Original Article

Quantitative Microbial Risk Assessment of *Salmonella* in Surface Water as a Source of Tap Water

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Abstract

Salmonella spp. is fecal-orally transmitted and readily spread into the environment. Little is known about risk of utilizing surface water in Thailand. Quantitative microbial risk assessment has been used to estimate the risk of waterborne salmonellosis. For hazard characterization, beta-Poisson model was employed to describe the relationship between dose of *Salmonella* from water exposure and salmonellosis. Prevalence and concentration of *Salmonella* in the surface water including water exposure were used to model probability of exposure. The probabilistic prevalence was described by beta distribution and the range of mean *Salmonella* prevalences of the surface water along rivers were between 8.33% and 33.33%. The mean concentrations of *Salmonella* in the surface water were between -4.03 and -3.45 log MPN/ml. The risk estimates from all sampling locations along rivers fell into 4 risk levels 1, 2, 3 and 4 of which the means of salmonellosis were 399, 526, 1,337 and 2,619cases/year, respectively. The risks of salmonellosis in the surface water upstream less fluctuated less than that downstream in Central Thailand. The risks of salmonellosis in the surface water samples nearby livestock farm areas were not clearly related to animal production.

Keywords: livestock, risk assessment, *Salmonella*, surface water,

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Introduction

According to Guidelines for Drinking-water Quality published by World Health Organization (WHO) (WHO, 2011), *Salmonella* spp. is among the waterborne pathogens potentially causing human adverse health effect as numerous waterborne outbreaks of salmonellosis have been reported in many countries. Waterborne salmonellosis has been one of the leading causes of waterborne disease outbreak in the United States during the late 19th and early 20th centuries and majority of these outbreaks were implicated with *Salmonella* Typhi, which has continued to occur (Craun et al., 2006). Unlike *Salmonella* Typhi, which is strictly transmitted among humans, non-typhoidal *Salmonella* also plays an important role in causing waterborne disease outbreaks attributed to either human or animal sources (Clark et al., 1996; Angulo et al., 1997).

Mode of transmission of *Salmonella* is usually by means of fecal-oral route. On one hand, one can directly be in contact with this pathogen as a result of inadequate personal hygiene. On the other hand, *Salmonella* can also spread into the environment by means of untreated raw sewage, livestock farming, municipal facility breakdown, and natural disasters. Therefore, susceptible population can indirectly be exposed to *Salmonella* by utilizing contaminated surface water from a variety of purposes such as drinking, bathing, agricultural irrigation, recreation, etc.

An approach to ensure the safety of drinking water is the integration of the risk assessment and risk management of the water supply (WHO, 2011). This approach is a systematic assessment of risks throughout a drinking water supply (from catchments and its source water to consumers) and also a part of the simplified framework for safe drinking water.

Microbiological risk assessment (MRA) has been used as scientific means to enhance consumer protection and also support international trades. Codex Alimentarius Commission (CAC) has recommended a structural approach to conduct MRA in estimation of risk: hazard identification, hazard characterization, exposure assessment, and risk characterization. The process of describing an agent that contaminates food of interest and brings some undesired consequences to consumers is defined as “hazard identification.” In the context of waterborne risk, the hazard could be pathogens that contaminate water for human utilization. This step is performed by a qualitative process via scientific evidence, expert opinion, waterborne outbreak investigation summarized by government agencies, and some clinical studies. By sequential event time line, the next step is to determine likelihood of obtaining pathogen and also the amount of pathogen obtained from the water. This step is called “exposure assessment.” After the exposure to a certain amount of pathogen so-called “dose”, likelihood of having adverse health effect (*e.g.* infection or illness) will be determined by means of relationship between “dose” of pathogens and “response” by dose-response assessment model. This step is called “hazard characterization” or historically “dose-response assessment.” The final step is to integrate the last two steps to determine likelihood of undergoing adverse health effect (dose-response assessment) as a result of exposure to the water contaminated with pathogen (exposure assessment) (CAC, 1999).

In Thailand, little is known about the risk of utilizing surface water potentially originated from either feces or manure, especially in the Central Thailand, as a source of tap water. It is essential to get insight into microbial contaminations, in surface water, upstream to downstream in terms of risk attributed to either human or animal. Therefore, the objectives of this study were to provide both extent and level of *Salmonella* contaminated in surface water and to quantitatively assess health risk of *Salmonella* as a result of utilizing surface water. Monitoring dynamics of *Salmonella* contaminated in surface water could be beneficial for implementing appropriate risk management accordingly. This monitoring scheme could also act as a template for water safety authorities to control other public health hazards associated with water. For the purpose of risk assessment, health risk derived from utilizing surface water is illustrated in form of number of illness among susceptible population. This information is essential for public health agencies to prepare either risk mitigation measures for long-term risk management plan or emergency plan in case of waterborne disease outbreaks.

Materials and methods

Surface water sampling: Two series of surface water samples were collected from Chao Phraya (Central) river and Mae Klong (West) river. These two rivers are the final sources of raw water of the water treatment plants supplying tap water for Bangkok metropolitan including its vicinity.

For the Central, the surface water samples were collected upstream of Chao Phraya rivers, *i.e.* from Yom river and Nan river in Phichit province. Tthe surface water samples were again collected downstream of Chao Phraya river running through many provinces such as Nakorn Sawan, Singburi, Angthong, Ayuthya and Pathumthani before getting into Bangkok water treatment plants, which are Bangkhen, Samsen, and Thonburi (Fig 1).

For the West, the surface water samples were collected along the downstream of Mae Klong river running through provinces such as Kanchanaburi, Nakorn Pathom and Nonthaburi before getting into Mahasawat water treatment plant in the Bangkok vicinity (Fig 2).

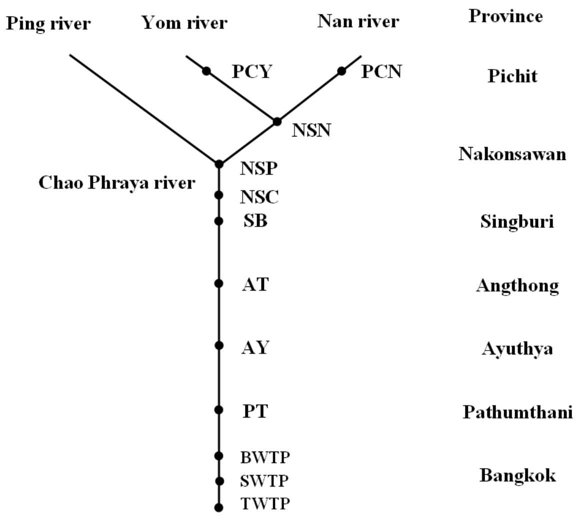

Figure 1 Sampling locations along the river in Central Thailand

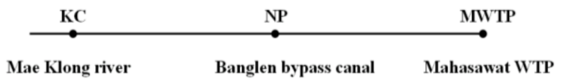

Figure 2 Sampling locations along rivers in West Thailand

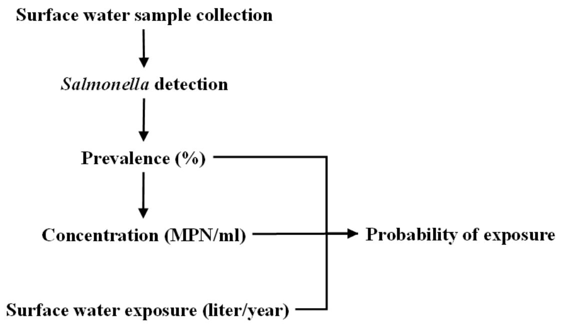

Figure 3 conceptual models for exposure assessment

All surface water samples were aseptically collected at least 30 centimeters below the water surface. At all sampling locations, duplicate samples of 1,000 ml were collected and repeated 5 times every other month. After collected, the surface water samples were kept under 10°C during transportation to the microbiological laboratory. Immediately after arriving at the laboratory, the surface water samples were filtrated through a filter with pore size and a diameter of 0.45 µm and 47 mm, respectively.

Since there were livestock farms that share the surface water with the community, Geographical locations of standard dairy, pig, broiler, layer, and duck farms officially registered to the Department of livestock and development (DLD), Ministry of Agriculture were mapped using the global information system (GIS). These standard livestock farms employ both waste and wastewater management to be entitled standard livestock farms, therefore these farms are not supposed to microbiologically pollute surface water nearby. For the fact that only a few livestock farms are officially registered with DLD, some other small-to-medium-sized livestock farms might not be aware of such environmental contamination. Therefore, it was assumed that the standard livestock farms would represent the densely populated livestock farms in the nearby areas. The surface water samples were then collected both before and after passing through these livestock farm areas in order to determine the level of *Salmonella* potentially as a result of manure contamination from these livestock farms.

Salmonella detection: Detection of *Salmonella* spp. followed the ISO 6579 (ISO, 2002). Briefly, the surface water membrane filters were transferred to Rappaport-Vassiliadis medium with soya (RVS) broth and incubated at 42°C for 24 h. Potential cultures from RVS broth were streaked onto the xylose lysine desoxycholate (XLD) agar and incubated at 37°C for 24 h. The presumptive *Salmonella* up to 5 colonies were confirmed by triple sugar iron (TSI) slant. Then, "White-Kauffman-Le Minor scheme" was used to assign the serovar. name of *Salmonella*.

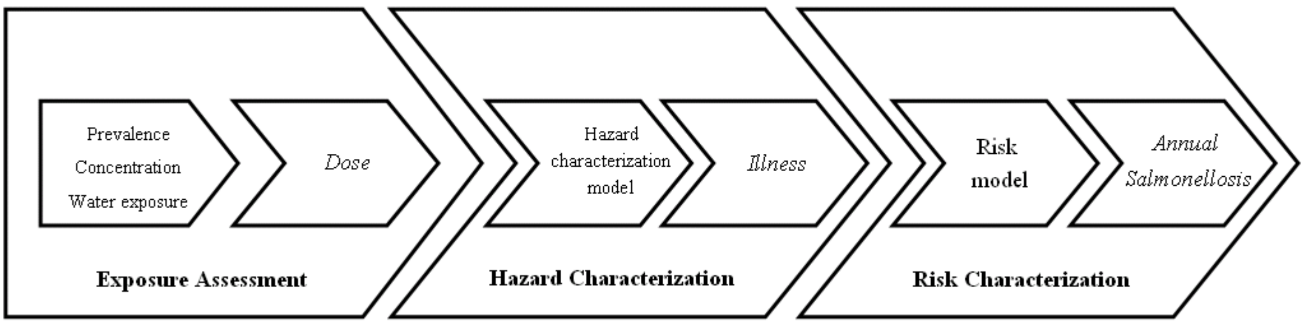

Figure 4 Conceptual model of hazard characterization

Water exposure: Nowadays, people living along river bank are in contact with the water mainly by swimming is 50 ml/h, 2.6 h/swim and 7 swims/year (Covello and Merkhofer, 1993). Therefore the water exposure (W) upon swimming is about 0.91 liter/year or 2.49 ml/day.

Risk assessment models:

Exposure assessment

In order to assess microbial exposure, frequency and extent of contamination of *Salmonella* in surface water samples at the point of water exposure should be considered. These two variables were interpreted as prevalence and concentration of *Salmonella* contaminated in surface water samples, respectively. Dose of microbial exposure was calculated by the product of both *Salmonella* concentration and water exposure. Conceptual model for the exposure assessment is presented in Figure 3.

1. Prevalence variable

Bayesian inference concept is essentially about weighted combining prior information and existing information altogether in order to better describe a variable distribution. The prior distribution provides probability distribution of a variable before any later information becomes available. The likelihood function represents the current probability distribution acquired. Beta distribution has been widely used to describe the prevalence (FAO/WHO, 2008). Since the range of prevalence is between zero (0%) and one (100%) inclusively, which is also applicable to the range of Beta distribution, the beta distribution is characterized by 2 parameters which are alpha and beta as shown in (1).

$$P = Beta\left(\alpha,\beta\right) \qquad (1)$$

In order to describe uncertainty of prevalence, alpha parameter is substituted by $s + \alpha$ and beta parameter is substituted by $n - s + \beta$, where s is the success trial(s) in the identical n trials of a binomial process as shown in (2). In this study, the success trials were the *Salmonella* contaminated (positive) samples where the identical n trials were the sample size.

$$P = Beta\left(s+\alpha, n-s+\beta\right) \qquad (2)$$

If the prior distribution is presumably an uninformed prior and likelihood function is binomial distribution in Bayesian inference, this notation is a posterior distribution. Interestingly, if beta(1,1) distribution, which is equivalent to uniform(0,1) distribution, is an uninformed prior (FAO/WHO, 2008), alpha and beta will be replaced by 1 as shown in (3).

$$P_1 = Beta\left(s+1, n-s+1\right) \qquad (3)$$

This approach is possible since beta distribution is a conjugate distribution to the binomial likelihood function in Bayesian inference. Therefore, parameters α and β become $s+1$ and $n-s+1$, respectively (FAO/WHO, 2008).

2. Concentration variable

2.1 Quantal assay

The basis of quantal assay is to determine whether a microorganism is present in a known volume of sample or not. A well-known technique applying quantal assay is the most probable number (MPN), where a series of volume of samples are inoculated into a selective broth. After incubated in an optimal condition, the selective broth is evaluated whether the microorganism is present or not (Haas et al., 1999).

A quantal assay could be set out by a series of sample volumes of V_1, V_2 and V_3 (usually 0.1, 0.01 and 0.001 ml) with n_1, n_2 and n_3 replicates (usually 3 or 5 equally), respectively. Results of the presence of microorganism, after incubated, for individual sets (replicates) are then read as s_1, s_2 and s_3, respectively (Table 1).

The probability of detecting replicate with the target microorganism in a sample volume (V_i) from all replicates (n_i) follows the binomial distribution. This probability also depends on the concentration of microorganism in a sample volume and the microorganism distribution in such sample volume is assumed to follow Poisson distribution. Then, Poisson distribution is substituted back into the binomial distribution. In order to account for a series of sample volumes, the probability of detecting positive of all sample volumes are multiplied together and transformed into a likelihood function as shown in equation (4) where μ is the mean concentration of microorganism in sample volume (Haas et al., 1999).

$$L = \prod_{i=1}^{r} \frac{n_i!}{s_i!(n_i-s_i)!}\left\{\left[1-e^{(-\mu V_i)}\right]^{s_i}\left[e^{(-\mu V_i)}\right]^{n_i-s_i}\right\} \qquad (4)$$

In a special case where a series of sample volumes are reduced to one single sample volume, the equation (4) can be differentiated with respect to μ. The concentration of microorganism (μ) in one single sample volume (V) with total replicates (n) and positive replicates (s) is simplified to be the equation (5).

$$\mu = -\frac{1}{V} ln\left(\frac{n-s}{n}\right) \qquad (5)$$

When all samples are negative, s will be decreased by one significant digit from 1 (which is 0.9) as a lower limit (FDA, 2007).

Salmonella concentrations at the point of exposure from the quantal assay was assumed to be log-normally distributed. Therefore, log of concentration is supposed to be normally distributed (FAO/WHO, 2008). Then, an approximately 95% confidence interval of log mean concentration is log μ ± 1.96 $SE_{\bar{X}}$. The standard error of log sampling mean concentration was calculated as shown in (6) (Haldane, 1939; Best and Rayner, 1985).

$$SE_{\bar{X}} = \left[\frac{sV^2 e^{(\mu V)}}{\left(e^{(\mu V)} - 1\right)^2} \right]^{-\frac{1}{2}} \qquad (6)$$

3. Probability of exposure (P_E)

Probability of exposure is the likelihood of experiencing at least one cell of *Salmonella* from water. Therefore, the input variables to model probability of exposure are concentration (C) & prevalence (P) of *Salmonella* and water exposure (W) as shown in (7) (Geng et al., 1983).

$$P_E = P\left(1 - e^{-CW}\right) \qquad (7)$$

Hazard characterization

The objective of this step of microbial risk assessment is to determine the dose-response relationship quantitatively (FAO/WHO, 2003). The dose is derived from the product of both *Salmonella* concentration and surface water exposure. These variables were obtained from the exposure assessment step. The conceptual model for hazard characterization related to exposure assessment previously and risk characterization is presented in Figure 4.

Responses upon exposure to a pathogen can be infection, illness, sequelae, or mortality (Haas et al., 1999). Even though dose-response models for *Salmonella* have been developed such as beta-Poisson model using human feeding trial of *Salmonella* and *Shigella* or weibull-gamma model using mixed bacterial pathogen feeding trials (FAO/WHO, 2002). The joint FAO/WHO on risk assessment of microbiological hazards in foods has recommended dose-response model based on outbreak data. This beta-Poisson model was derived from fitting candidate distributions from real-world outbreaks worldwide where α and β are parameters determining the shape of this model as shown in (8).

$$P_D = 1 - \left(1 + \frac{CW}{\beta}\right)^{-\alpha} \qquad (8)$$

In order to incorporate the uncertainty into the expected value of this model parameters, the upper and lower bounds and 2.5th and 97.5th percentiles are assigned to the probability distribution of α and β as shown in Table 2 (FAO/WHO, 2002).

Risk characterization

The essence of risk characterization is to estimate the probability of illness from *Salmonella* upon the surface water exposure. The risk characterization is a two-step linked process where the non-zero *Salmonella* exposure happens before the illness develops in the host. Therefore, the probability of illness (P_I) is a conditional probability where the probability of illness (P_D) is estimated, given that the probability of exposure is non-zero (P_E). Assuming that illness development and hazard exposure are independent, the model for risk estimate or probability of illness is basically the product of probability of illness due to dose of water exposure from the best-fitted dose-response model and the probability of non-zero exposure as shown in (9). The risk estimate is calculated as probability of illness from water exposure per annum (CAC, 1999).

$$P_I = P_D \times P_E \qquad (9)$$

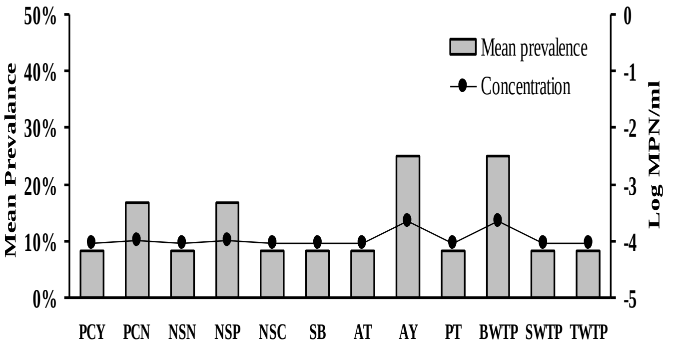

Figure 5 Mean *Salmonella* prevalence and concentration in surface water along rivers in Central Thailand

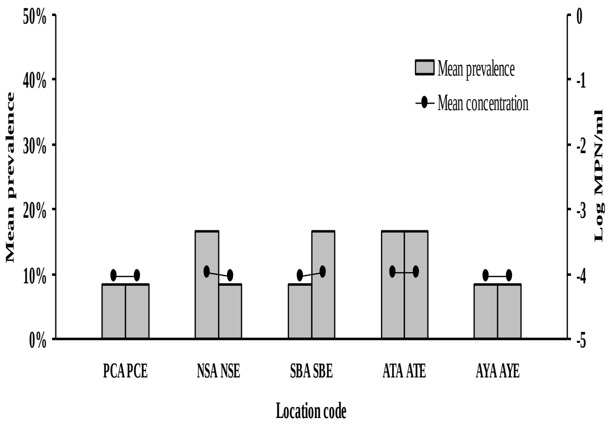

Figure 6 Mean Salmonella prevalence in surface water before and after passing through nearby livestock farm areas along rivers in Central Thailand

Table 1 Quantal assay to determine presence of microorganism in series of sample volumes

Set	Sample volume	Replicate	Result
1	V_1	n_1	S_1
2	V_2	n_2	S_2
3	V_3	n_3	S_3

Table 2 Uncertainty of model parameters of beta-Poisson model

Uncertainty	α	β
Lower bound	0.0763	38.49
2.5th percentile	0.0940	43.75
Expected value	0.1379	50.07
97.5th percentile	0.1817	56.39
Upper bound	0.2274	57.96

Monte Carlo simulation

The models used in this study are composed of variables while the uncertainty of these variables is generally described by a probability distribution within the models. Therefore, the output of the simulation model is dependent upon various possible values within the domain range of the probability distribution. The possible output of the model will be even more complicated or diversified if the model has more than one single probability distribution. Aside from the mathematical operation used in the model, the possible output of simulation model could be infinite particularly when the model variables are continuous. Therefore, it is very difficult or sometimes even impossible to manually calculate the output of the model having some probability distributions by only a spreadsheet software. To resolve this difficulty, the simulation software is used to randomly sample value from the probability distributions in the models. Each sampling or so-called "iteration" designated in the simulation software will generate one possible output of such model at an iteration. In order to cover all possible range of possible output of the model, the values will be reiteratedly sampled again and again sometimes up to a few thousands iterations. A commercial simulation software, @Risk 4.5.3 in the Decision Tools Suite 4.5 (Palisade corporation), was used in this study.

Results

Exposure assessment:

Contamination frequency

As the surface water was derived from two major sources, which were the Chao Phraya river and the Mae Klong (Dam) river in the Central and West Thailand, respectively. The prevalences of *Salmonella* in the sample locations were categorized by the geography and the provinces along the two rivers. Among 10 surface water samples from the rivers in Central Thailand, positive results were only 0, 1 or 2. Therefore, the range of mean *Salmonella* prevalences of the surface water along the rivers in Central Thailand was between 8.33% and 25.00%.In contrast, there was no positive result of *Salmonella* in the surface water in the river in West Thailand. Even though there was no positive result at all, the mean *Salmonella* prevalence from beta distribution was not close to zero percent depending heavily on the extent of sample size. The mean *Salmonella* prevalences of surface water along the rivers in Central and West Thailand are summarized in Table 3 and Figure 5.

Contamination level

The mean concentration of *Salmonella* in the surface water was derived from the quantal assay particularly by the most probable number (MPN) technique. Like contamination frequency, the concentrations of *Salmonella* in the sample locations were categorized by the geography and then descending sorted along the provinces by those rivers. Therefore, it was easier to compare the prevalence and concentration all along. The mean concentrations of *Salmonella* in the surface water from rivers in Central Thailand were between -4.03 and -3.98 log MPN/ml except that those of *Salmonella* in Ayuthaya and Bangkhen water treatment plant in Bangkok were -3.65 log MPN/ml. Therefore, it might be generalized that the contamination level of *Salmonella* in the upper stream of river is lower than that of *Salmonella* in the lower stream. While the mean concentrations of *Salmonella* in the surface water from the rivers in West Thailand were as constant as -4.03 log MPN/ml. Therefore, the contamination levels of *Salmonella* in the surface water in the West and upper stream of Central Thailand were approximately the same.

Salmonella contamination attributed to nearby livestock areas

The *Salmonella* contaminations in the surface water samples both before and after passing through nearby livestock farm areas along the rivers in Central Thailand were mostly constant except in Nakorn Sawan and Singburi as shown in Table 4 and Figure 6. For Singburi, the *Salmonella* contamination in the affluent livestock farm area was lower than that from effluent livestock farm area. Interestingly the *Salmonella* contamination in the surface water sample from affluent livestock farm area of Nakorn Sawan turned out to be higher than that from effluent livestock farm areas. In contrast, the *Salmonella* contaminations in the surface water samples passing through nearby livestock farm areas along the rivers in West Thailand were unpredictable even in the same province and the results are summarized in Table 4.

Risk characterization:

Risk of salmonellosis

According to the exposure assessment models, the probability of exposure is a function of both prevalence and concentration. However, in this study the concentration was rather depended on the prevalence by means of the quantal assay. The mean prevalences in this study were 8.33%, 16.67%, 25.00% and 33.33%. Therefore, in order to avoid the confusion of many locations and samples, the risks of salmonellosis were ranked as risk levels 1 to 4 corresponding to those 4 mean prevalences. The risk levels from the model simulation were the probability

Table 3 Mean *Salmonella* prevalence and concentration in surface water along rivers in Central and West Thailand

Province	Location / Description	Code	Mean	
			Prevalence (%)	Concentration (Log MPN/ml)
Phichit	Yom river	PCY	8.3	-4.03
	Nan river	PCN	16.67	-3.98
Nakon Sawan	Yom rivers merges Nan river	NSN	8.3	-4.03
	Nan river merges Ping river	NSP	16.67	-3.98
	Chao Phraya river	NSC	8.33	-4.03
Singburi	Chao Phraya river	SB	8.33	-4.03
Angthong	Chao Phraya river	AT	8.33	-4.03
Ayuthya	Chao Phraya river	AY	25.00	-3.65
Pathumthani	Samlae untreated water pumping system	PT	8.33	-4.03
Bangkok	Bangkhen water treatment plant	BWTP	25.00	-3.65
	Samsen water treatment plant	SWTP	8.33	-4.03
	Thonburi water treatment plant	TWTP	8.33	-4.03
Kanchanaburi	Mae Klong river	KC	8.33	-4.03
Nakorn Pathom	Banglen bypass canal	NP	8.33	-4.03
Nonthaburi	Mahasawat water treatment plant	MWTP	8.33	-4.03

Table 4 Mean *Salmonella* prevalence in surface water before and after passing through nearby livestock farm areas along rivers in Central and West Thailand

Province	Description	Code	Mean	
			Prevalence (%)	Concentration (Log MPN/ml)
Phichit	Affluent livestock areas	PCA	8.33	-4.03
	Effluent livestock areas	PCE	8.33	-4.03
Nakon Sawan	Affluent livestock areas	NSA	16.67	-3.98
	Effluent livestock areas	NSE	8.33	-4.03
Singburi	Affluent livestock areas	SBA	8.33	-4.03
	Effluent livestock areas	SBE	16.67	-3.98
Angthong	Affluent livestock areas	ATA	16.67	-3.98
	Effluent livestock areas	ATE	16.67	-3.98
Ayuthya	Affluent livestock areas	AYA	8.33	-4.03
	Effluent livestock areas	AYE	8.33	-4.03
Nakorn Pathom	Affluent livestock areas	NPA1	8.33%	-4.025
	Effluent livestock areas	NPE1	33.33%	-3.448
	Affluent livestock areas	NPA2	16.67	-3.98
	Effluent livestock areas	NPE2	8.33	-4.03

Table 5 No. of salmonellosis cases among Thai population[a] per year attributed to surface water exposure using model simulation

Risk level	Min	5th percentile	Mean	95th percentile	Max
1	0.005[b]	2	399	1,861	26,745
2	0.3[c]	14	526	2,096	29,565
3	5	77	1,337	4,747	30,901
4	16	210	2,619	9,079	67,179

[a] Thai population at 65 million, [b,c] Cases could have been integer if Thai population was 10^{11} or 10^{9}, respectively.

of illness among susceptible population. In order to gain a better sense of human adverse health effect, the probability of risk was multiplied by total Thai population at 65 million. The results of simulation in terms of number of salmonellosis cases are shown in Table 5 and Figures 7-8.

Risk of surface water exposure

The mean prevalence and contamination together with the surface water exposure of various locations along the rivers in Central and West Thailand were substituted into the simulation models. As mentioned previously, the risks of salmonellosis of sampling locations along the rivers were ranked by 4 risk levels and are summarized in Table 6

The risk levels of the surface water were nearly constant from upstream of Chao Phya river to downstream particularly in front of the water treatment plants, where the mean salmonellosis was about 399 cases/year. Likewise, the risk levels of the surface water were stable along the river in the West Thailand. Therefore, this risk level could be generalized as the background mean salmonellosis of surface water in Thailand. However, the mean salmonellosis in Nan river (PCN) and Nan river merging Ping river (NSP) was 127 cases/year higher than the background mean salmonellosis. Furthermore, the mean salmonellosis in Ayuthya (AY) and in front of Bangkhen water treatment plant (BWTP) was 938 cases/year higher than the background mean salmonellosis.

Risk of surface water exposure attributed to nearby livestock areas

The overall risk levels of salmonellosis of surface water exposure to nearby livestock areas fluctuated between 1 and 2. Therefore, the risk of salmonellosis was right around the background risk attributed to the community contamination per se (Table 7). Comparing the risk of salmonellosis of surface water before and after passing through nearby livestock areas, the risk was the same in Phichit, Angthong and Ayuthya. On the other hand, the mean salmonellosis cases after passing through nearby livestock areas in Singburi (SBA) increased by 127 cases/year (SBE). Interestingly, the mean salmonellosis cases after passing through nearby livestock areas in Nakorn Sawan (NSA) in turn dropped by 127 cases/year (NSE).

Discussion

The range of mean prevalence of *Salmonella* in the surface water along the rivers in Central and West Thailand was from 8.33% to 25.00%. These data are essential since to our knowledge this might be the first scientific evidence to demonstrate the *Salmonella* contamination frequency in the surface water consecutively along the rivers in both West and Central Thailand. Therefore, this information could be employed as a background prevalence of *Salmonella* to compare with prevalence of *Salmonella* in the surface water in case of disasters, *e.g.* flood, draught, waterborne disease outbreak, etc., in the future.

Since one of the primary aims of this study

Table 6 Risk level of salmonellosis in the surface water along rivers in Central and West Thailand

Province	Location / Description	Code	Risk level
Phichit	Yom river	PCY	1
	Nan river	PCN	2
Nakon Sawan	Yom rivers merges Nan river	NSN	1
	Nan river merges Ping river	NSP	2
	Chao Phraya river	NSC	1
Singburi	Chao Phraya river	SB	1
Angthong	Chao Phraya river	AT	1
Ayuthya	Chao Phraya river	AY	3
Pathumthani	Samlae untreated water pumping system	PT	1
Bangkok	Bangkhen water treatment plant	BWTP	3
	Samsen water treatment plant	SWTP	1
	Thonburi water treatment plant	TWTP	1
Kanchanaburi	Mae Klong river	KC	1
Nakorn Pathom	Banglen bypass canal	NP	1
Nonthaburi	Mahasawat water treatment plant	MWTP	1

Table 7 Mean *Salmonella* prevalence in surface water before and after passing through nearby livestock farm areas along rivers in Central Thailand

Province	Description	Code	Risk level
Phichit	Affluent livestock areas	PCA	1
	Effluent livestock areas	PCE	1
Nakon Sawan	Affluent livestock areas	NSA	2
	Effluent livestock areas	NSE	1
Singburi	Affluent livestock areas	SBA	1
	Effluent livestock areas	SBE	2
Angthong	Affluent livestock areas	ATA	2
	Effluent livestock areas	ATE	2
Ayuthya	Affluent livestock areas	AYA	1
	Effluent livestock areas	AYE	1

Table 8 Effect of sample size on mean and variance of probabilistic prevalence

Sample size (n)	Positive (s)	Prevalence		
		Deterministic	Probabilistic	
			Mean	Variance
10	1	10.0	16.7	0.010684
50	5	10.0	11.5	0.001926
100	10	10.0	10.8	0.000934
500	50	10.0	10.2	0.000181
1,000	100	10.0	10.1	0.000090
2,000	200	10.0	10.0	0.000045

was to determine the *Salmonella* contamination in the surface water comprehensively from upstream to downstream of the rivers as the source of tap water, the sample sizes of individual sampling locations were not large enough. Sample sizes played an important role in particularly the mean prevalence described by beta distribution, especially, when sample size is small. As the parameter of beta distribution is determined directly by both the positive sample and the corresponding sample size. This effect is usually less pronounced when the sample size gets larger as shown in Table 8. Therefore, in order to address the true mean prevalence of *Salmonella* in the surface water in the sampling locations in this study, more in-depth researches for individual province or location (*e.g.* increasing the sample size) are needed to improve the accuracy and variance from this background information.

Considering that this study should have had a larger sample size, the range of mean *Salmonella* prevalence was still in line with prevalence from some previous studies. One study demonstrated that the prevalence of *Salmonella* in river was between 0.6% (1/195) and 20.1% (8/39) (Hendricks, 1971). Another report evaluated the risk of salmonellosis from fresh produce using irrigation water and found that the prevalence of *Salmonella* was around 6.2% (89/1,429) (Johnson et al., 2003). From a review of *Salmonella* occurrence in comparable surface waters, the prevalence of *Salmonella* ranged from 3% (*n* = 32) in surface runoff or agricultural water, 8.5% (*n* = 342) from rainfall runoff and drainage from agricultural land to 57% (*n* = 14) from runoff, agricultural land and pastures (Levantesi et al., 2012). Therefore, the prevalence of *Salmonella* mainly varied depending upon the functions of the area nearby the river.

From Table 3 and Figure 5 the *Salmonella* prevalence and corresponding concentration in the surface water taken upstream fluctuated less than taken downstream of rivers in Central Thailand. Partly this could be explained by the population density. The denser the population, the more activities by the river bank, resulting in more shedding of the pathogen into the water source. This finding agreed with a previous report showing that *Salmonella* prevalence in the upper stream was about 2.6% (1/39) and in the lower stream was about 20.1% (8/39) of the same river.

Since *Salmonella* in the surface water, *e.g.* river or watershed system, is associated with manure possibly from animal farms (Levantesi et al., 2012), it is more intuitive to expect that surface water passing through nearby livestock farm areas will have higher *Salmonella* contamination. By collecting surface water sample both before and after passing through nearby livestock farm area, the comparative *Salmonella* prevalence of effluent water is supposed to be higher as a result of runoff water. However in this study the result showed that *Salmonella* contaminations in the surface water samples both before and after passing through nearby livestock farm areas along the rivers in Central Thailand were mostly constant (Table 4 and Fig 6), except in Singburi and Nakorn Sawan.

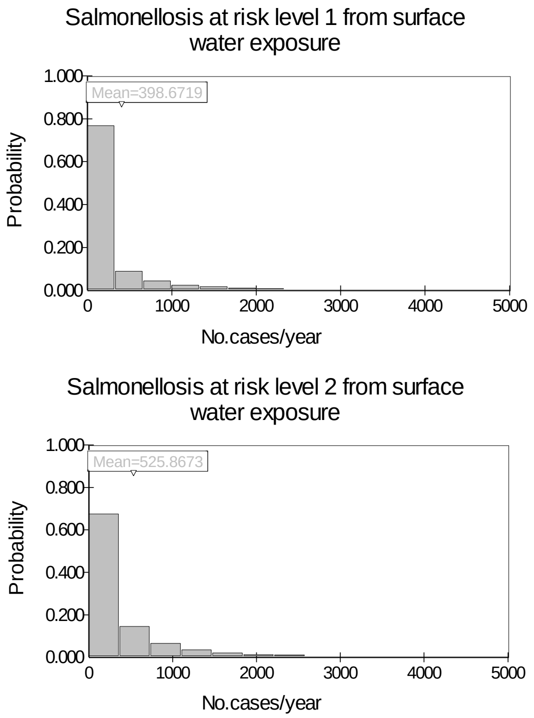

Figure 7 Distribution of salmonellosis at risk levels 1-2 from surface water exposure

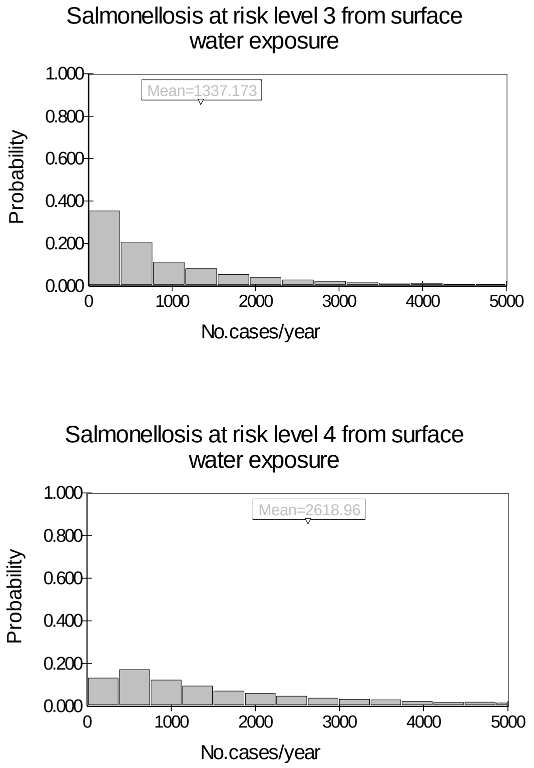

Figure 8 Distribution of salmonellosis at risk levels 3-4 from surface water exposure

Therefore, this unpredictable finding could be a result of either the normal range of variability of *Salmonella* contamination from the nearby livestock areas or some other factors that were not addressed. Moreover, the result of this study was applicable to one previous study indicating that either *Salmonella* spp. or *E. coli* O157:H7 prevalence was not directly associated with bovine, swine, and poultry feeding operations (Johnson et al., 2003).

The risk estimate as an output from the model simulation was usually reported in the form of probability of getting illness from a certain pathogen. However, the form of probability to the general is not as intuitive as risk in the form of number of cases. Therefore, in this study the risk estimates of salmonellosis of all Thai population upon exposure to surface water were reported as number of *Salmonella* cases per annum. Additionally the risk estimates were also ranked into 4 risk levels depending on the prevalence of *Salmonella* in the water samples. This risk level simplified the comparison of risk from various sampling locations. Note that in some cases where the risk level is low the minimum number of cases/year is not an integer. Assuming that the model and calculation were correct, this occurrence is because the risk was so low that among a certain amount of population the cases was still a fraction. If the risk of a much larger population was considered, even the very low level of cases would have been an integer.

In order to recognize the magnitude of risk of salmonellosis from surface water exposure, it has been generally accepted that cases of a certain pathogen associated with surface water supplies are less than the 1: 10,000 risk of infection per year according to the goal for surface water supplies set by US Environmental Protection Agency (Regli et al., 1991). Taking this as an acceptable level of risk for 65 million Thai population, around 6,500 salmonellosis should cause by the water exposure in this study. After comparing mean of 4 risk levels in this study with the acceptable level of risk (Table 5), means of 4 risk levels, which were 399, 526, 1,337 and 2,619 cases of salmonellosis, were much lower than 6,500 cases per year. Strictly speaking, when the maximum cases of salmonellosis were compared, all risk levels were much higher than acceptable cases. This seemed to be 2 extreme scenarios of risk comparison. Therefore, 95th percentile might be a good candidate since risk levels 1, 2 and 3 passed the acceptable cases, while 95th percentile of cases of salmonellosis of risk level 4 (9,079) was higher than the acceptable cases. Consequently one might opt for 95th percentile as a parameter to compare with the acceptable level of risk. Note that the susceptible population in this study considered the entire Thai population. In fact only a certain fraction of Thai population is applicable to this surface water exposure. Therefore, the risk of salmonellosis upon the surface water exposure could have been even lower and more realistic if the susceptible population of interest was confined to only Thai population living by the riverbank.

In conclusion, this study provided essential information regarding the health risk levels derived from surface water exposure and demonstrated it in the form of number of salmonellosis cases. This scientific evidence is crucial for both national and local authorities that take care of public health directly, *e.g.* Ministry of public health as a national health care provider, or indirectly, *e.g.* Metropolitan and Provincial Waterworks Authorities as clean and safe water providers. This background information is ready to be used to prepare either risk mitigation measures for long-term risk management plan or emergency plan in case of waterborne disease outbreaks.

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บทคัดย่อ

การประเมินความเสี่ยงซัลโมแนลลาในน้ำผิวดินซึ่งเป็นแหล่งของน้ำประปา

ปวีรนุช บานไม่รู้โรย[1] ภัทรภร ชัยชนะ[2] ชัยวัฒน์ พูลศรีกาญจน์[2]
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ซัลโมแนลลาเป็นแบคทีเรียที่ติดต่อผ่านทางอุจจาระและแพร่กระจายไปในสิ่งแวดล้อมได้ กอปรกับข้อมูลความเสี่ยงของการอุปโภคบริโภคน้ำผิวดินยังมีน้อยมาก จึงนำการประเมินความเสี่ยงจุลชีพเชิงปริมาณมาใช้ในการประมาณความเสี่ยงโรคซัลโมแนลโลซิสจากการรับสัมผัสน้ำผิวดิน โดยขั้นตอนการอธิบายอันตรายใช้การแจกแจง Beta Poisson เพื่ออธิบายความสัมพันธ์ระหว่างปริมาณซัลโมแนลลาจากการรับสัมผัสน้ำผิวดินและการเกิดโรคซัลโมแนลโลซิส และใช้ความชุกและความเข้มข้นของซัลโมแนลลาในน้ำผิวดินรวมทั้งปริมาณการรับสัมผัสน้ำผิวดิน ในการหาความน่าจะเป็นในการสัมผัสกับซัลโมแนลลา ความชุกเชิงความน่าจะเป็นใช้การแจกแจง Beta ความชุกเฉลี่ยของซัลโมแนลลาในน้ำผิวดินจากแม่น้ำอยู่ระหว่างร้อยละ 8.33 และ 33.33 ความเข้มข้นเฉลี่ยของซัลโมแนลลาในน้ำผิวดินอยู่ระหว่าง -4.03 และ -3.45 log MPN/มล. ค่าประมาณความเสี่ยง ณ จุดเก็บตัวอย่างตามแม่น้ำแบ่งได้เป็น 4 ระดับ คือ ระดับที่ 1, 2, 3 และ 4 มีจำนวนผู้ป่วยโรคซัลโมแนลโลซิสเฉลี่ย 399, 526, 1,337 และ 2,619 คน/ปี ตามลำดับ การศึกษาครั้งนี้พบว่า ความเสี่ยงโรคซัลโมแนลโลซิสที่บริเวณต้นน้ำมีความผันแปรน้อยกว่าความเสี่ยงบริเวณปลายน้ำในภาคกลางของประเทศไทย

คำสำคัญ: การประเมินความเสี่ยง ซัลโมแนลลา น้ำผิวดิน ปศุสัตว์

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