Quantitative Risk Assessment of *Salmonella* spp. in Fermented Pork Sausage (Nham)

Sukhuntha Osiriphun¹, Adisak Pongpoolponsak² and Kooranee Tuitemwong³

ABSTRACT

The Quantitative Microbial Risk Assessment (QMRA) of *Salmonella* spp. in fermented pork sausage, Nham, was carried out. It was to evaluate the public health impact of one of the favorite local foods on Thai people from exposure to *Salmonella* spp. The QMRA employed secondary data available from published papers and articles from government agencies in Thailand. *Salmonella* spp. was the major cause of gastroenteritis in Thailand. The most frequently found strains are *S. Enteritidis* and *S. Typhimurium*. It was found that probability of *Salmonella* contaminated in Nham was 0.186. The probability of *Salmonella* prevalence in Nham was 0.035. The average value of *Salmonella* found in Nham ranged 105 to 195 CFU/25g. However, the amount of *Salmonella* in raw pork was 205-250 CFU/25g with the probability of 0.045. It appeared that the number of *Salmonella* decreased when the raw pork was processed to Nham which has the low pH of 4.5. The maximum contamination level of *Salmonella* in Nham was 20% with the range of 10-40%. Nham, stored at 4°C for 14 days, had *Salmonella* increased to 10 to 10⁵/piece or 1 to 500 CFU/g. The risk of becoming ill due to the consumption of Nham was 1.9% if one consumes Nham with *Salmonella* of 120-170 CFU/25g. It is recommended to cook Nham before consumption to reduce the risk.

**Key words:** QMRA/ risk assessment/ *Salmonella* spp./fermented pork sausage/ Nham

INTRODUCTION

In 1995, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) assembled an expert drafting committee, Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standard Issues. The relationship of risk analysis, assessment, management and communication is displayed in Figure 1 (Lammerding, 1997). Risk assessment has been used in several major food commodities and pathogens such as *Salmonella* in shell eggs and *Campylobacter* in broiler chicken meat. Evaluating the microbial safety of the food typically requires consideration of multiple factors that influence the prevalence and numbers of a microbial pathogen in the product (Lammerding et al., 1999). As a tool for strategic decision making, the scope of an assessment should be incorporated such activities that support relevant information

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for the risk manager. The approach was taken to develop “from farm to fork to illness” risk assessment for *Salmonella* in locally produced fermented pork sausage or Nham which deems important to Thai people who usually consume it uncooked.

More than 16.4 million slaughtered pigs have been produced in 2001. The pork production has been expanding over the year both in the size and numbers of the farms (Agriculture Information Center, 2002). Traditional fermented pork products or Nham has been popular among consumers in Thailand although originally it was a mere food in the North of the country. However, the product is usually consumed raw, and occasionally with a minimal heat treatment. The raw meat was frozen to destroy parasites before processing. Lactic acid fermentation was employed to control bacterial pathogens from growing and produce toxins. In this regards, *Salmonella* manage to survive the fermentation and cause adverse health effects to consumers (Chalermchaikit, 2001). The contamination rates of the organisms were high during the processes in slaughter house and distribution. Dressing and transportation are also contributing to the problems.

Food safety risk analysis is still a relatively new discipline, and the concept of food safety risk analysis is still in its infancy (Commission of the European Communities, 2000). One approach is the development of a quantitative risk model in combination with Monte Carlo simulation (Vose, 1998). The basic elements of quantitative microbial risk assessment are hazard identification, exposure assessment, hazard characterization or dose response assessment and risk characterization (Figure 1). In this study, we attempted to estimate risk of getting *Salmonella* from consuming locally fermented pork sausage or Nham. This was to evaluate the public health impact on the one of the favorite local foods in Thailand. The model for the outcome of a statistical distribution of risk is developed based on the risk of *Salmonella* in pork and pork sausage. The main goal of this study was to evaluate the relationship between the factors that affect the presence and behavior of *Salmonella* and the probability of human illness.

**Figure 1** Schematic representation of relationships between risk assessment, risk management, and risk communication (http://compepid.tusk.edu/riskanalysis/course/module2.htm)
MATERIALS AND METHODS

1. Review and collect epidemiology data related to the outbreaks of *Salmonella* spp. in Thailand. Risk groups were categorized as small children under 5, elderly over 60, and immunocompromised individuals such as AIDS patients. The rest was considered low-risk group.

2. Review and collect epidemiology data related to the outbreaks of *Salmonella* spp. that caused by Nham.

3. The risk assessment was conducted as follows: hazard identification, exposure assessment, dose response, and risk characterization (FAO 2002). Exposure assessment of *Salmonella* spp. in pig farm, feed, transportation, slaughter house, and pork fermentation were performed using exponential growth model, Exponential dose-response model, Poisson and Beta distribution models, and Beta-Poisson dose-response models using data available from local literatures for simulation on @RISK4.5 (Palisade Co., Newfield, NY.) with Monte Carlo simulation for 10,000 iterations. Sensitivity analysis employed step characteristic equation and Spearman rank test (Table 1).

4. Distribution and estimates of risk and uncertainties were simulated by models (Table 1) and using @RISK4.5 with Monte Carlo simulation for 10,000 iterations.

**Models for simulations**

They were often considered to be appropriate for the explanation of each circumstance by other workers (Whiting and Buchanan, 2001). The data used for simulations

<table>
<thead>
<tr>
<th>Process step</th>
<th>Parameters</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential Dose - response Model</td>
<td>Probability of illness calculated from number of infected population (P)</td>
<td>( P = 1 - e^{-RN} )</td>
</tr>
<tr>
<td>Poisson Distribution</td>
<td>Probability of receiving <em>Salmonella</em> spp. at least 1 CFU/g (R)</td>
<td>( R = - \left[ \ln \left( 1 - P \right) \right] / N )</td>
</tr>
<tr>
<td>Exponential Dose - response Model</td>
<td>Probability of illness simulated from animal study data (P)</td>
<td>( P = 1 - e^{-rD} )</td>
</tr>
<tr>
<td>Beta –Poisson Dose - response Model</td>
<td>Probability of illness simulated from prevalence of <em>Salmonella</em> in Nham (P)</td>
<td>( P = 1 - (1 + \lambda / \beta)^{-\alpha} )</td>
</tr>
<tr>
<td>Risk Characterization</td>
<td>Risk of consumers of eating contaminated Nham (P)</td>
<td>( P = \sum_{i=1}^{\infty} p(x) * I(x) )</td>
</tr>
<tr>
<td>Sensitivity test</td>
<td>Step Characteristic (SC)</td>
<td>( (SC) = (\mu_t k_i) / \ln 10 )</td>
</tr>
</tbody>
</table>

Table 1 Models and parameters for the simulation of distributions and assessment of risk of *Salmonella* spp. in Nham (FAO, 2002; Vose, 1998)

**Figure 2** Nham traditionally produced and wrapped tightly to create microaerophilic conditions with banana leaves. Some manufacturers use plastic tubes to replace the leaf.
were collected from hospitals, literatures and personal communications with government agencies. The notations, specific values, and the clarifications of these terminologies were described and used frequently elsewhere (Whiting and Buchanan, 2001; Schlundt, 1999).

The key assumption in the model is that the dose-response relationship for foodborne human *Salmonella* infection fits the exponential dose-response model. This model relates the number of cells of a biological agent consumed with the probability of an adverse effect in the consuming population (Buchanan *et al.*, 1997). The probability of illness calculated from the number of infected population. The equation for this probability was

$$P = 1 - e^{-RN}$$  \hspace{1cm} (1)

where \(P\) is the probability of an adverse effect, \(N\) is the number of biological agent consumed (CFU), and \(R\) is a constant specific to each pathogen that helps define the shape of dose-response curve. This model and the closely related Beta-Poisson model were effectively used to describe dose-response relationship for a number of food-borne and waterborne infection agents (Buchanan *et al.*, 1997). Concepts of purposefully conservative dose-response relationship are, *Salmonella* dose-response relation can be described by the exponential model. Mathematically, this is a simple transformation of equation 1 (Buchanan *et al.*, 1997). The Poisson distribution, probability of receiving *Salmonella* spp. at least 1 cfu/g, was shown in (2) (Buchanan *et al.*, 1997).

$$R = -\frac{\ln(1 - P)}{N}$$  \hspace{1cm} (2)

Originally, dose-response relations were largely described using single value estimates of biological endpoints. For example, the simple technique of Reed and Muench (1938) has been used for over 60 years to estimate LD\(_{50}\) values, a mean describing the relationship between levels of pathogenic microorganisms and the frequency of mortality (Buchanan *et al.*, 2000). More recently a number of non-threshold mathematical models have been used to describe the entire sigmoidal dose-response curve. Using curve fitting software, it is relatively easy to take experimental data and fit it to one or more of these models. However, it is important to note that all of the models are empirical and cannot be used to infer the underlying physiological basis for pathogenicity. Two of the more widely used models for fitting dose-response data are the exponential and beta-Poisson models that were initially introduced by Haas (1983) (Buchanan *et al.*, 2000). The Exponential Dose -response Model; Probability of illness simulated from animal study data was

$$P = 1 - e^{-rD}$$  \hspace{1cm} (3)

where: \(P=\)probability of infection at dose \((d)\) (CFU), \(r=\)model parameter specific for each pathogen. For the Beta –Poisson dose - response Model; probability of illness was simulated from prevalence of *Salmonella* in Nham. The equation for this probability is as follow.

$$P = 1-(1+\lambda/\beta)^{-\alpha}$$  \hspace{1cm} (4)

The model has been used by several investigators to describe dose-response relations for a number of different classes of biological agents, including extrapolating to the ingestion of low levels similar to what would be expected in food and water (Buchanan *et al.*, 1997, 2000). The exponential model assumes that the probability of a cell causing infection is independent to dose, whereas the beta-Poisson assumes that infectivity is dose dependent. Both equations are non-threshold sigmoidal functions. The non-threshold character of the equations is more evident when the probability of a response is transformed to log values so that log dose versus log response plots are used instead of the more traditional log dose
versus response plots.

The final stage in a microbial food safety risk assessment is the development of the risk characterization. Risk characterization is the integration of the exposure and dose-response assessments to provide an overall evaluation of the likelihood that the population will suffer adverse effects as a result of the hazard. Mathematically, the exposure assessment serves as the input for the dose-response assessment, which, when ‘solved’, provides the risk estimate (i.e. probability of an adverse effect).

Risks also depend upon the depth of salmonellae penetration in to Nham. If products were made from pork contaminated with Salmonella spp., Risk Characterization, the expected risk of consumers to become ill caused by eating contaminated Nham, is obtained from equation (5) (Buchanan et al., 2000).

\[ P = p \sum_{i=1}^{\infty} p(x) * I(x) \quad (5) \]

Where \( P \) = proportion of sample infected, \( I(x) \) = percentage of people infected by Salmonella spp. n CFU, \( p(x) \) = probability of Salmonella spp. in Nham.

The sensitivity analysis is a method used to examine the behavior of a model by measuring the variation in its outputs resulting from changes to its inputs. It employed Step Characteristic as shown in (6): where \( \mu_k \) = specific growth rate at 32°C = 0.63 and \( t_k \) = production time (h), which indicated that Salmonella decreased in numbers during fermentation period

\[ (SC) = (\mu_k t_k)/\ln10 \quad (6) \]

**RESULTS AND DISCUSSION**

Nham is the popular food product to Thai people. It can be consumed either uncooked or partially or fully cooked. The production is mostly at small scale, such as cottage industry, to small industry. Thai FDA declared GMP as a mandatory program for industries covering 57 food groups, including meat products, effective by July 24, 2003. Contamination of pathogens such as Staphylococcus aureus and Salmonella spp. are possible, especially Salmonella Anatum, which is the most prevalent in Nham product. This serovar is tolerable to lactic acid produced during the fermentation process (Swetwiwathana et al., 1994).

Hazard identification: Salmonella spp. are Gram negative, short rod intracellular foodborne pathogens. They are ubiquitous and are commonly found in poultry and pork meats. The disease, salmonellosis, is divided into 3 groups according to the pathogenicity. They are diarrhea, bacteremia, and enteric fever. The transmission is mainly through oral. The main cause of exposure to the pathogens was the consumption of contaminated foods and water. The illness is apparent after the ingestion of contaminated food with Salmonella of about 10^4 to 10^6 cells (Swetwiwata et al., 1994, 1997; Bangtrakulnonda, 2002). The organisms passed from stomach to intestine where they multiply. They can also survive and grow in M cells and, later, destroy the M cells before they proceed to glands and polymorphous phagocytic cells.

The relationship of Salmonella spp. and time of growth in Nham using square root growth model (Figure 3).

The probability of Salmonella spp. contamination in sample \( (p) \) employed beta distribution as described by Vose (1998)

\[ p = Beta (\alpha, \beta) \quad (7) \]

where \( \alpha = (\text{the number of contaminated sample} +1) \) and \( \beta = (\text{total number of sample} - \text{number of positive sample contaminated} +1) \).

In this study, the selected growth models were those reported by Whiting and Buchanan (1994). The models covered 3 levels; primary, secondary, and tertiary growth models.
The exponential growth model was used as the primary model as described by Gerwen et al. (2000) in (8).

\[
\log \frac{N_t}{N_0} = \frac{\mu t}{2.303}
\]

(8)

where \(N_t\) and \(N_0\) were numbers of \(Salmonella\) spp. in (CFU/g) at time \(t\) and time \(0\), \(\mu\) is specific growth rate of \(Salmonella\) spp. at the process temperature.

The Secondary Growth Model employed a square root Gamma in (9) and CTPM (the Cardinal Temperature and pH Model) in (10) (Rosso et al., 1995).

\[
\mu = \mu_{opt} \gamma(T) \gamma(pH) \gamma(a_w)
\]

(9)

where \(\gamma(T) = \left( \frac{T-T_{min}}{T_{opt}-T_{min}} \right)\)

\[
\mu = \frac{\mu_{opt}[(T-T_{max})(T-T_{min})^2]}{(T_{opt}-T_{min})(T_{opt}-T_{min})(T-T_{opt}) - (T_{opt}-T_{max})(T_{opt}+T_{min}-2T)}
\]

(10)

where

\(\mu_{max}\) = The maximum of specific growth rate at optimal growth temperature (37°C = 1.65), \(T\) is Temperature (min, optimum, or max).

From equation 2, \(T_{swine} = 40°C\), \(T_{opt} = 37°C\), \(T_{max} = 46.2°C\), and \(T_{min} = 5.2°C\). \(\mu_{max}\) could be calculated using the equation from Delignette-Muller et al. (2000) (11)

\[
\mu = \frac{\mu_{max}[T-T_{max})(T-T_{min})^2]}{(T_{opt}-T_{min})(T_{opt}-T_{min})(T-T_{opt}) - (T_{opt}-T_{max})(T_{opt}+T_{min}-2T)}
\]

(11)

The number of \(Salmonella\) spp. in pork reaches its maximum at about 225 CFU/25g with the probability of 0.035 and with cell concentrations range from 105 to 190 CFU/25g (Figure 4).

Levels of contamination of \(Salmonella\) in Nham (Figure 5) has the maximum level of 25 CFU with probability 0.185. The distribution exhibited as Binomial distribution characteristics.

**Exposure assessment**

The consumption rates of Nham by Thai consumers were 0.1 to 0.4 g/week (Figure 6). The binomial distribution (mean 100, SD 0.0162) showed that probability of Thai people of getting ill, caused by \(Salmonella\) in Nham, was 0-6 persons/
Figure 4  Prevalence of *Salmonella* spp. in raw pork simulated by binomial distribution model (mean of 331 and SD of 0.69).

Figure 5  Levels of *Salmonella* spp. in Nham using Binomial distribution model (mean of 800 and SD of 0.186).

100 capita with the probability of getting illness (per 100 capita) of 0.32 (Figure 7).

Dose response model simulated from animal model, estimated cases of illness of Thai people, and contamination of *Salmonella* in Nham data.
Thai people. The risk group referred to children under 5 and elderly over 60 years old as well as the immuno-compromised individuals.

Figures 9 and 10 displayed simulations of exponential and Beta-Poisson distribution models with Monte Carlo simulation of 10,000 iterations using animal model and levels of contamination of *Salmonella* in Nham reported in local literatures. Figure 9 indicated that the exponential model predicted a smaller number of *Salmonella* ($10^5$CFU) in the foods that cause illness ($P=1.0$). The Beta-Poisson distribution indicated $10^{10}$ that has the probability of causing illness at 1.0. The exponential model appeared to fit better with the reported dose response reported by Brooks *et al.* (2001). However, the differences were not evident with those simulated from contamination data in Figure 10. The numbers of *Salmonella* that cause

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**Figure 6** Probability of the consumption of Nham up to 1 serving size/week using consumption data.

**Figure 7** Probability of illness of Thai population (100 capita) caused by *Salmonella* spp. in Nham using binomial distribution model in @ RISK program with Monte Carlo simulation of 10,000 iteration.
Figure 8  Beta-Poission dose response curves for *Salmonella* spp. in Nham simulated from estimated illness of risk and normal populations.

Table 2  Numbers of illness of Thai people caused by salmonellosis (Bangtrakulnonth, 1994).

<table>
<thead>
<tr>
<th>Types of salmonellosis</th>
<th>Number of affected persons (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>789,168.06</td>
</tr>
<tr>
<td>Dysentery</td>
<td>136.42</td>
</tr>
<tr>
<td>Food poisoning</td>
<td>2,531.45</td>
</tr>
<tr>
<td>Enteric fever</td>
<td>124.48</td>
</tr>
<tr>
<td>Pyrexia (unknown cause of origin)</td>
<td>232,172.33</td>
</tr>
<tr>
<td>Total</td>
<td>1,024,132.74</td>
</tr>
</tbody>
</table>

Figure 9  Dose response relationship between probability of illness and log numbers of *Salmonella* spp. using exponential and Beta Poisson distribution models.
illness in consumers (P=1.0) were in the range of 15-17 CFU. It could be seen that if the contamination data were used (Figure 10), the numbers that caused illness was very low compared to those used other sources of data. This is clearly indicated that the contamination data could not describe the occurrence of illness in human effectively. In other words, this suggested differ tolerance levels of *Salmonella* of Thai people. The numbers were very much lower than that predicted by using the number of illness population (Figure 8). This finding also indicated that after fermentation the numbers of *Salmonella* were decreased. This could be the results of low pH or the production of lactic acid after the Nham was being fermented. Swethiwatana and Lothong (1997) reported that number of *Salmonella* Anatum (100CFU/g) reduced to 8% after day 5 of the Nham fermentation. The survivors, about 62%, appeared to be in the injured state. Cooking or partially cooking, in support to the low pH of Nham, would effectively reduce and prevent *Salmonella* from infecting Thai people.

**Risk characterization and uncertainty**

The maximum probability of risk estimate of illness due to the consumption of Nham contaminated with *Salmonella* was at 1.9% or 0.019 (Figure 11). From data published by Swetwiwathana *et al.* (1994), the numbers of *Salmonella* declined with fermentation time of Nham (Figure 12). If Nham was stored at

![Figure 10](image)

**Figure 10** Exponential and Beta-Poisson distributions of *Salmonella* spp. in Nham using contamination data of the organisms on Nham. The two models suggested similar results.

![Figure 11](image)

**Figure 11** Risk estimate of illness caused by *Salmonella* spp. The maximum risk of 0.019 was observed at about 170 cells of *Salmonella*. 
refrigeration temperature at 4°C for 14 days (with temperature varied from 1 to 7), the numbers of Salmonella could survive (Figure 15).

Sensitivity analysis

The sensitivity analysis is a method used to examine the behavior of a model by measuring the variation in its outputs resulting from changes to its inputs. It employed Step Characteristic \[ \text{(SC)} = (\mu_k t_k) / \ln 10 \] where \( \mu_k \) = specific growth rate at 32°C = 0.63/h and \( t_k \) = production time (h)] which indicated that Salmonella decreased in numbers during fermentation period (Table 3). The number decreased from about log 4 in day 1 to about log 2 in day 7, the last day of its fermentation. Generally the Nham were stored at room temperature but most of them that sold in supermarkets were displayed at refrigeration temperature (8 to 10°C).

Uncertainty of Salmonella spp. in Nham stored in a refrigerator

Uncertainties analysis is a method used to estimate the uncertainty associated with model inputs, assumptions and structure and/or form. The prevalence of Salmonella spp. in Nham varied. The maximum likelihood of detecting Salmonella in Nham was at 20% (Figures 13 and 14). The range of detecting Salmonella in Nham was 10 to 40%. Salmonella in Nham ranged from 1 to 33 CFU. At the likelihood of detecting Salmonella from 10-40% the numbers of Salmonella at

Table 3 Relationships of risk of fermentation process that could lead to the growth/contamination of Salmonella in Nham.

<table>
<thead>
<tr>
<th>Fermentation time (day)</th>
<th>pH</th>
<th>SC</th>
<th>N (Log CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>-</td>
<td>3.8</td>
</tr>
<tr>
<td>1</td>
<td>5.5</td>
<td>-</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>6.56</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>4.3</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>4.1</td>
<td>-</td>
<td>2.1</td>
</tr>
</tbody>
</table>

SC=step characteristics
maximum were 6, 9, 13, and 18 CFU, respectively. The numbers of *Salmonella* in Nham also varied during the storage at a refrigeration temperature. The refrigerator temperatures were about 1 to 7°C but the reported growth temperature was 6-10°C. Therefore, the numbers of *Salmonella* in Nham at 4°C for 14 days would be 1-500 CFU/g. The numbers of *Salmonella* were 10 to 105 CFU/piece or 1 to 500 CFU/g. *Salmonella* could be at maximum of 100 CFU/piece or 0.2 CFU/g.

**Figure 13** The maximum probability of Nham of being contaminated by *Salmonella* at 20 CFU was 0.8.

**Figure 14** Probability of *Salmonella* contamination in Nham at 10-40% (from left to right) respectively.

**Figure 15** Numbers of *Salmonella* in Nham stored at 4°C for 1 to 5 day. The number appeared to decrease with storage time (data from Swetwiwathana *et al.*, 1994).
CONCLUSIONS

This study suggested that the probability of Salmonella spp. presenting in Nham was 0.186. The probability of Salmonella prevalence in Nham was 0.035. Average value of Salmonella found in Nham was from 105 to 195 CFU/25g. However, the value for raw pork was 205-250 CFU/25g with the probability 0.045. It appeared that the number decreased when the meat was processed to Nham which has low pH, 4.5. The risk probability of Nham was 1.9% with the range of cells from 120 to 170 CFU/25g. The maximum contamination level of Salmonella in Nham was 20% with the range 10-40%. Nham, stored at 4°C for 14 days, had Salmonella of 10-10⁴ /piece or 1-500 CFU/g. The maximum risk of getting Salmonella in Nham was 10² CFU/piece or 0.2 CFU/g.

LITERATURE CITED


