การประเมินความเสี่ยงเชิงปริมาณซัลโมแนลลาในน้ำผิวดินในลุ่มน้ำภาคกลางของประเทศไทย

นางสาวปวีรนุช บานไม่รู้โรย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

QUANTITATIVE RISK ASSESSMENT OF SALMONELLA SPP. IN SURFACE WATER OF THE RIVER, CENTRAL PART THAILAND.

Miss Paweeranut Banmairuroy

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Public Health
Department of Veterinary Public Health
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2013

Thesis Title	QUANTITATIVE RISK ASSESSMENT OF SALMONELLA SPP. IN SURFACE WATER OF THE RIVER, CENTRAL PART THAILAND.
By	Miss Paweeranut Banmairuroy
Field of Study	Veterinary Public Health
Thesis Advisor	Associate Professor Suphachai Nuanualsuwan, D.V.M., Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Veterinary Science

(Professor Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D.)

THESIS COMMITTEE

.....Chairman

(Associate Professor Rungthip Chuancheun, D.V.M., Ph.D.)

.....Thesis Advisor

(Associate Professor Suphachai Nuanualsuwan, D.V.M., Ph.D.)

.....Examiner

(Associate Professor Alongkorn Amonsin, D.V.M., Ph.D.)

.....Examiner

(Taradon Luangtongkum, D.V.M., Ph.D.)

.....External Examiner

(Associate Professor Adisorn Swetwiwathana, Ph.D.)

ปวีรนุช บานไม่รู้โรย : การประเมินความเสี่ยงเชิงปริมาณซัลโมแนลลาในน้ำผิวดินในลุ่มน้ำภาคกลางของประเทศไทย. (QUANTITATIVE RISK ASSESSMENT OF SALMONELLA SPP. IN SURFACE WATER OF THE RIVER, CENTRAL PART THAILAND.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร.ศุภชัย เนื้อนวลสุวรรณ, 46 หน้า.

ซัลโมแนลลาเป็นเชื้อที่มีการติดต่อผ่านทางอุจจาระและสามารถแพร่กระจายสู่สิ่งแวดล้อม เนื่องจากข้อมูลความเสี่ยงของการอุปโภคบริโภคน้ำผิวดินซึ่งเป็นแหล่งของน้ำประปาที่มีการปนเปื้อนสิ่งปฏิกูลในเขตภาคกลางและภาคตะวันตกของประเทศไทยมีจำกัด การประเมินความเสี่ยงจุลชีพเชิงปริมาณจึงถูกนำมาใช้ในการประมาณความเสี่ยงของการเจ็บป่วยด้วยโรคซัลโมแนลโลซิส การแจกแจงแบบเบต้า-พัวซองใช้ในการอธิบายความสัมพันธ์ระหว่างปริมาณเชื้อจากการสัมผัสกับน้ำและการเกิดโรคซัลโมแนลโลซิสในขั้นตอนการอธิบายอันตราย ความชุกและความเข้มข้นของซัลโมแนลลาในน้ำผิวดินรวมทั้งปริมาณการสัมผัสน้ำใช้ในการสร้างแบบจำลองความน่าจะเป็น การอธิบายความเสี่ยงเป็นบูรณาการระหว่างการอธิบายอันตรายและการประเมินการสัมผัส ความน่าจะเป็นของความชุกอธิบายโดยใช้การแจกแจงแบบเบต้า จากการเก็บตัวอย่างน้ำผิวดิน ซึ่งเป็นแหล่งที่มาของน้ำประปาจากการประปานครหลวง ปริมาตร 1000 มิลลิลิตร แล้วนำมาวิเคราะห์หาเชื้อซัลโมแนลลาโดยวิธี ISO 6579 พบว่า ค่าเฉลี่ยความชุกของซัลโมแนลลาในน้ำผิวดินจากแม่น้ำอยู่ระหว่างร้อยละ 8.33 และ 33.33 ค่าเฉลี่ยความเข้มข้นของซัลโมแนลลาในน้ำผิวดินอยู่ระหว่าง -4.03 ถึง -3.45 log MPN/มิลลิลิตร การสัมผัสน้ำของประชากรบริเวณริมฝั่งแม่น้ำ เท่ากับ 0.91 ลิตรต่อปี หรือ 2.49 มิลลิลิตร/วัน ค่าความเสี่ยงจากจุดเก็บตัวอย่างตามแม่น้ำแบ่งออกเป็น 4 ระดับ คือ ระดับ 1,2,3 และ 4 ซึ่งมีค่าเฉลี่ยของการป่วยโรคซัลโมแนลโลซิสเป็น 399, 526, 1,337 และ 2,619 คน/ปี ตามลำดับ การศึกษาครั้งนี้พบว่า ค่าความเสี่ยงโรคซัลโมแนลโลซิสที่ต้นน้ำความผันแปรน้อยกว่าค่าความเสี่ยงโรคที่ปลายน้ำ นอกจากนี้จากผลการศึกษาซีโรวาร์ของซัลโมแนลลาโดยวิธี Kauffman-White serotyping พบ Non typhoidal Salmonella ปนเปื้อนอยู่ในแหล่งน้ำมากที่สุด แต่ซัลโมแนลลาซีโรวาร์เดียวกันจากต้นน้ำไปปลายน้ำที่พบไม่ได้เกิดในรอบการเก็บตัวอย่างเดียวกัน การศึกษาในครั้งนี้รายงานความเสี่ยงเชิงสุขภาพจากการสัมผัสน้ำผิวดิน ซึ่งเป็นข้อมูลพื้นฐานและจำเป็นสำหรับหน่วยงานภาครัฐบาลและท้องถิ่นในการจัดการความเสี่ยงที่เกิดขึ้นและเตรียมการในกรณีเกิดโรคระบาดที่มีน้ำเป็นสื่อ

ภาควิชา สัตวแพทยสาธารณสุข ลายมือชื่อนิสิต .....

สาขาวิชา สัตวแพทยสาธารณสุข ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2556

5575310831 : MAJOR VETERINARY PUBLIC HEALTH
KEYWORDS: SALMONELLA SPP. / SURFACE WATER / RISK ASSESSMENT

PAWEERANUT BANMAIRUROY: QUANTITATIVE RISK ASSESSMENT OF SALMONELLA SPP. IN SURFACE WATER OF THE RIVER, CENTRAL PART THAILAND.. ADVISOR: ASSOC. PROF. SUPHACHAI NUANUALSUWAN, D.V.M., Ph.D., 46 pp.

Salmonella spp. is transmitted via fecal oral route and readily spread into the environment. The information regarding risk of utilizing the surface water in the Central and West Thailand as a source of tap water associated with either feces or manure was limited. Quantitative microbial risk assessment has been used to estimate the risk of waterborne salmonellosis. For hazard characterization, the beta Poisson model was employed to describe the causal relationship between dose of Salmonella from water exposure and its effect as salmonellosis. Prevalence and concentration of Salmonella in the surface water including water exposure were used to model the probability of exposure. The risk characterization was the integration of both hazard characterization and exposure assessment. The probabilistic prevalence was described by beta distribution. The surface water as a resource of MWA tap water were collected 1000 ml and then detected Salmonella spp. by ISO 6579. The range of mean Salmonella prevalence of the surface water along rivers was between 8.33% and 33.33%. Whereas the mean concentrations of Salmonella in the surface water were between -4.03 and -3.45 log MPN/ml. The water exposure of population by the river bank was about 0.91 liter/year. The risk estimates from all sampling locations along rivers fell into 4 risk levels where the mean of salmonellosis of risk levels 1, 2, 3 and 4 was 399, 526, 1,337 and 2,619 cases/year, respectively. The risks of salmonellosis in the surface waters from upstream were less fluctuating than those in the surface water from the downstream of rivers of Central and West Thailand. The majority of serovar was non-Typhiodal Salmonella. Although the same Salmonella serovar was identified from upstream to downstream of the same river yet across sampling rounds. This study provided essential and background health risk levels from surface water exposure and were crucial for the national or local authorities to prepare risk mitigation measures for a long term risk management plan or emergency plan in case of waterborne disease outbreaks.

Department: Veterinary Public Health Student's Signature .....

Field of Study: Veterinary Public Health Advisor's Signature .....

Academic Year: 2013

ACKNOWLEDGEMENTS

I am very honored and grateful to be endowed with The H.M. King Bhumibol Adulyadej's 72nd Birthday Anniversary Scholarship and the Thailand Research Fund (TRF : RDG5520019) for the financial supports.

I thank my advisor, Dr. Suphachai Nuanualsuwan for his invaluable time, excellent consultancy, kindness and encouragement during the time of this study. I thank Associate Professor Adisorn Swetwiwathana, Associate Professor Dr. Alongkorn Amonsin, Associate Professor Dr. Rungtip Chuancheun and Instructor Dr. Taradon Luangtongkum as members of thesis committee for their precious and practical suggestions.

I thank Dr.Chaiwat Pulsrikarn, Ms.Phattharaporn Chaichana and all member staffs at the WHO National Salmonella and Shigella Center, Department of Medical Science, Ministry of Public Health for their kindly supports in terms of the laboratory equipment and the microbiology trainings.

I thank all member staffs and graduate students at the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University for their friendship, encouragement and assistance during the entire course of this study.

I am also very thankful to, the last but not the least, my lovely family and friends. As they are always supportive and cheerful whenever I have encoutered the obstacle.

CONTENTS

Page

LIST OF TABLE

Page

LIST OF FIGURE

Page

CHAPTER I

INTRODUCTION

According to the 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. Salmonellosis in the European Union (EU) and the United States were about 100,000 and 42,000 human cases every year, respectively (CDC, 2011; EFSA, 2011). Although, in Thailand, up to 3,083 isolates have been confirmed as *Salmonella* cases in 2008 (NCSS, 2008). Waterborne salmonellosis was 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 to cause waterborne disease outbreaks attributable to either human or animal sources (Clark et al., 1996; Angulo et al., 1997).

Salmonella is gram-negative, non-spore forming, rod, facultative anaerobe bacterium (Hendriksen, 2003). Clinical symptoms are acute gastroenteritis such as watery diarrhea, abdominal pain, vomit, nausea and fever (CDC, 2011; EFSA, 2011). Mode of transmission of *Salmonella* is usually by means of fecal oral route. On one hand, one could directly contact with this pathogen as a result of inadequate personal hygiene. On the other hand, *Salmonella* could also be spread into the environment by means of untreated raw sewage, livestock farming, municipal facility breakdown, and natural disasters. Therefore, the susceptible population could indirectly be exposed to *Salmonella* by utilizing the contaminated surface water

from a variety of purposes such as drinking, bathing, agricultural irrigation or 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 the systematic assessment of risks throughout a drinking water supply (from catchments and its source water to the consumers) and also a part of the simplified framework for safe drinking water.

Microbiological risk assessment (MRA) has been used as a scientific means to enhance the consumer protection and also support the international trades. The Codex Alimentarius Commission (CAC) has recommended a structural approach to conduct MRA in the estimation of risk: hazard identification, hazard characterization, exposure assessment and risk characterization. The process of describing an agent resulting in some undesired consequence to the consumers and also contaminated in a food of interest is defined as “hazard identification”. In the context of waterborne risk, the hazard could be pathogens that contaminated the water for human utilizations. 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 the sequential event time line, the next step is to determine the likelihood of taking pathogen and also the amount of pathogen that is taken from the water. This step is called “exposure assessment”. After the exposure of a certain amount of pathogen so called “dose”, the likelihood of having adverse health effect (e.g. infection or illness) will be determined by means of the 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 later two steps to determine the likelihood of undergoing the adverse health effect (dose response assessment) as

a result of exposing to the water contaminated with pathogen (exposure assessment) (CAC, 1999).

In the Central Thailand, inhabitants by the river bank make use of the surface water from the river as a drinking water source, a wastewater drainage and a recreation water. The micro-organisms from the communities in the upstream maybe runoff through the downstream and finally presented in the surface or even tap water if the microbial load was overwhelmed (Levantesi et al., 2012). Moreover, flooding and rainfall are the risk factors of microbial contamination in the water resources (Ahmed et al., 2009). Bangkok and vicinity areas are overcrowded and the major population utilizes the tap water supplied by Metropolitan Waterworks Authority (MWA). The raw water of MWA comes from two sides. The Central Thailand is originally derived from Yom, Nan, and then Chao Phraya rivers which altogether pass through many provinces e.g. Nakhon Sawan, Singburi, Angthong, Ayutthaya, Pathumthani and Nonthaburi provinces, respectively. Whereas, the West Thailand of tap water is initially derived from Mae Klong river passing through Kanchanaburi and Nakhon Pathom provinces, respectively. In order to track the common source of *Salmonella* contamination in surface water, *Salmonella* serovars determination will apply.

Little is known about the risk of utilizing the surface water, especially in the Central Thailand, as a source of tap water and related information about risk estimate of such surface water potentially originated from feces. It is essential to get insight into the microbial contaminations in the surface water from upstream to downstream in terms of risk from human. Therefore, the objectives of this study were to estimate the risk of exposure of *Salmonella* spp. as a result of utilizing the surface water in the rivers of the Central Thailand by the probabilistic approach and Monte Carlo simulation and also to determine serotype of *Salmonella* contaminating

the surface water in the rivers of the Central Thailand. Monitoring dynamic of *Salmonella* contaminated in the surface water as a result of human origin ranging from the upstream to the downstream of the river as a source of tap water could be beneficial by implementing the appropriate risk management options accordingly. This monitoring scheme could also acts as a template for water safety authorities to control some other public health hazards associated with water. For the purpose of risk assessment, the health risk derived from utilizing surface water is illustrated in the 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.

CHAPTER II
LITERATURES REVIEW

Salmonella spp.

Salmonella is a bacterial genus in the family *Enterobacteriaeae*. *Salmonella* is identified as Gram negative, rod shaped and motile micro-organism which range from 0.7-1.5 to 2-5 μm in size. This non-spore forming rod, facultative anaerobic, peritrichous flagella micro-organism prefer glucose to sucrose and lactose as an energy source. As a mesophilic pathogen, the optimum temperatures for growth of *Salmonella* are 32-37°C. *Salmonella* has been differentiated up to 2,579 serovarieties (serovars) based on the Kaufmann-White scheme, using somatic, flagella and capsular antigens to identify the serovarieties. *Salmonella enterica* is the major species of foodborne pathogen throughout the world (Quinn et al., 2002).

Since, *Salmonella* has been found among humans animals and environment so the mode of transmission was complicated. *Salmonella* could be transmitted either directly or indirectly among humans and animals. Modes of transmission were through food and from person to person so-called the "fecal-oral" route and contamination indirectly via food or water consumption (EFSA, 2011). *Salmonella* survived in a wide variety of animal reservoirs including humans (Winfield and Groisman, 2003). Some serovars are highly adapted to a specific animal species such as *S*. Choleraesuis in swine, *S*. Dublin in cattle and *S*. Enteritidis in poultry (Sanchez et al., 2002; Chiu et al., 2004). These animal reservoirs can asymptomatically and unknowingly shed *Salmonella* resulting in the contamination of food and produces via the irrigation water and the manure used as a fertilizer (Teunis et al., 2010).

Clinical symptoms of salmonellosis are usually fever, abdominal pain, diarrhea, nausea and vomit after the incubation period of 12-72 hours. Illness usually

lasts up to 4-7 days. Healthy adult do not require any medical treatment to recover from salmonellosis. However, in the young, elder or immunocompromised, illness might require some antimicrobial treatments when septicemia was present (CDC, 2011).

To detect *Salmonella* in foods, ISO 6579 (2002) has been widely used as an international standard for *Salmonella* detection by means of culture technique (ISO, 2002). This step-wise standard initially recovers *Salmonella* from the specimen or sample, biochemically confirms suspected colonies and serotypes (Hendriksen, 2003). Lastly, *Salmonella* serotypes are designated according to the Kauffmann-White Scheme maintained by WHO Collaborating Centre for Reference and Research on *Salmonella* and also employed by most public health laboratories worldwide (Grimont and Weill, 2007).

Salmonellosis as Waterborne disease

Salmonella spp. has been implicated as the major waterborne disease pathogen throughout the world (Levantesi et al., 2012). The drinking water (Johnson et al., 2003; Falco and William, 2009; Laine et al., 2010), the surface water (Arvanitidou et al., 1997; Jokinen et al., 2011; Henriette et al., 2012; Levantesi et al., 2012), ground water used as drinking-water source (Knight et al., 1990), tank water used as a drinking water (Angulo et al., 1997; Taylor et al., 2000), recreational water (Hlavsa et al., 2011) and irrigation water (Melloul et al., 2011; Ndiaye et al., 2011; Benjamin et al., 2013) have been involved as the water-related sources of *Salmonella*. Since the surface water could be inherently contaminated by *Salmonella* (Angulo et al., 1997). Therefore, the presence of *Salmonella* in drinking water could be inevitable as a result of the insufficient disinfection of surface water

or even a cross contamination between the raw sewage and drinking water supplies (Laine et al., 2010).

The *Salmonella*-contaminated manure may lead to the contamination of surface water so the untreated manure slurry might flow directly into the ponds or canal system and finally reach the river thus resulting in the pollution of water source for inhabitants and livestock (Angulo et al., 1997; Taylor et al., 2000). In addition, the untreated sewages or wastewaters from the communities, industrial or agricultural areas are among the other sources of *Salmonella* contamination of the water in the environment (Waage et al., 1999; Johnson et al., 2003; Sahlstrom et al., 2006). The same *Salmonella* spp. isolated from both patient and also from sewage sludge indicates a possible spread of *Salmonella* into the environment via water (Sahlstrom et al., 2006). *Salmonella* contaminated in water in the environmental water signifies the likelihood of waterborne disease transmission via drinking water source and poses the risk of *Salmonella* infection in human (Sahlstrom et al., 2006; Ahmed et al., 2009; Jokinen et al., 2011).

Quantitative microbial risk assessment

In 1995, Food and Agriculture Organization (FAO) and World Health Organization (WHO) assembled an expert drafting committee, Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standard Issues for management of public health risks for hazard in food and water. Risk analysis is a structural model advancing the food safety with the aims of promoting the safety food, decreasing foodborne and waterborne illnesses and facilitating domestic and international trades in foods. Risk analysis consists of three components; risk assessment, risk management and risk communication (FAO/WHO, 2003). Risk management is the process of evaluating the management options in order to

diminish the assessed risks and to select and implement such options. Risk communication is the mutual co-ordination among risk assessor, risk manager and other stakeholders in terms of information regarding the risk to the susceptible population (FAO/WHO, 1995).

Microbiological risk assessment (MRA) is a tool for the appraisal of safety of food and water supplies providing an estimate of the probability of adverse health effects from microbiological hazard that was potentially presented in food or water by science-based identification. Four steps of MRA are hazard identification, exposure assessment, hazard characterization and risk characterization (Lammerding and Fazil, 2000).

The scope of a risk assessment is dependent upon the risk manager specifying risk question(s). Risk profile is recommended to do in the first place to render a mutual understanding and an underlying problem between risk assessor and risk manager including the scope of information. Generally choices of microbial risk assessment are either qualitative or quantitative approaches depending on the available information, risk question, underlying objectives and the expertise of risk assessor. Qualitative risk assessment is descriptive information with much subjective opinions whereas quantitative is numerical and mathematical analyses (Lammerding and Fazil, 2000). Although preferable approach is a quantitative risk assessment, the qualitative risk assessment should not be discounted (CAC, 1999).

Hazard identification

Hazard identification is a qualitative information examining the sufficient evidence to recognize a certain pathogen presented in the food or water as a hazard causing adverse health effect. The hazard in microbial risk assessment is usually identified as the ability of causing human illness before performing risk assessment

(Lammerding and Fazil, 2000). The crucial element of this step is the availability of scientific information and the extent of hazard (Forsythe, 2002). Epidemiological investigations are probably the best source of information since they are based on true human populations experiencing illness in the real outbreak scenarios (Jaykus, 1996). Additionally clinical and microbiological evidences were also used to support epidemiological data (Lammerding and Fazil, 2000).

Exposure assessment

The primary aim of exposure assessment is to estimate the "likelihood" that an individual or population will be exposed to a microbial hazard, which was identified in the hazard identification step, and the "dose" of pathogen (as hazard) to which population may be exposed in food or water (Forsythe, 2002). The unit of exposure is usually a meal serving size (Lammerding and Fazil, 2000). The assessment should be in line with such a unit of exposure at the moment of consumption or a specified volume of water consumed daily (Forsythe, 2002). Overall, exposure assessment describes the entire food supply chain (from production to consumption) to which the hazard contaminates the commodity of interest e.g. food or water. Furthermore, the consumption behavior of population and factors affecting the growth and survival of pathogen in food or water e.g. temperature, pH, water activity and oxygen level should also be incorporated (Lammerding and Fazil, 2000; Forsythe, 2002).

Binomial process describes the uncertainty of some positive samples (*p*) from all samples (*n*) that has only two outcomes, positive or negative (FAO/WHO, 2003). In terms of microbiology this could refer to prevalence of pathogen where *n* is the number of samples, *p* is the probability that is found to be positive and *s* is the number of positives found. Each of the quantities *n*, *p*, *s*, can be estimated when the

other two were known. Beta distribution is often used to describe the uncertainty of a probability value (*p*) when both *n* and *s* are available (FAO/WHO, 2008). Prevalence's uncertainty decreases and then the prevalence approaches the true value when sample size increases (FAO/WHO, 2003). These relationships can be summarized mathematically in Beta(α_1,α_2) where α_1 is number of *Salmonella* spp. positive sample(s) +1 and α_2 is number of negative samples (n-s) +1 (FAO/WHO, 2008).

Pathogen concentration in the commodity usually occurs with very low number (FAO/WHO, 2003). The actual concentration cannot be measured directly so the mathematical model will use. Concentration will be evaluated from qualitative data to quantitative data by maximum likelihood estimator (MLE) and the quantal assay (FAO/WHO, 2003).

The water consumption is the volume of water in which population intake. Nowadays, one rarely drinks the water directly from the river. While, water recreational activities such as swimming, bathing, canoeing, fishing, kayaking, motor boating, and rowing but only swimming or bathing have higher chance of water ingestion (Dorevitch et al., 2011). The uncertainty of water volume ingested by people during swimming or bathing was described by probability distribution (Haas et al., 1999).

Hazard characterization

Hazard characterization, previously known as dose-response assessment, examines the probability of population "responding" to pathogen of interest at "dose" evaluated from the exposure assessment step (FAO/WHO, 2003). So, another role of the exposure assessment is to provide the relevant information for the dose-response assessment step (Buchanan et al., 2000). The response of the population

to pathogen is the interaction among host, pathogen and food matrix (Buchanan et al., 2000; FAO/WHO, 2003).

Beta-Poisson model has been used as dose-response model when pathogen in food or water was low. Beta-Poisson posses a sigmoidal dose-response relationship which population can be infected after exposure to even a single cell of pathogen (Forsythe, 2002). Dose-response model of *Salmonella* proposed by Teunis et al in 2010 from the epidemiological data in Japan was also beta-Poisson. No difference of outbreak models between serotypes and susceptible categories was found where the most frequent serotypes are *S*.Typhimurium and *S*.Enteritidis. As doses and responses were positively related, this relationship showed that response in terms of the probability of illness among infected subjects is a function of ingested doses (Teunis et al., 2010). Nuanualsuwan (2011) suggested a dose-response model for *Salmonella* spp. for Thai population with beta-Poisson model where α and β are 0.3681 and 0.01065, respectively (Nuanualsuwan, 2011).

Risk characterization

Risk characterization is the last step of the MRA that integrated exposure assessment step and dose-response assessment step to obtain the likelihood of adverse health effect to the population at risk (FAO/WHO[b], 2003).

Point-estimation, traditionally reported, represents only one single value among all possible values of a random variable. This obviously overlooks both the "viability" which captures natural diversity and the "uncertainty" which is primarily caused by the lack of knowledge in that random variable and altogether so-called "total uncertainty." In turn, the probabilistic approach for risk assessment characterizes both variability and uncertainty in the form of the probability distribution that describe the range of all possible values that could happen together

with the corresponding frequency of each value in such a range. Probabilistic risk assessments are inherently applied for quantitative risk assessment and can be estimated by the mathematically analytical techniques. Monte Carlo simulation is an analytical technique which is based on randomly selecting a single point estimate from the whole ranges of possible values of a probability distributions assigned for a random variable. This tool makes the calculation of the risk assessment model having some random variables described by probability distribution possible (Lammerding and Fazil, 2000). Therefore, the accuracy and scientific rigor of quantitative microbial food safety risk assessments has been augmented (Jaykus, 1996; Buchanan et al., 2000).

CHAPTER III
MATERIALS AND METHODS

Water sample locations

Two series of surface water samples were collected from the Chao Phraya river (Central Thailand) and the Mae Klong river (Western Thailand) as geographical water sources between June 2012- February 2013. These two rivers are finally the sources of raw water of the water treatment plants supplying tap water for Bangkok metropolitan including its vicinity.

For the Central Thailand, the surface water samples were collected from the upstream of Chao Phraya rivers (Figure 2) which are Yom river and Nan river in Phichit (Figure 3). Then the surface water samples were again collected along the downstream of Chao Phraya river running through many provinces such as Nakorn Sawan (Figure 4), Singburi (Figure 5), Angthong, Ayutthaya and Pathumthani before getting into the Bangkhen, Samsen, and Thonburi water treatment plants in Bangkok.

Figure 1: River of Thailand

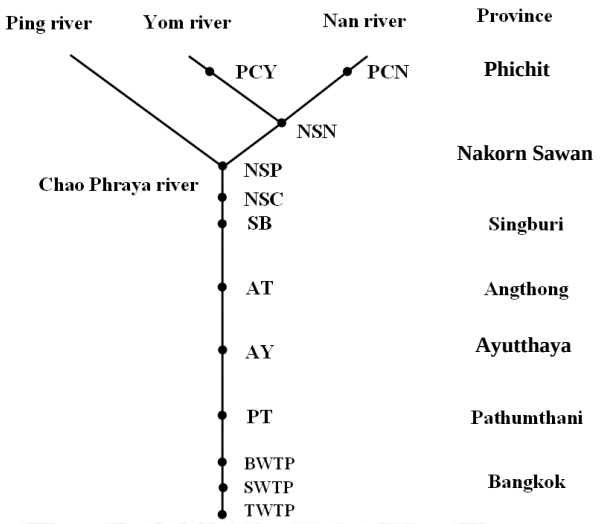

Figure 2: Sampling locations before and along the Chao Phraya river

1. PCY is Yom river in Phichit, 2. PCN is Nan river in Phichit, 3. NSN is Yom merge into nan river in Nakorn Sawan, 4.NSP is Ping river in Nakorn Sawan, 5. NSC is Chao Phraya river in Nakorn Sawan, 6. SB in Chao Phraya river in Singburi, 7. AT is Chao Phraya river in Angthong, 8. AY is Chao Phraya river in Ayutthaya, 9. PT is Pumping station in Pathum Thani, 10. BWTP is Bangkhen water treatment plant, 11. SWTP is Samsen water treatment plant and 12. TWTP is Thonburi water treatment plant

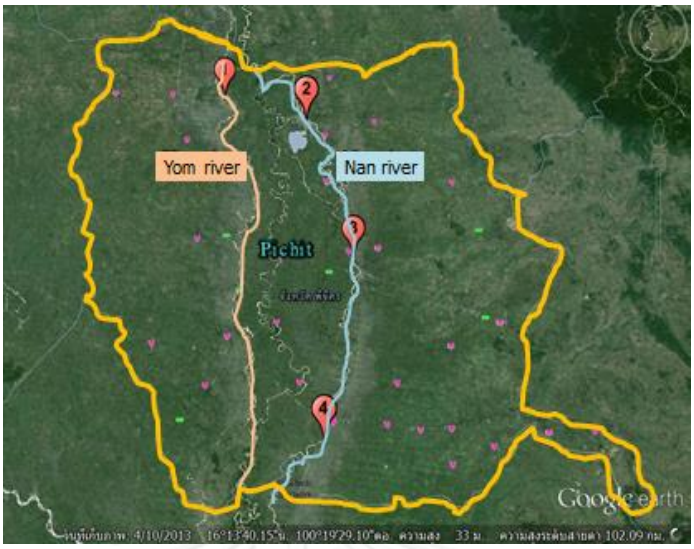

Figure 3: Sampling points in Phichit province

1. Upstream of Yom river (PC1), 2.Upstream of Nan river (PC2), 3.Midstream area in Nan river (PC3) and 4. Downstream of Nan river (PC4)

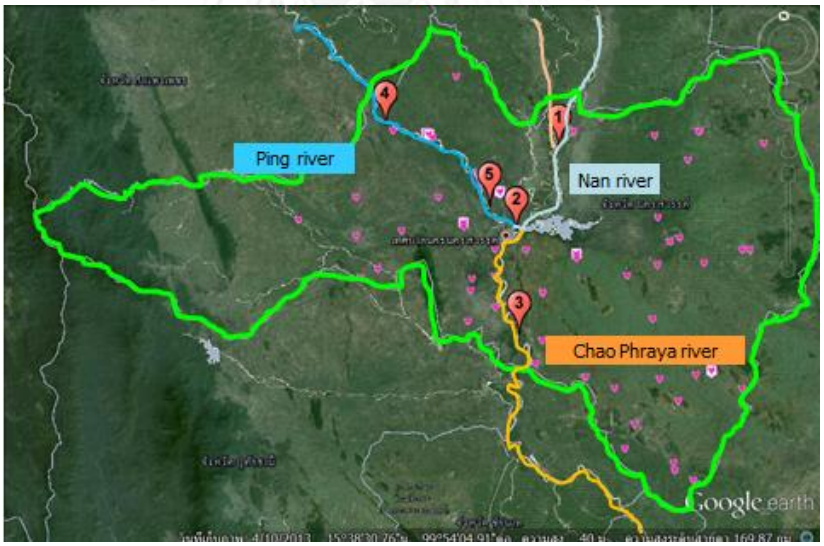

Figure 4: Sampling points in Nakorn Sawan province

1. Yom and Nan rivers merging area (NS1), 2.Nan and Ping rivers merging area (NS2), 3.Downstream in Chao Phraya river in Nakorn Sawan (NS3), 4.Upstream of Ping river (NS4) and 5.Downstream of Ping river (NS5)

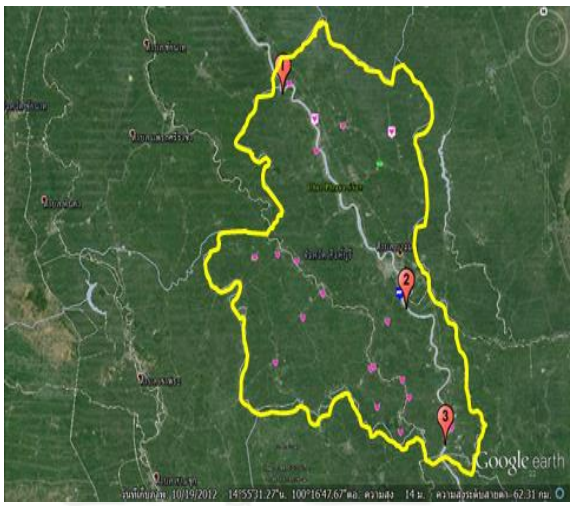

Figure 5: Sample points in Singburi province

1. Upstream of Chao Phraya river at Singburi (SB1), 2. Midstream at Chao Phraya river (SB2) and 3. Downstream of Chao Phraya river at Singburi (SB3)

Table 1: Sample location in the eastern surface water

Province	River	Location	Purpose	Code	Sample size
Phichit	Yom	Pho Thale	Upstream	PC1	10
	Nan	Muang	Upstream	PC2	10
	Nan	Muang	Midstream	PC3	10
	Nan	Muang	Downstream	PC4	10
Nakhon Sawan	Yom+ Nan	Chumsai	Upstream	NS1	10
	Nan+Ping	Paknumpo	Upstream	NS2	10
	Chao Phraya	Paknumpo	Downstream	NS3	10
	Ping	Banphot Phisai	Upstream	NS4	10
	Ping	Banphot Phisai	Downstream	NS5	10

Province	River	Location	Purpose	Code	Sample size
Singburi	Chao Phraya	In Buri	Upstream	SB1	10
	Chao Phraya	In Buri	Midstream	SB2	10
	Chao Phraya	Phrom Buri	Downstream	SB3	10
Angthong	Chao Phraya	Phamok	Upstream	AT1	10
	Chao Phraya	Chaiyo	Midstream	AT2	10
	Chao Phraya	Chaiyo	Downstream	AT3	10
Ayutthaya	Chao Phraya	Bangsai	Upstream	AY1	10
	Chao Phraya	Bangphli	Midstream	AT2	10
	Chao Phraya	Bangsai	Downstream	AY3	10
Pathum Thani	Chao Phraya	Samlae	Pumping station	PT	10
Bangkok	East canal	Samsen	Water treatment plant	SWTP	10
		Bangkhen	Water treatment plant	BWTP	10
		Thonburi	Water treatment plant	TWTP	10

For the West Thailand, 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 the Mahasawat water treatment plant in the Bangkok vicinity. (Table 2)

Table 2: Sample location in the western surface water

Province	River	Location	Purpose	Sample size
Kanchanaburi	Mae Klong	Ta muang	Upstream	10
Nakorn Pathum	Banglen by pass canal	Banglen	Downstream	10
	Tha Cheen	M.6 Hinmool	Upstream	10
	Tha Cheen	M.1 Hinmool	Midstream	10
	Tha Cheen	M.3 Banglen	Downstream	10
	Banglen by pass canal	Banglen	Upstream	10
Nonthaburi	West canal	Mahasawat	Water treatment plant	10

Water sample collection

All surface water samples were collected at least 30 centimeters below the water surface by aseptic technique to avoid the cross contaminations (Ahmed et al., 2009). For each sampling site, duplicate samples of 1,000 ml were collected and repeated 5 times for every other month (Jokinen et al., 2011). After collected, all water samples were stored below 10°C at all times to minimize *Salmonella* growth during the transportation from the sampling sites to the microbiological laboratory (Ahmed et al., 2009).

Salmonella detection

Water samples in the amount of 1,000 ml were filtered through a membrane filter having pore size 0.45 µm and diameter 47 mm. (Ahmed et al., 2009; Jokinen et al., 2011). The filters were then detected for *Salmonella* by ISO 6579 (2002) as shown in Figure 6 and the detail was described in the appendix. After applying a non-selective pre-enrichment onto the filters, a combination of two selective enrichments and plating on two selective media are performed to minimize the growth of other competing bacteria, Later the colonies resembling *Salmonella* spp. on Xylose Lysine Deoxycholate (XLD) agar were confirmed by biochemical test. ISO 6579(2002) recommended Triple Sugar Iron (TSI) and Lysine Indole Motility (LIM).

In order to identity *Salmonella* spp. contamination in the source of surface water, Kauffman-White serotyping scheme was used for serological confirmation (Grimont and Weill, 2007).

Number of *Salmonella* spp. positive sample was used in exposure assessment step to estimate prevalence of *Salmonella* spp. in surface water.

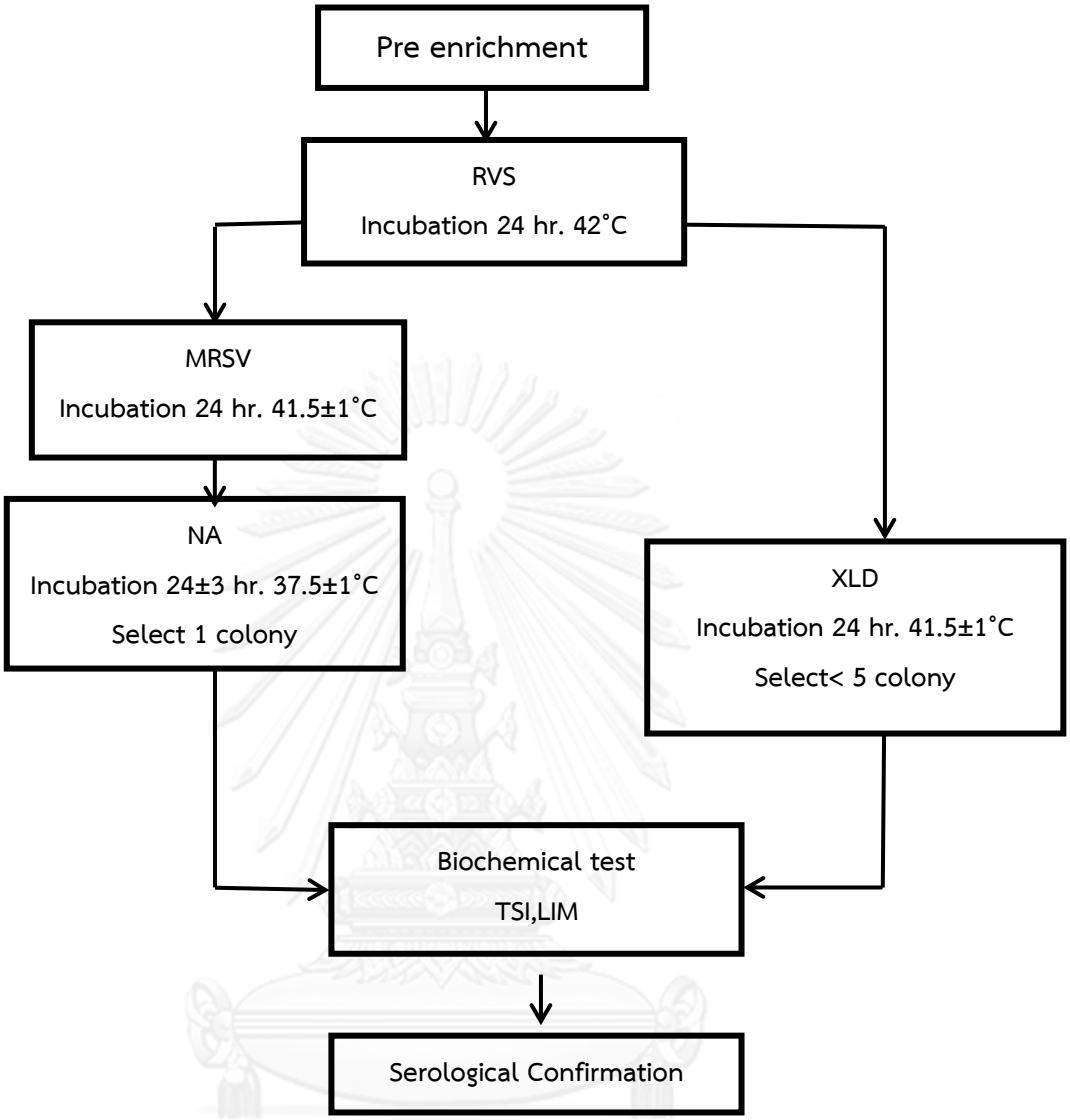

Figure 6: *Salmonella* isolation and serotype identification

Surface water exposure

Nowadays, the water exposure of population by the river bank could be mainly from swimming. It was estimated that the contact rate of surface water from swimming is 50 ml/hr, 2.6 hr/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.

Quantitative microbiological risk assessment

Exposure assessment

In order to assess the microbial exposure, the 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 the prevalence and concentration of *Salmonella* contaminated in surface water samples, respectively. However, the dose of microbial exposure was calculated by the product of both *Salmonella* concentration and surface water exposure. The conceptual model for the exposure assessment was presented in Figure 7.

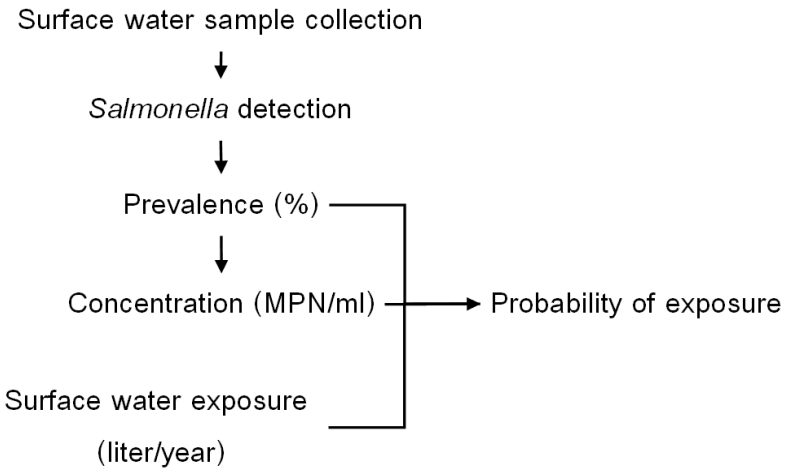

Figure 7: Conceptual model for exposure assessment

1. Prevalence variable

Bayesian inference concept is essentially about weightedly combining prior information and existing information altogether in order to better describe a variable distribution. The prior distribution provided probability distribution of a variable before any new information became available. Then the likelihood function represents the probability distribution currently 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).

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

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.

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

If the prior distribution is presumably an uninformed prior and likelihood function is Binomial distribution in Bayesian inference. Then 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). Then alpha and beta will be replaced by 1 as shown in (3).

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

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

Quantal assay

The basis of quantal assay is to determine whether a microorganism was 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 sample were inoculated into a selective broth. After incubated in an optimal condition, the selective broth is evaluated whether the microorganism was present or not (Haas et al., 1999).

A quantal assay could be set out by a series (*r*) 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. The results of presence of microorganism, after incubated, for individual sets (replicates) are then read as s_1, s_2 and s_3, respectively (Table 3).

Table 3: A quantal assay to determine the presence of a microorganism in a series of sample volumes

Set (*r*)	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

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 (L) as shown in equation (4) where μ is the mean concentration of microorganism in sample volume (Haas et al., 1999).

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

In a special case, where a series of sample volumes are the same and then the equation (4) could be differentiated with respect to μ. The concentration of microorganism (μ) in one single sample volume (V) in this study is 1000 ml with total replicates (n) and positive replicates (s) is simplified to be an equation (5).

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

Furthermore in a special case, where all samples were negative, *s* would be decreased by one significant digit from 1.0 (which is 0.9) as a lower bound (FDA, 2007). Examples of concentration calculation were shown in the appendix.

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

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

3. Probability of exposure (P_E)

Probability of exposure is the likelihood of experiencing at least one cell of *Salmonella* from water exposure. 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).

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

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 was presented in Figure8.

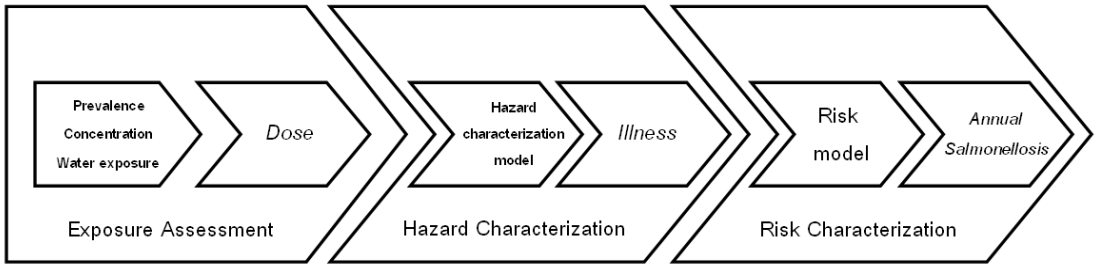

Figure 8: The conceptual model of hazard characterization

The responses can be infection, illness, sequelae, and 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 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 shape of this model as shown in (8).

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

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 4 (FAO/WHO, 2002).

Table 4: 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

Risk characterization

The essence of risk characterization is to estimate the probability of illness from *Salmonella* due to the surface water exposure. The risk characterization is a two-steps linked process where the non-zero *Salmonella* exposure had happened before the illness developed in the host. Therefore, the probability of illness (P_I) is a conditional probability where the probability of illness (P_D) was 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 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 was calculated as probability of illness from water exposure per annum (CAC, 1999).

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

Monte Carlo simulation

The models used in this study are composed of random variables while either the uncertainty or variability of these random variables is collectively described by a probability distribution within the models. Therefore, the output of the simulation model is dependent upon various possible values within the domain ranges of the probability distributions. The possible output of the model was 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 the model variables were continuous. Therefore, it is difficult or sometimes even almost impossible to manually calculate the output of the model unless using a spreadsheet software. To resolve this difficulty, the simulation software has been 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 in that iteration. In order to cover all possible range of possible outputs of the model, the iteration was repeatedly done 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.

CHAPTER IV
RESULT

Exposure assessment

Contamination frequency

As the surface water was derived from two major sources which are the Chao Phraya and the Mae Klong (Dam) rivers in the Central and West Thailand, respectively. The prevalences of *Salmonella* in the sample locations were categorized by the geography and then descendingly sorted along the provinces by two rivers. Among 290 surface water samples from rivers in the Central Thailand, the positive results were 0, 1 or 2. Therefore, the mean *Salmonella* prevalences of the surface water along rivers in the Central Thailand were 8.33%, 16.67% and 25.00%, respectively, while positive result of *Salmonella* in the surface water in the river in the West Thailand were 0, 1 and 3. So, the *Salmonella* prevalences in the West Thailand were 8.33%, 16.67 and 33.33%. Mostly *Salmonella* prevalences in midstream and downstream are higher than upstream. Except the Nan river in Phichit (PC2), Ping river in Nakorn Sawan (NS4), and Chao Phraya river in Ayutthaya (AY1) which have *Salmonella* prevalences in upstream more than midstream and downstream. The mean *Salmonella* prevalences of surface water along rivers in the Central and West Thailand were summarized in Tables 5-6 and Figure 9.

Table 5: Mean *Salmonella* prevalence and concentration in the surface water along rivers in the Central Thailand

Province	Location / Description	Code	Mean	
			Prevalence (%)	Concentration (Log MPN/ml)
Phichit	Yom river / Upstream	PC1	8.33	-4.03
	Nan river / Upstream	PC2	16.67	-3.98
	Nan river / Midstream	PC3	8.33	-4.03
	Nan river / Downstream	PC4	8.33	-4.03
Nakhon Sawan	Yom rivers merges Nan river	NS1	8.33	-4.03
	Nan river merges Ping river	NS2	16.67	-3.98
	Chao Phraya river / Downstream	NS3	8.33	-4.03
	Ping / Upstream	NS4	16.67	-3.98
	Ping / Downstream	NS5	8.33	-4.03
Singburi	Chao Phraya river / Upstream	SB1	8.33	-4.03
	Chao Phraya river / Midstream	SB2	8.33	-4.03
	Chao Phraya river / Downstream	SB3	16.67	-3.98

Province	Location / Description	Code	Mean	
			Prevalence (%)	Concentration (Log MPN/ml)
Angthong	Chao Phraya river / Upstream	AT1	8.33	-4.03
	Chao Phraya river / Midstream	AT2	16.67	-3.98
	Chao Phraya river / Downstream	AT3	16.67	-3.98
Ayutthaya	Chao Phraya river/ Upstream	AY1	25.00	-3.65
	Chao Phraya river / Midstream	AY2	8.33	-4.03
	Chao Phraya river / Downstream	AY3	8.33	-4.03
Pathumthani	Samlae untreated water pumping	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

Table 6: Mean *Salmonella* prevalence and concentration in the surface water along rivers in the West Thailand

Province	Location / Description	Code	Mean	
			Prevalence (%)	Concentration (Log MPN/ml)
Kanchanaburi	Mae Klong river	KC	8.33	-4.03
Nakorn Pathom	Banglen bypass canal / Downstream	NP1	8.33	-4.03
	Tha Cheen / Upstream	NP2	8.33	-4.03
	Tha Cheen / Midstream	NP3	33.33	-3.45
	Tha Cheen / Downstream	NP4	16.67	-3.98
	Banglen bypass canal / Upstream	NP5	8.33	-4.03
Nonthaburi	Mahasawat water treatment plant	MWTP	8.33	-4.03

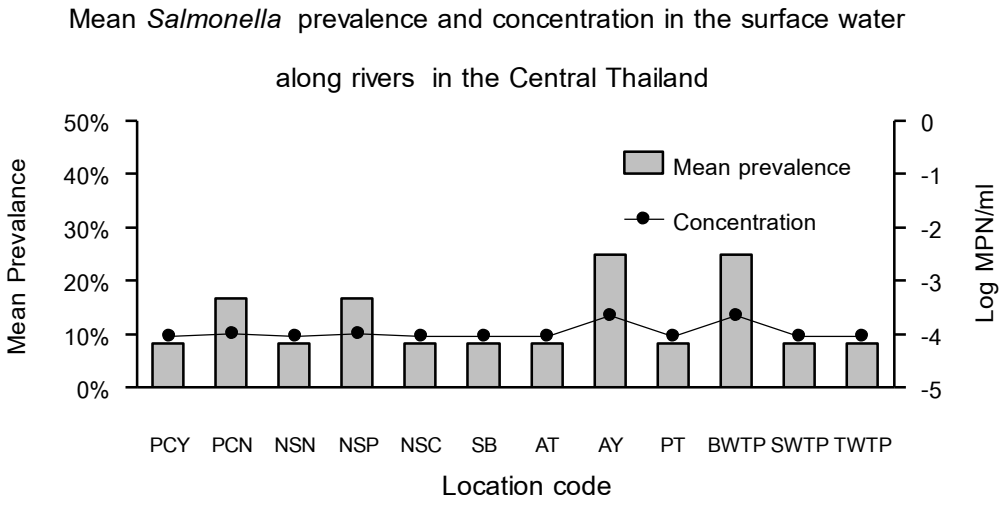

Figure 9: Mean *Salmonella* prevalence and concentration in the surface water along rivers in the Central Thailand

Contamination level

The mean concentration of *Salmonella* in the surface water was derived from the quantal assay particularly most probable number (MPN). Like contamination frequency, the concentrations of *Salmonella* in the sample locations were categorized by the geography and then descendingly sorted along the provinces by those rivers. Therefore, it is easier to compare between prevalence and concentration all along. The mean concentrations of *Salmonella* in the surface water from rivers in the Central Thailand were -4.03 and -3.98 log MPN/ml except that those of *Salmonella* in Ayutthaya province (AY1) and Bangkhen water treatment plant in Bangkok province (BWTP) were -3.65 log MPN/ml. So, it might be generally speaking that the contamination level of *Salmonella* in the upper stream of river is lower than that of *Salmonella* in the lower stream of the same river. While, the mean concentrations of *Salmonella* in the surface water from rivers in the West Thailand were -4.03, -3.98 and -3.45 log MPN/ml. Mostly *Salmonella* concentration in the surface water in upstream lower than midstream and downstream but the Nan river in Phichit (PC2), Ping river in Nakorn Sawan (NS4), and Chao Phraya river in Ayutthaya (AY1) have *Salmonella* concentration in upstream more than midstream and downstream.

Risk characterization

Risk of salmonellosis

According to the exposure assessment models, the probability of exposure is a function of both prevalence and contamination. While in this study, the concentration was inherently depending on the prevalence by means of the quantal assay. The mean prevalences in this study were among 8.33%, 16.67%, 25.00% and 33.33%. Therefore, in order to avoid the confusion of numerous locations and

samples, the risks of salmonellosis would be ranked as risk levels 1 to 4 corresponding to those 4 mean prevalences. The risk levels from the model simulation were the probability of illness among susceptible population. Then, 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 were shown in Table 7 and Figures 10-11.

Table 7: No. of salmonellosis cases among Thai populationa per year attributable to surface water exposure using the model simulation

Risk level	Min	5^{th} percentile	Mean	95^{th} 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.

Table 8: No. of salmonellosis cases among population at province that sample were collecteda per year attributable to surface water exposure using the model simulation

Risk level	Min	5th percentile	Mean	95th percentile	Max
1	0.001	0	77	358	5,138
2	0.1	3	101	403	5,681
3	1	15	257	912	5,938
4	3	40	503	1,745	12,909

[a] Population at Phichit, Nakorn Sawan, Singburi, Angthong, Ayutthaya, Pathum Thani, Kanchanaburi, Nakorn Pathom, Nonthaburi and Bangkok province 12,490,000 (DOPA, 2012)

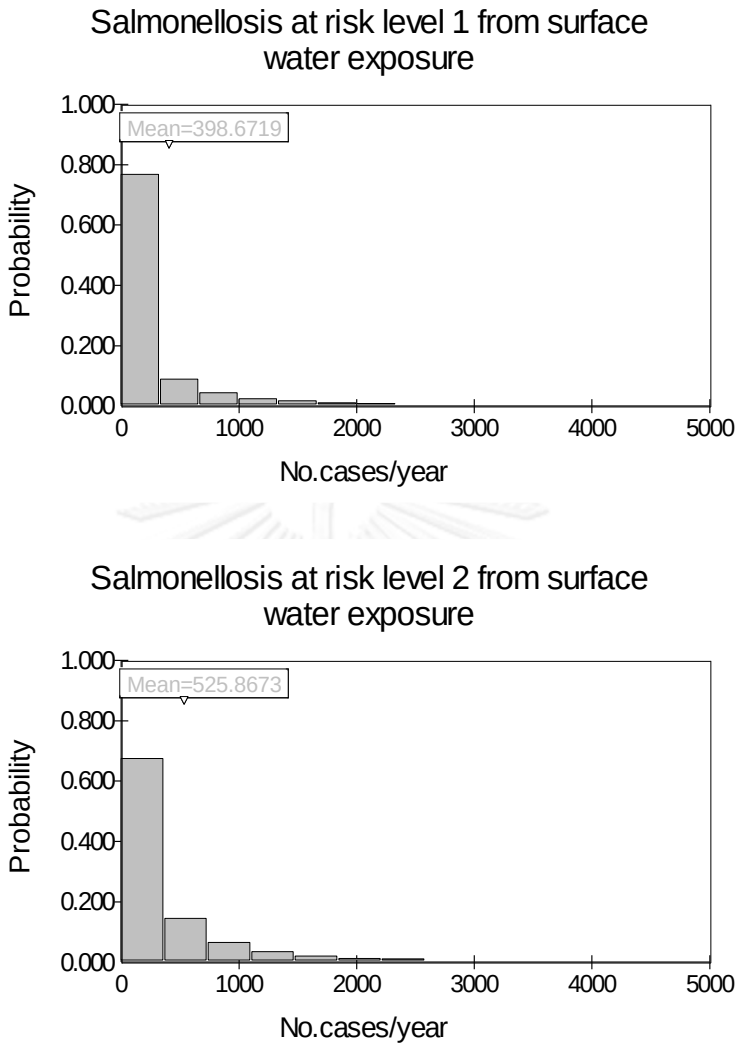

Figure 10: Distribution of salmonellosis at risk levels 1-2 from surface water exposure

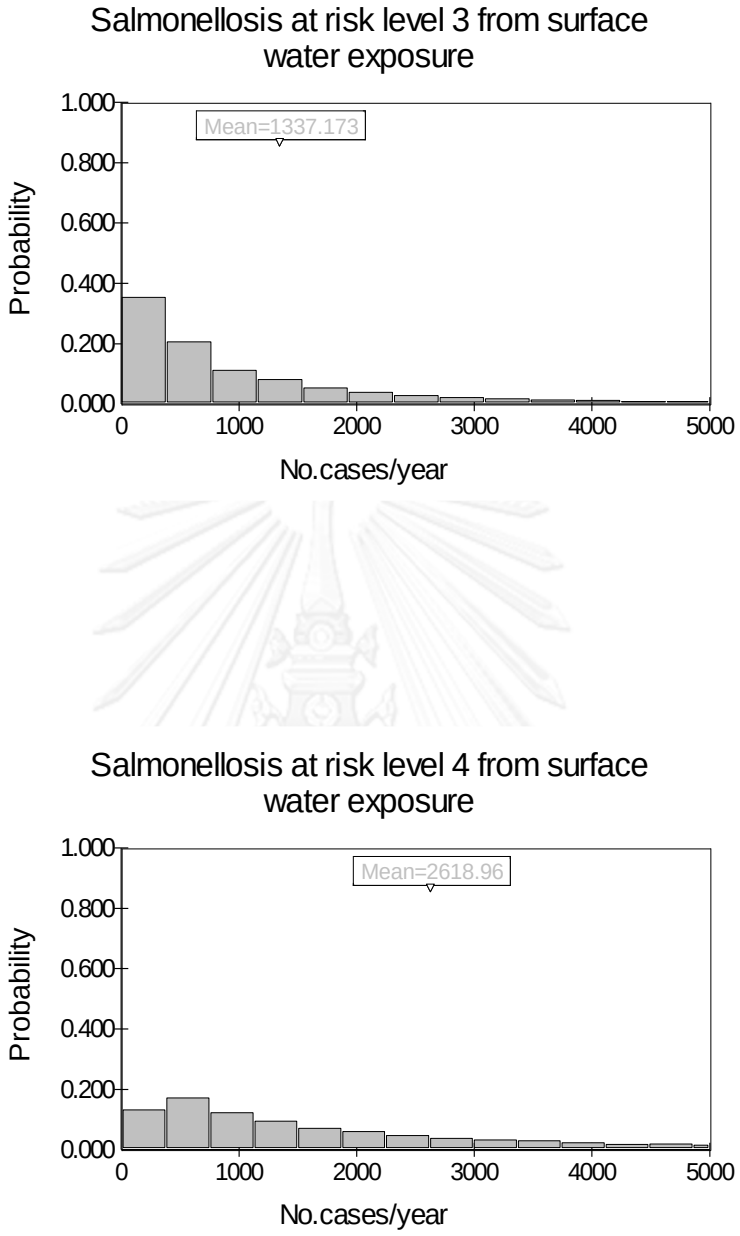

Figure 11: Distribution of salmonellosis at risk levels 3 4 from surface water exposure

Table 9: Risk level of salmonellosis in the surface water along rivers in the Central Thailand

Province	Location / Description	Code	Risk level
Phichit	Yom river / Upstream	PC1	1
	Nan river / Upstream	PC2	2
	Nan river / Midstream	PC3	1
	Nan river / Downstream	PC4	1
Nakhon Sawan	Yom rivers merges Nan river	NS1	1
	Nan river merges Ping river	NS2	2
	Chao Phraya river / Downstream	NS3	1
	Ping / Upstream	NS4	2
	Ping / Downstream	NS5	1
Singburi	Chao Phraya river / Upstream	SB1	1
	Chao Phraya river / Midstream	SB2	1
	Chao Phraya river / Downstream	SB3	2
Angthong	Chao Phraya river / Upstream	AT1	1
	Chao Phraya river / Midstream	AT2	2
	Chao Phraya river / Downstream	AT3	2
Ayutthaya	Chao Phraya river / Upstream	AY1	3
	Chao Phraya river / Midstream	AY2	1
	Chao Phraya river / Downstream	AY3	1
Pathumthani	Samlae untreated water pumping	PT	1

Province	Location / Description	Code	Risk level
Bangkok	Bangkhen water treatment plant	BWTP	3
	Samsen water treatment plant	SWTP	1
	Thonburi water treatment plant	TWTP	1

Table 10: Risk level of salmonellosis in the surface water along rivers in the West Thailand

Province	Location / Description	Code	Risk level
Kanchanaburi	Mae Klong river	KC	1
Nakorn Pathom	Banglen bypass canal / Upstream	NP1	1
	Tha Cheen / Upstream	NP2	1
	Tha Cheen / Midstream	NP3	4
	Tha Cheen / Downstream	NP4	2
	Banglen bypass canal / Downstream	NP5	1
Nonthaburi	Mahasawat water treatment plant	MWTP	1

The risk levels of the surface water were almost constant from upstream of Chao Phraya river to the downstream in front of the water treatment plant 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, mean salmonellosis in Nan river (PC1) and Nan river merging Ping river (NS2) was 127 cases/year lower than background mean salmonellosis. Furthermore, mean salmonellosis in Ayutthaya (AY1), Bangkhen water treatment

plant (BWTP) and Nakhon Pathom (NP3) were 938 cases/year lower than background mean salmonellosis.

Table 11: *Salmonella* serovars isolated from surface water in Central and West Thailand sorted from upstream to downstream.

Province	Location / Description	Code	Sampling round	Serovariety
Phichit	Nan river / Upstream	PC2	3	*S*. enterica subsp. enterica ser. 4,5,12:i:-
Nakorn Sawan	Nan river merges Ping river	NS2	5	*S*. Virchow (6,7:r:1,2)
	Ping / Upstream	NS4	3	*S*. Rissen (6,7:fg:-)
Singburi	Chao Phraya river / Downstream	SB3	2	*S*. Thompson (6,7:k:1,5)
Angthong	Chao Phraya river / Midstream	AT2	4	*S*. Agona (4,12:f,g,s:-)
	Chao Phraya river / Downstream	AT3	4	*S*. Give (3,10:lv:1,7)
Ayutthaya	Chao Phraya river/ Upstream	AY1[a]	4	*S*. Weltevreden (3,10:r:z_6)
		AY1[a]	4	*S*. Weltevreden (3,10:r:z_6)
Bangkok	Bangkhen water treatment plant	BWTP	2	*S*. Weltevreden (3,10:r:z_6)
		BWTP	2	*S*. enterica subsp. diarizonae ser 60:z_{52}:z_{53}

Province	Location / Description	Code	Sampling round	Serovariety
Nakorn Pathom	Tha Cheen / Midstream	NP3	1	*S*. enterica subsp. enterica ser. 9,12:-:1,5
		NP3	2	*S*. enterica subsp. houtenae ser 43:z_4z_{23}:-
		NP3	4	*S*. Hadar (6,8:z_{10}:e,n,x)
	Tha Cheen / Downstream	NP4	3	*S*. Corvallis (8,20:z4z23:-)
	Banglen bypass canal / Downstream	NP5	4	*S*. Hvittingfoss (16:b:e,n,x)

[a] Duplicate sample

CHAPTER V
DISCUSSION

The range of mean prevalence of Salmonella in surface water along the rivers in the Central and West Thailand was from 8.33% to 33.33%. These data were 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 the 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 was to determine the Salmonella contamination in the surface water as a tap water comprehensively from the upstream to the downstream of the rivers otherwise, the sample size of individual sampling locations could have been larger than this. The sample size played an important role to particularly the mean prevalence described by beta distribution chiefly when sample size was small. As the parameter of beta distribution was determined directly by both the positive sample and the corresponding sample size. This effect is usually less pronounced while the sample size is getting larger as shown in Table 12. Therefore, in order to address the true mean prevalence of Salmonella in the surface water in 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.

Table 12: The effect of sample size on the 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

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

From Tables 5-6, *Salmonella* prevalence and corresponding concentration in the surface waters taken from the upstream of rivers were less

fluctuating than those in the surface water from more downstream of rivers of Thailand. Partly this could be explained by the population density. The higher dense population would have a higher likelihood of activities by the riverbank and incidentally shed the pathogen into the surface water source. This finding agreed with a previous report where *Salmonella* prevalence in the upstream was about 2.6% (1/39) which is lower than that in the downstream at 20.1% (8/39) of the same river (Hendricks 1971). *Salmonella* prevalences and concentrations in the surface water in the river of Thailand in upstream are less than in midstream and downstream because inhabitants by the river bank make use of the surface water from the river for many purposes such as a drinking water source, a wastewater drainage, a recreation water. The micro-organisms from the communities in the upstream maybe runoff through downstream (Levantesi et al., 2012). In the other hand, both *Salmonella* prevalences and concentrations in upstream were higher than those in midstream and downstream in Nan river in Phichit province (PC2), Ping river in Nakorn Sawan province (NS4), and in Chao Phraya river in Ayutthaya province (AY1). Therefore, in order to justify this unusual finding, aside from increasing the sample size, one might consider tracking the source of *Salmonella* contamination of these three sampling locations.

Infected animals as a carrier has a certain degree of consequences against public health. For the fact that *Salmonella*-contaminated manure may lead to the contamination of surface water. So, the untreated manure slurry might flow directly into the ponds or canal system and lastly reach to the river thus resulting in the pollution of water source for inhabitants and livestock (Angulo et al., 1997; Taylor et al., 2000). In addition, the untreated sewages or wastewaters from the communities, industrial or agricultural areas are the other sources of *Salmonella*

contamination to the water in the environment (Waage et al., 1999; Johnson et al., 2003; Sahlstrom et al., 2006).

As the low *Salmonella* concentration in water, *Salmonella* concentration was calculated from equation 5 that generally used in food. If high volume,Equation 5 can apply for the water. From that equation when high volume, the concentration will decrease whereas, low volume the concentration will increase. According to the result of *Salmonella* serovars in Table 11, the majority of serovar in this study was non-typhoidal *Salmonella*. But the most frequency serotype which found in the river or fresh water were *Salmonella* Virchow (Polo et al.,1999). No evidence indicated that *Salmonella* serovar in surface water from upstream are same as that from downstream. *Salmonella* serovars in upstream was not correlated with *Salmonella* serovar in the midstream and downstream of the same province.

Salmonella Weltevreden was identified in the upstream of Chao Phraya river in Ayutthaya province (AY1) and again identified from surface water in front of the Bangkhen water treatment plant (BWTP). It is tempting to conclude that *S*. Weltevreden in Bangkok has been directly derived long distance from Ayutthaya province. But, *S*. Weltevreden in both provinces were in different round of sampling with time interval of 4 months. So, it could be loosely indicated that source of contamination of *Salmonella* in Bangkok and Ayutthaya had release the same serovar of *Salmonella*. However, one could also track the common source of *Salmonella* from both provinces by using DNA finger printing molecular techniques e.g. pulse field gel electrophoresis to differentiate the DNA patterns of *S*. Weltevreden from both provinces. If the DNA patterns were different then one might be more confident to conclude that *S*. Weltevreden isolated from Bangkok might not be derived from Ayutthaya. In contrary, if the DNA patterns were identical then risk

management options in the Ayutthaya province would justify to eliminate *Salmonella* contamination from upstream in Ayutthaya province to be effective along the Chao Phraya river down to Bangkok province.

Salmonella has been repeatedly isolated from Tha Cheen river in Nakorn Pathom province. This result is not surprising since Nakorn Pathom has been known for huge swine production. Since *Salmonella* in the surface water e.g. river or watershed system has been associated with manure possibly from animal farms and this case is swine farms (Levantesi et al., 2012). The same *Salmonella* spp. isolated from both human and also from sewage sludge indicates a possible spread of *Salmonella* to the water in the environment (Sahlstrom et al., 2006). Therefore, it is more intuitive to expect that surface water passing through nearby livestock farm areas would have higher *Salmonella* contamination.

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 then even the very low level of cases would have been an integer.

In order to compare Salmonellosis cases per year in Thailand with Bureau of epidemiology, 65 million Thailand populations were used as population at risk. According to Bureau of epidemiology, The secondary foodborne pathogen is *Salmonella* spp. (41.47%) (BOE, 2012).

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 would be 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; EPA, 1994). Taking this as an acceptable level of risk for 65 million Thai populations, around 6,500 salmonellosis should have been caused by the water exposure in this study. After comparing mean of 4 risk levels in this study (Table 7) with the acceptable level of risk, 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 lower than acceptable cases. This seemed to be 2 extreme scenarios of risk comparison. Then 95^{th} percentile might be a good candidate since risk levels 1, 2 and 3 passed the acceptable cases. While 95^{th} percentile of cases of salmonellosis of risk level 4 (9,079) was higher than the acceptable cases. Therefore, one might opt for 95^{th} 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, 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. But the population at risk is the population in the province where water were collected (12,450,000), Salmonellosis

cases per year in all of risk level lower than the salmonellosis cases per year in Thai population.

In conclusion, this study has provided essential information regarding the health risk levels derived from surface water exposure and demonstrated 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 readily used to prepare either risk mitigation measures for long-term risk management plan or emergency plan in case of waterborne disease outbreaks. For the future studies, some other waterborne disease pathogens such as *Campylobacter*, *Shigella*, Norovirus, Hepatitis A virus, *Cryptosporidium*, *Giardia* or *Entamoeba histolytica* are recommended to investigate.

==========

REFERENCES

Ahmed W, Sawant S, Huygens F, Goonetilleke A and Gardner T 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. Water Res. 43(19): 4918-4928.

Angulo FJ, Tippen S, Sharp DJ, Payne BJ, Collier C, Hill JE, Barrett TJ, Clark RM, Geldreich EE, Donnell HD and Swerdlow DL 1997. A Community Waterborne Outbreak of Salmonellosis and the Effectiveness of a Boil Water Order. American Journal of Public Health. 87(4): 580-584.

Arvanitidou M, Papa A, Constantinidis TC, Danielides V and Katsouyannopoulos V 1997. The occurrence of *Listeria* spp. and *Salmonella* spp. in surface waters. Microbiol Res. 152(4): 395-397.

Benjamin L, Atwill ER, Jay-Russell M, Cooley M, Carychao D, Gorski L and Mandrell RE 2013. Occurrence of generic *Escherichia coli*, *E*. coli O157 and *Salmonella* spp. in water and sediment from leafy green produce farms and streams on the Central California coast. Int J Food Microbiol. 165(1): 65-76.

Best DJ and Rayner JCW 1985. A Comparison of the MPN and Fisher-Yates Estimators for the Density of Organisms. Biometrical Journal. 27(2): 167-172.

Buchanan RL, Smith JL and Long W 2000. Microbial risk assessment: dose-response relations and risk characterization. Int J Food Microbiol. 58(3): 159-172.

BOE 2012. Foodborne and waterborne disease. Annual Epidemiological Surveillance Report." [Online]. Available: http://www.boe.moph.go.th/Annual/AESR2012/index.html

CAC 1999. Principles and guidelines for the conduct of microbiological risk assessment. Vol. 30. In: CAC, Geneva, Switzerland.

CDC 2011. *Salmonella* surveillance Overview. 1-12.

Chiu CH, Su LH and Chu C 2004. *Salmonella enterica* Serotypes Cholerasuis: Epidemiology, Pathogenesis, Clinical disease and treatment. Clin. Microbiol. Rev. 17(2): 311-322.

Clark RM, Geldreich EE, Fox KR, Rice EW, Johnson CH, Goodrich JA, Barnick JA, Abdesaken F, Hill JE and Angulo FJ 1996. A waterborne *Salmonella* typhimurium outbreak in Gideon, Missouri: Results from a field investigation. International Journal of Environmental Health Research. 6(3).

Covello VT and Merkhofer MW 1993. Risk assessment methods : Approaches for assessing health and environmental risks In: Plenum Press, The United States of America.

Craun MF, Craun GF, Calderon RL and Beach MJ 2006. Waterborne outbreaks reported in the United States. J Water Health. 4(2): 19-30.

DOPA 2011. "Thailand population." [Online]. Available: http://stat.bora.dopa.go.th/stat/

Dorevitch S, Panthi S, Huang Y, Li H, Michalek AM, Pratap P, Wroblewski M, Liu L, Scheff PA and Li A 2011. Water ingestion during water recreation. Water Res. 45(5): 2020-2028.

EFSA 2011. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA journal. 9(3): 1-378.

EPA 1994. Health Effects Support Document for Acanthamoeba. In: EPA, The United States of America.

Falco R and William SI 2009. Salmonella outbrek in Alamosa, Colorado, March and April 2008. Colorado Department of Health and the Enviroment. 4-9.

FAO/WHO 2002. Risk assessments of *Salmonella* in eggs and broiler chickens In: Microbiological Risk Assessment. FAO/WHO, Geneva. Switzerland.

FAO/WHO 2003. Hazard characterization for pathogens in food and water: Guideline. In: Microbiological Risk Assessment. FAO/WHO Geneva. Switzerland. 27-39.

FAO/WHO 2008. Exposure assessment of microbiological hazards in food. In: Microbial Risk Asessment. FAO/WHO, Geneva, Switzerland. 1-32.

FDA 2007. Guide for the control of Mollusc shellfish In: FDA, The United State of America.

Forsythe SY 2002. The microbiological risk assessment of food. 111 ed. In: Blackwell science Ltd, The United Kingdom.

Geng S, Campbell RN, Carter M and Hills FJ 1983. Quality-control program of seedborr. Plant disease. 67(2).

Grimont PAD and Weill FX 2007. Presents of the WKL scheme symbols. In: Antigenic formulae of the Salmonella serovars. 9ed. WHO collaborating center for reference and research on Salmonella (ed). Paris: Pasteur institue.

Haas CN, Rose JB and Gerba CP 1999. Quantitative microbial risk assessment. In: John Wiley & Sons, Inc. , The United States of America.

Haldane JBS 1939. Sampling errors in the determination of bacterial or virus density by the dilution method. J Hyg (Lond). 39(3): 289-293.

Hendricks CW 1971. Increased Recovery Rate of Salmonellae from Stream Bottom Sediments Versus Surface Waters. APPL. MICROBIOL. 21(2): 379-380.

Hendriksen RS 2003. Laboratory Protocol level 1: Training course isolation of *Salmonella.* A global *Salmonella* surveillance and laboratory support project of the World Health Organization. WHO Geneva. Switzerland.

Henriette AB, Ebiane NM, Olive NE, Blandine TK, Elvis TR, Christel MJ, Moïse N and Thomas N 2012. Occurrence of *Salmonella* spp. in surface waters of Yaoundé, Cameroon. JESWR. 1(10): 243-250.

Hlavsa MC, Roberts VA, Anderson AR, Hill VR, Kahler AM, Orr M, Garrison LE, Hicks LA, Newton A, Hilborn ED, Wade TJ, Beach MJ and Yoder JS 2011. Surveillance for waterborne disease outbreaks and other health events associated with recreational water --- United States, 2007--2008. MMWR Surveill Summ. 60(12): 1-32.

ISO 2002. ISO 6579 Microbiology of Food and Animal Feeding Stuffs—Horizontal method for detection of Salmonella spp. In: ISO, Geneva. Switzerland.

Jaykus LA 1996. The application of quantitative risk assessment to microbial food safety risks. Crit Rev Microbiol. 22(4): 279-293.

Johnson JY, Thomas JE, Graham TA, Townshend I, Byrne J, Selinger LB and Gannon VP 2003. Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. Can J Microbiol. 49(5): 326-335.

Jokinen C, Edge TA, Ho S, Koning W, Laing C, Mauro W, Medeiros D, Miller J, Robertson W, Taboada E, Thomas JE, Topp E, Ziebell K and Gannon VP 2011. Molecular subtypes of *Campylobacter* spp., *Salmonella enterica*, and *Escherichia coli* O157:H7 isolated from faecal and surface water samples in the Oldman River watershed, Alberta, Canada. Water Res. 45(3): 1247-1257.

Knight IT, Shults S, Kaspar CW and Colwell RR 1990. Direct detection of *Salmonella* spp. in estuaries by using a DNA probe. Appl Environ Microbiol. 56(4): 1059-1066.

Laine J, Huovinen E, Virtanen MJ, Snellman M, Lumio J, Ruutu P, Kujansuu E, Vuento R, Pitkanen T, Miettinen I, Herrala J, Lepisto O, Antonen J, Helenius J, Hanninen ML, Maunula L, Mustonen J and Kuusi M 2010. An extensive gastroenteritis outbreak after drinking-water contamination by sewage effluent, Finland. Epidemiol Infect. 1-9.

Lammerding AM and Fazil A 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. Int J Food Microbiol. 58(3): 147-157.

Levantesi C, Bonadonna L, Briancesco R, Grohmann E, Toze S and Tandoi V 2012. *Salmonella* in surface and drinking water: Occurrence and water-mediated transmission. Food Research International 45: 587-602.

Melloul A, Hassani L and Rafouk L 2011. *Salmonella* contamination of vegetable irrigated with untreated water. World Journal of Microbiology and Biotechnology. 17: 207-209.

NCSS 2008. Annual report of confirmed *Salmonella* and *Shigella* in Thailand 2008. 1-28.

Ndiaye M, Dieng Y, Niang S, Pfeifer H, Tonolla M and Peduzzi R 2011. Effect of irrigation water on the incidence of *Salmonella* spp. on lettuces produced by urban agriculture and sold on the markets in Dakar, Senegal. Afr. J. Microbiol. Res. 5(19): 2885-2890.

Nuanualsuwan S 2011. Third progress report under research project “Hazard characterization of *Salmonella* spp. and Vibrio parahaemolyticus” supported by National Science and Technology Development Agency (NSTDA).

Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC and Maguire D 2002. Enterobacteriaceae, In: Veterinary microbiology and microbial disease. In: Blackwell science Ltd, Cornwall, United Kingdom. 113-123.

Regli S, Rose JB, Haas CN and Gerba CP 1991. Modeling the risk from Giardia and viruses in drinking water. J. Amer. Water Works Assoc. 83: 76-84.

Sahlstrom L, de Jong B and Aspan A 2006. Salmonella isolated in sewage sludge traced back to human cases of salmonellosis. Lett Appl Microbiol. 43(1): 46-52.

Sanchez S, Hofacre CL, Lee MD, Maurer JJ and Doyle MP 2002. Animal sources of Salmonellosis in human. Vet Med today: Zoonosis update. 221(4): 492-497.

Taylor R, Sloan D, Cooper T, Morton B and Hunter I 2000. A waterborne outbreak of Salmonella Saintpaul. Commun Dis Intell. 24(11): 336-340.

Teunis PFM, Kasuga F, Fazil A, Ogden LD, Rotariu O and Strachan NJC 2010. Dose-response modeling of Salmonella using outbreak data. Int J Food Microbiol. 144(2): 243-249.

Waage AS, Vardund T, Lund V and Kapperud G 1999. Detection of low numbers of Salmonella in environmental water, sewage and food samples by a nested polymerase chain reaction assay. J Appl Microbiol. 87(3): 418-428.

WHO 2011. Guidelines for drinking-water quality. In: WHO, Geneva. Switzerland.

Winfield MD and Groisman EA 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli.* Appl Environ Microbiol. 69(7): 3687-3694.

APPENDIX

APPENDIX A

Detection of Salmonella spp. by ISO 6579 (2002)

1. Pre-enrichment in non-selective medium : The filters were enriched in 225 ml. of Buffered Peptone Water (BPW) at 37°C for 24 hr.

2. Enrichment in selective medium : Sample of 0.1 ml from BPW was transferred to 10 ml. of Rappaport Vasiliadis Soy (RVS) broth at 42°C for 24 hr.

3. Streak 10 µl loop full from RVS to selective media: Xylose Lysine Deoxycholate (XLD) and Modified Semi-Solid Rappaport-Vassiliadis Agar (MSRV).

3.1 The XLD plate: A typical Salmonella colony has a slightly transparent zone of reddish colour and a black centre, a pink-red zone may be seen in the media surrounding the colonies.

3.2 The MSRV plate: The presence of an opaque halo centered on the point of inoculation is a presumption for Salmonella. Subcultures can be prepared by removing a fraction of culture from the outer edge of the halo to confirm purity and conduct additional biochemical and serological tests.

4. Biochemical test: Triple Sugar Iron (TSI) and Lysine Indole Motility (LIM) at 37°C for 24 hr.

4.1 Triple Suger Iron

Procedure:

- A sterilized straight inoculation needle was touched the top of a well-isolated colony
- Stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant.
- Incubated with caps loosen at 35°C and examine after 18-24 hours

Results:

- If lactose (or sucrose) was fermented, a large amount of acid was produced, which turned the phenol red indicator both in butt and in the slant. Some organisms generated gases, which produced bubbles/cracks on the medium.
- If lactose was not fermented but the small amount of glucose is, the oxygen deficient butt was yellow (remember that butt comparatively have more

glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) was oxidized to carbondioxide and water by the organism and the slant will be red (alkaline or Neutral pH).

- If neither lactose/sucrose nor glucose was fermented, both the butt and the slant was red. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids.
- If H2S was produced, the black color of ferrous sulfide was seen.
- Salmonella: Slant was Alkaline, Butt was acid and Produced H2S

4.2 Lysine Indole Motility

When inoculated with an organism that ferments dextrose, acids are produced that lower the pH, causing the indicator in the medium to change from purple to yellow. The acidic pH also stimulates decarboxylase enzyme activity. Organisms that possess a specific decarboxylase degrade the amino acid provided in the medium, yielding a corresponding amine. Lysine decarboxylation yields cadaverine. The production of these amines elevates the pH and causes the medium in the bottom portion of the tube to revert to a purple color. The medium in the upper portion of the tube remains acidic because of the higher oxygen tension. If the organism being tested does not produce the required decarboxylase, the medium remains yellow (acidic) throughout or yellow with a purple or red reaction near the top. Lysine deamination produces a colour change in the upper portion of the medium. Oxidative deamination of lysine yields a compound that reacts with ferric ammonium citrate, producing a burgundy red or red-brown color in the top centimeter of the medium (the bottom portion of the medium remains acidic). This reaction can only be detected if lysine decarboxylase is not produced, which is the case with Proteus, Morganella and Providencia species. Indole is produced in this medium by organisms that possess the enzyme tryptophanase. Tryptophanase degrades typtophan present in the casein peptone, yielding indole. It can be detected in the medium by adding Kovacs reagent to the agar surface. Indole combines with the p-imethylaminobenzaldehyde of Kovacs reagent and produces a red complex. Cultures are stab-inoculated and incubated at 37°C for 18-24 hours. Motility, lysine deamination and lysine decarboxylation reactions are read before testing indole reaction, since addition of Kovacs reagent causes the colour of the medium to change to yellow. Therefore, positive lysine decarboxylase reaction could be misinterpreted as negative

Salmonella is motility positive No indole production, Lysine deaminase negative, Lysine decarboxylase positive (Purple colour)

7. Serology test by Salmonella antiserum

All strains identified as Salmonella were serotyped according to the Kauffman White serotyping scheme (Grimont et al., 2007). Salmonella antisera (S & A Reagent Laboratory LMT, Bangkok, Thailand) were used for serotype identification.

Sample colonies from TSI slant were placed on the glass slide around 1 ml by straight wire then a drop of antiserum was added on to slide, mixed and rocked for about 1 minute. The agglutination will be generally expected for Salmonella.

Appendix B

Salmonella concentration calculation example

Example 1: The 2 water samples that have volume each 500 ml and 1 positive bacteria sample. What are the bacterial concentration in the water sample?

From equation (5)

V = Volume, 500 ml

n= Total sample, 2 samples

s= Positive sample, 1 sample

$\mu = -1/500 \ln \{(2-1)/2)\}$

$\mu = 0.001386 \text{ ml}^{-1}$

$= 1.386 \times 10\text{-}3 \text{ ml}^{-1} = -2.86 \log \text{ml}^{-1}$

Example 2: The 2 water samples that have volume each 2000 ml and 1 positive bacteria sample. What are the bacterial concentration in the water sample?

From equation (5)

V = Volume, 2000 ml

n= Total sample, 2 samples

s= Positive sample, 1 sample

$\mu = -1/2000 \ln \{(2-1)/2)\}$

$\mu = 0.00003466$ ml-1

$= 3.466 \times 10\text{-}5 \text{ ml}^{-1} = -3.46 \log \text{ml}^{-1}$

VITA

Ms. Paweeranut Banmairuroy was born on November 26th, 1987 in Suphanburi province, Thailand. She has been graduated with Bachelor Degree of Veterinary Science (D.V.M.) in academic year 2011 from the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. After that she became a graduate student in Veterinary Public Health Program, Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University during academic years 2011-2013. She has been a research assistant (RA) for the "Quantitative microbial risk assessment (QMRA) of water in the flood area of the Central Thailand" and "Risk analysis for water Quality" projects both supported by Thailand Research Fund (TRF : RDG5520019 and RDG5620033). After graduating in Spring 2014, she is expecting to work with a poultry production company of Charoen Pokaphan (CP) group.