive, Texas). Comparison between pathogen concentrations from different sampling points as well as correlation between the concentration of each pathogen with temperature and pH were conducted. @Risk version 5.0.1 of Palisade Corporation 2008 was used for fitting the probability distribution function and running Monte Carlo simulations to calculate infection risk,

Results

Microbial Contamination in Wastewater and Vegetables

The mean pathogen concentration from each sampling point is described in Table 1. In the household sewers, *G. lamblia* varied from 0 to 3,051 cysts/L whereas no *E. histolytica* was found. In both canals, the middle sampling points had the highest concentrations of *G. lamblia* (3,040 and 1,081 cysts/L for canals 1 and 2) and *E. histolytica* (13,215 and 2,503 cysts/L for canals 1 and 2). Morning glory vegetables collected from canals 1 and 2 contained a comparable number of *G. lamblia* but have a large difference in *E. histolytica* between the two canals (Table 1). Finally, no protozoa were found in the agriculture field.

There was a significant difference in *E. histolytica* ($P = 0.03$, Mann-Whitney test) between the two canals. *E. histolytica* concentration was significantly higher in the canal water than in water from household sewer ($P = 0.04$, Mann-Whitney test). *E. histolytica* and *G. lamblia* were significantly correlated ($P < 0.003$, $R^2 = 0.47$). No correlation was found between pH and temperature with the concentration of each pathogen ($P > 0.05$). As the focus of the study was to estimate the infection risk in the

Table 1. Concentration of *G. lamblia* and *E. histolytica* in water and vegetables from each sampling location

Sampling point	*Giardia lamblia* (cysts/L)		*Entamoeba histolytica* (cysts/L)	
	Mean	SD	Mean	SD
Household sewerage				
Sewer 1	589	±1,316	0	±0
Sewer 2	610	±1,365	0	±0
Sewer 3	109	±244	0	±0
Canal 1				
Point 1	297	±261	6,422	±6,039
Point 2	3,043	±5,270	13,215	±9,786
Point 3	0	±0	4,315	±7,474
Canal 2				
Point 1	300	±264	4,501	±4,553
Point 2	1,081	±1,010	2,503	±4,336
Point 3	0	±0	801	±991
Vegetables (cyts/100 g)				
Morning glory in canal 1	2,380	±1,611	65,778	±90,912
Morning glory in canal 2	2,883	±3,425	16,820	±10,563
Agriculture field	0	±0	0	±0

SD standard deviation.

Table 2. Average concentration of *G. lamblia* and *E. histolytica* in water from each sampled location

Sampling point	*Giardia lamblia* (cysts/L)		*Entamoeba histolytica* (cysts/L)	
	Mean	SD	Mean	SD
Sewerage household	436	±1,049	0	±0
Canal	787	±2,149	5,293	±6,588
Vegetables (cysts/100 g)	2,631	±2,410	41,299	±63,794
Agriculture field	0	±0	0	±0

SD standard deviation.

community where people were in contact with wastewater, mean values of protozoa concentration were calculated for each sampling point category, meaning household sewer, canal, vegetable, and agricultural field (Table 2).

Exposure to Wastewater

Surveys were conducted in Klong Luang municipality using closed-end questions, with a multistage cluster sampling. The results are summarized in Table 3, where we show the frequency of exposure and percentage of the population who report partaking in each of the exposure activities. Despite the extensive growth of morning glory on the water surface of the canals, relatively few people (7.5% of respondents) reported collecting these vegetables. 6.2% of population reported eating raw morning glory as a traditional salad. Contact with canal water (washing, cleaning), swimming in the canal, and fishing in the canal were more prevalent in the community (Table 3). The frequency columns showed the number of times during 1 year that people are exposed to wastewater in corresponding scenario. Finally, the volume of involuntarily ingested wastewater and the quantity of vegetable consumed in corresponding scenario were based on the interviews and on literature (Covello and Merkhofer 1993; Haas et al. 1999; Steyn et al. 2004; Surinkul and Koottatep 2009) (Table 3). We did not include the drinking water exposure to really focus on the wastewater impact although this is an important exposure for studying diarrhea in developing settings.

Risk of Diarrhea Infection

Risk of infection by a single exposure scenario is presented in Table 4. Infection risk by both *G. lamblia* and *E. histolytica* per single exposure with specific activity varies from 44 to 100%. The highest risk of infection from one of the pathogens was due to consuming morning glory. Eating morning glory collected from canal represented an infection risk of 100% by *G. lamblia* and 67% by *E. histolytica*. In general, infection risk by *G. lamblia* was higher than *E. histolytica* when people were exposed to wastewater from the canals through swimming and fishing and eating vegetables. However, *E. histolytica* caused higher risk than *G. lamblia* when people were exposed to wastewater through contact with wastewater and collecting vegetables (Table 4).

When looking at the comparative infection risk in Klong Luang, based on the percentage of population exposed to pathogens from wastewater, *G. lamblia* had the highest infection risk caused by swimming in the canal with a risk of 9.2% in the whole population, followed by contact with canal water (7.2%) and fishing (6.9%). Similar trend were observed by *E. histolytica* (data not shown).

Table 5 presents the yearly infection risk in different scenarios. All scenarios represented a yearly high infection varying from 0 to 100%. In general, *E. histolytica* caused

Table 3. Exposure assessment in different scenarios

Practice	Frequency/year[a]	Ingestion dose of water or vegetable	Percentage of population (%)	Exposed group of people
Contact with canal water	192	50 mL/time[b]	16.4	Farmer
Swimming in the canal	85	100 mL/time[b]	15.1	Farmer, children
Fishing in the canal	47	100 mL/time[b]	11.3	Farmer
Collecting morning glory	57	50 mL/time[b]	7.5	Farmer
Raw eaten vegetables	156	100 g/time[a]	6.2	Household

[a]From interview of this study.
[b]Steyn et al. (2004).

Table 4. Probability of infection by single exposure from different scenarios

Scenario	PDF for concentration of protozoa in water or vegetables		Volume or quantity	Mean infection risk by a single exposure (%)	
	Giardia lamblia	*Entamoeba histolytica*		*Giardia lamblia*	*Entamoeba histolytica*
Contact with canal water	Negative binomial (1, 0.0012694)	Negative binomial (1, 0.0001889)	50 mL/time	44	46
Swimming in the canal	Negative binomial (1, 0.0012694)	Negative binomial (1, 0.0001889)	100 mL/time	61	49
Fishing in the canal	Negative binomial (1, 0.0012694)	Negative binomial (1, 0.0001889)	100 mL/time	61	49
Collecting morning glory	Negative binomial (1, 0.0012694)	Negative binomial (1, 0.0001889)	50 mL/time	44	46
Eating raw vegetables	Negative binomial (2, 0.0007594)	Negative binomial (1, 0.0000242133)	100 g/time	100	67

Probability distribution function (*PDF*), fitted with all replicate values of real measurements of protozoa in water and vegetable.
Values in parentheses are values of two parameters r (>0) and P ($\in 0, -1$) of the negative binomial distribution.

higher yearly risk than *G. lamblia* did in each scenario. The highest infection risk is attributed to eating raw morning glory at 100%. However, the early risks of other activities such as collecting morning glory, contact with canal water, swimming, and fishing in the canal was also very high for both *G. lamblia* and *E. histolytica*, reaching the yearly risk almost at 100% (Table 5).

Discussion

Quality of Wastewater in Klong Luang

In Klong Luang, canal 1 is known as the most polluted among eight canals of the municipality. Indeed, canal 1 was much more polluted than canal 2; the concentration of both protozoan species was almost three times higher in canal 1 (113 cysts/100 mL) compared with canal 2 (46 cysts/100 mL). This observation is in agreement with other studies conducted on the microbial contamination of wastewater in the same areas (Anceno et al. 2007b; Diallo et al. 2008). The reason for this pollution could be that canal one receives discharges from Talad Thai market, the biggest market surrounding Bangkok. Canal 1 also receives wastewater along the canal from the ramification of the Chao Phraya River and canal Rapipat. The load of both protozoa coming from Talad Thai market (point 2) in this study was 3,043 and 13,215 cysts/L for *G. lamblia* and *E. histolytica*, respectively (Table 1), suggesting that the outlet of wastewater from Talad Thai constitutes an important polluting source for canal 1. According to local regulation, water from canal 1 is not allowed for irrigation, whereas water from canal 2 is. In principle the canal 2 receives only

Table 5. Yearly risk of infection for different scenarios

Scenario	*Giardia lamblia* (%)				*Entamoeba histolytica* (%)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Contact with canal water	0	100	99.3	6.26	0	100	99.98	1.42
Swimming in the canal	0	100	99.2	6.59	0	100	99.98	1.42
Fishing in the canal	0	100	98.6	8.49	0	100	99.97	1.47
Collecting morning glory	0	100	97.8	10.60	0	100	99.96	1.58
Raw eaten vegetables	100	100	100	0	100	100	100	0

SD standard deviation.

water from the river. Although the concentration of pathogens was relatively low (from 0 to 436 cysts/L for *G. lamblia* and *E. histolytica*), according to our observation, wastewater from households and other sources were also discharged into the canal 2 but to a lesser extent compared to the canal 1 (Anceno et al. 2007b; Diallo et al. 2008).

Water samples in the agriculture field were negative for both protozoa. It was observed that irrigation water in the agricultural field, during that period of the year (May–June 2008), was mainly rain water and did not seem to come from canal 1, which could explain the absence of protozoa in the fields. The absence of protozoa could be explained also by mechanisms of pathogen removal in the fields and in other water bodies, like sedimentation, solar irradiation or even predation of protozoa by greater microorganisms (Arias et al. 2003). Observations by Anceno et al. (2007a, b) on the fluctuations of flow rate and pathogen density along canals revealed that while flow decreases from up to downstream, pathogens density also decreases (Anceno et al. 2007a). The large variety of removal mechanisms from canals reduces the quantity of pathogens ending up in rivers, indicating that canals have the capacity to remove pathogens (Diallo et al. 2008).

Contrary to the irrigation water in the agricultural field, morning glory collected from canals 1 and 2 were heavily contaminated with both *G. lamblia* and *E. histolytica* (Tables 1, 2). Morning glory growing in the canals therefore retained protozoa from canal water on their surface or even inside the leaf and stems as commonly observed in other plant species. A study found in the same study area, that *Escherichia coli* and *Salmonella* concentrations on the surface of lettuce decreased 4 log (99.99%) after exposure to light in 1 day (Surinkul and Koottatep 2009). However, cysts of protozoa are generally much more resistant than bacteria *E. coli* and *Salmonella,* and thus, survive longer on vegetables.

Thailand has a wide coverage of onsite sanitation across the country. However, pathogens are constantly released from onsite sanitation to the soil or through effluent pipes to nearby drainage systems or canals, because of poor sealing or inappropriate emptying of the sanitation systems (Surinkul and Koottatep 2009). In general, sanitation systems have been improved in Thailand but are still quite poor in certain places like Klong Luang municipality, leading to poor microbial quality of water in the canals. Health risks may occur when the sanitation systems containing dangerous pathogens are not properly managed. Exposure depends on people's behavior toward potentially contaminated sources and on how they protect themselves from exposure.

Health Risk Related to Wastewater and Method Used

This study looked at different scenarios in which people were exposed to wastewater and at the risk of infection. From a comparative point of view, being exposed to wastewater in the canal and through consumption of vegetables from the canals resulted in each exposure pathway a high risk of infection. Indeed, the yearly infection risk by both *G. lamblia* and *E. histolytica* were all much higher than the acceptable risk proposed by WHO at 10^{-4} person per year (1 case per 10,000 person per year) (WHO 2006b). Unfortunately, no reported data were found for the diarrheal disease in 2008 to compare to with the risk provided by this study. However, in Klong Luang municipality, 744 diarrhea cases were reported in 2005 by the Thai_government (2005). Usually, the reported cases of diarrhea decreases from one year to another, then it is possible that in 2008 the number of reported cases of diarrhea was less than 744. The risk estimated by QMRA from our study was already much higher than the reported cases. This

phenomenon has been observed in other studies in Thailand (Diallo et al. 2008; Yajima and Koottatep 2010).

A methodological limitation of QMRA is that the dose–response models (exponential and Beta-poisson model) for almost all pathogens were developed and validated in developed countries, which may not be accurate when applying it in developing countries (Haas et al. 1999; Haas and Eisenberg 2001). Moreover, the current status of the art of QMRA is that this risk assessment method does not yet account for other factors that could influence the infection such as degree of immunity of targeted people, or the distribution of infection over time due to the initial exposure (Huang and Haas 2009). Other issues with QMRA are related to the data availability on exposure assessment in developing countries. For instance, the assumptions about the volume of wastewater ingestions when exposed to water or quantity of food consumption are usually from studies conducted in developed countries. For further application of QMRA in the developing world, local data on exposure need to be generated. Despite the current limitations related to the method of QMRA, the latter is being increasingly used not only to assess health risk in many contexts and become a useful tool as the scientific basis for setting health-based target but also to manage the quality of water (drinking and waste) and food safety with a risk-based approach (WHO 2006a, b; Mataragas et al. 2010). The net advantage of QMRA compared to epidemiology relies on the fact that QMRA usually needs less financial, human, and time resources and can give quickly information on health risk of considered pathogens then this seems to be appropriate for its application in developing countries. Finally, as QMRA works on the dose–response of each pathogen then can characterize the health of each specific pathogen and it can detect very low levels of infection or disease risk and can complement epidemiological studies.

The exposure assessment showed that all people exposed to wastewater according to the scenarios will suffer diarrhea at least once a year (Table 5). It is difficult to know if there is a small group of people with such exposure, who constantly suffer from infection. In addition, considering the swimming scenario, only 15.1% of the population (n = 7,444 persons) were in contact with canal water 85 times a year (Table 3). For further studies, it would be useful to stratify the population depending on the frequency of exposure through their behavior, since it cannot be assumed that the 15.1% of the population swimming in the canals do this exactly 85 times per year.

Morning glory consumption was found to have a high infection risk, which is in agreement with Surinkul and Koottatep (2009) who defined agricultural products as the main transmission route of excreta-related pathogens in the peri-urban area of Thailand. However, in this study, we assumed that no removal of pathogens in vegetables takes place before consumption. Thus, from a public health point of view, a farmer selling highly contaminated vegetables in the market could have an extended impact on the health of consumers. Nevertheless, an appropriate action such as proper washing by sellers or consumers could largely reduce this health impact, as concluded also by other studies (Ensink et al. 2007; Keraita et al. 2007). Another way to mitigate health risk from vegetable consumption is to well cook vegetables before eating them. For people having exposure activities such as contact with water while collecting vegetables in the canals and fishing, it is recommended that they use protective conditions (e.g., gloves, boots, etc.) to reduce health risk. It is also recommended that people living along the canal to limit or not to bath or swim in these canals. These findings then can support policies and practices that would improve health status of people living in similar settings in Thailand.

In conclusion, this study shows high concentration of *G. lamblia* and *E. histolytica* in wastewater of Klong Luang municipality, Thailand. The risk of infection and diseases through contact with wastewater and vegetable consumption was much higher than the acceptable risk defined by WHO wastewater reuse (10^{-4} pppy). Exposure to wastewater from different sources constitutes an important element for risk management options. Actual exposure of the population to wastewater and vegetable consumption was assessed by determining the frequency of these practices at the local level. This is an advantage of the study as many other QMRA studies have usually extracted this information of exposure from the literature, which might be not relevant for the local context. The study also shows that QMRA is appropriate to study health risks in developing countries since it can detect very low levels of infection or disease risk and can complement epidemiological studies.

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