

Exposure assessment and process sensitivity analysis of the contamination of *Campylobacter* in poultry products

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ABSTRACT Studies were conducted in a Thai poultry plant to identify the factors that affected numbers of *Campylobacter jejuni* in chicken carcasses. The concentrations of *Campylobacter* were determined using the SimPlate most probable number and modified charcoal cefoperazone deoxycholate plating methods. Results indicated that the mean concentrations of *C. jejuni* in carcasses after scalding, plucking, and chilling were 2.93 ± 0.31 , 2.98 ± 0.38 , 2.88 ± 0.31 , and 0.85 ± 0.95 log cfu, whereas the concentrations of *C. jejuni* in the scalding tank water, plucked feathers, and chicken breast portion were 1.39 ± 0.70 , 3.28 ± 0.52 , and 0.50 ± 1.22 log cfu, respectively. Sensitivity analysis using tornado order correlation analysis showed that risk parameters affecting the contamination of *C. jejuni* in the chicken slaughter and processing plant could be ranked as chilling water pH, number of pathogens in the scald

tank water, scalding water temperature, number of *C. jejuni* on plucked feathers, and residual chlorine in the chill water, respectively. The exposure assessment and analysis of process parameters indicated that some of the current critical control points were not effective. The suggested interventions included preventing fecal contamination during transportation; increasing the scalding temperature, giving the scalding water a higher countercurrent flow rate; reducing contamination of feathers in the scalding tank to decrease *C. jejuni* in the scalding water; spraying water to reduce contamination at the plucking step; monitoring and maintaining the chill water pH at 6.0 to 6.5; and increasing the residual chlorine in the chill water. These interventions were recommended for inclusion in the hazard analysis and critical control point plan of the plant.

Key words: broiler slaughterhouse, *Campylobacter jejuni*, chicken meat product, exposure assessment, probabilistic risk assessment modeling

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INTRODUCTION

Campylobacteriosis is one severe form of enteric disease in humans that occurs worldwide (Buncic, 2006). Many scientific data show that the source of *Campylobacter* infection in humans in developed and developing countries is associated with the consumption of chicken contaminated with these pathogens because of unhygienic conditions, improper handling, and improper cooking (Oyarzabal et al., 2004, 2005; ACMSF, 2005). *Campylobacter* has been isolated from 55.25% of the chilled chicken meat and 49.63% of the frozen chicken meat in markets (Kanarat et al., 2004; Padungtod et

al., 2008). Strategic targets for the reduction of *Campylobacter* in poultry production have been established worldwide, including in the United States, United Kingdom, and European countries such as Norway and Sweden, as well as in others (ACMSF, 2008; Lindqvist and Lindblad, 2008). The main objective of these strategic targets is to reduce the number of cases of human *Campylobacter* infection.

The hazard analysis and critical control point (HACCP) guidelines and other food safety management systems focus on the safety and wholesomeness of food products. The identification of critical control points (CCP) relies on risk assessment to understand the fate of pathogens during the production, storage, and sale of food. To this end, the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) have developed a risk assessment framework for *Campylobacter* spp. in broiler chickens (FAO/WHO, 2001a). It is hoped that

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quantitative risk assessment methods will become increasingly useful for the control of *Campylobacter* in chicken meat products to systematically identify the proper control steps in the areas of poultry production with more specific prevalence and concentration data. The quantitative risk assessment model has been used to assess the effects of interventions to control *Campylobacter* and to reduce the incidence of campylobacteriosis in the Netherlands (Nauta et al., 2005).

With respect to quantitative microbial risk assessment in Thailand, the Agricultural Commodity and Food Standards (ACFS) agency first announced the Principles and Guidelines for the Conduct of Microbiological Risk Assessment in 2007 (ACFS, 2007), which was quoted from FAO/WHO guidelines (FAO/WHO, 2001a). The ACFS also provided common guidelines of Good Manufacturing Practice (GMP) for poultry abattoirs in Thailand (ACFS, 2006). These guidelines cover 3 aspects of the process: 1) receipt of live poultry at the slaughterhouse; 2) the stunning and killing, scalding, defeathering, evisceration, and inside-outside washing steps with a high risk of carcass contamination; and 3) the chilling, portioning, weighing, and packaging steps with a lower risk of carcass contamination.

Although the GMP and HACCP are being implemented in Thailand and elsewhere, incidences of pathogen contamination of raw chickens still exist (Saengthongpinit et al., 2005; Vindigni et al., 2007; Iamtaweejaloen et al., 2009; Kooprasertying et al., 2009; Osiriphun, 2009). Therefore, process analysis at the slaughterhouse level to verify that an adequate HACCP program is being used is very important. The present work focused on assessment of the exposure to *C. jejuni* and analysis of the process sensitivity of broiler chicken processing in Thailand. The quantitative microbial risk assessment work was performed to collect data on the prevalence and concentrations of *C. jejuni* at each processing step in a Thai poultry plant and to incorporate these data into an exposure assessment to better understand the critical process steps that affect the contamination of poultry products with *Campylobacter*. The outputs from the assessment were used to evaluate the existing food safety management systems, GMP, and HACCP, and to identify proper intervention at potential problematic steps in the process.

MATERIALS AND METHODS

Sample Collection and Analysis

Samples were collected from a commercial chicken slaughter and processing plant in Samutprakarn Province, Thailand. The processing steps at the selected plant are as follows. After slaughter and blood draining, the chickens enter a continuous countercurrent scalding chamber, which is followed by plucking, evisceration, and washing the exterior and interior of carcasses with

chlorinated water. After that, the chicken carcasses enter a continuous countercurrent chiller of cold aqueous hypochlorite solution (less than 2°C and approximately 1.5 ppm of residual chlorine). The mean residence time of the chickens in the multitank chiller was approximately 58 to 60 min.

Twenty samples of carcasses rinse after each step (scalding, plucking, washing, and chilling; a total of 80 samples) were randomly collected to determine the amount of *C. jejuni* present, using the carcass rinse techniques described by the USDA Food Safety and Inspection Service (USDA/FSIS, 2009) and NACMCF (2007). Briefly, the carcass was put into a plastic bag (30 × 60 cm) and 100 mL of Butterfield's phosphate buffer was added. The carcass was rinsed inside and outside, using a rocking motion, for 1 min. This was done by grasping the carcass in the bag with one hand and holding the closed top of the bag with the other hand; the carcass was then removed. The remaining fluid was kept in an icebox and then sent to the laboratory for analysis of *C. jejuni*. The carcass wash solution represented a 10⁰ dilution (USDA/FSIS 2009). Twenty samples of chicken meat products (20 breasts, 20 legs, 20 thighs, and 20 wings), and 20 samples of cooked product (karaage, or restructured fried chicken) were collected to determine the amount of *C. jejuni*. At scalding and chilling, approximately 500 mL of water was collected every 5 min until 20 samples were obtained, and the samples were stored in sterile plastic bags. All samples were placed in an insulated cooler filled with flaked ice and brought to the laboratory for analysis of *C. jejuni*. The enumeration of *C. jejuni* was made by plating samples on modified charcoal cefoperazone deoxycholate (mCCDA) and subjecting them to a SimPlate most probable number (MPN) device, as described below (NACMCF, 2007; USDA/FSIS, 2009). These data were used for model development and simulation.

On a different day, 60 carcass rinses of samples were collected after the scalding and chilling steps, and the amount of *C. jejuni* was examined in the same manner. Fifteen samples of chicken meat products (5 breasts, 5 wings, and 5 thighs) and 15 samples of a cooked meat product (steamed chicken breast) were collected to determine the concentrations of *C. jejuni*. These data were used in the process of model validation.

Enumeration with Modified CCDA Plating Method

For the carcass rinses, scald water, and chill water, serial dilutions of the samples were made in PBS, and four 0.25-mL diluents were plated onto the surface of mCCDA plates (Oxoid, Hampshire, UK) (NACMCF, 2007) and spread with a sterile glass "hockey stick." Plates were incubated at 42°C for 48 h in a microaerobic environment (5% O₂, 10% CO₂, and 85% N₂). Col-

ony-forming units with the characteristics of *Campylobacter* were counted, and 10% of colonies (but not more than 10 colonies/plate) were picked for biochemical confirmation as described by Bohaychuk et al. (2009) and FSIS/USDA (2009). This included microscopic examination of the typical corkscrew motility that exhibited spiral rod morphology. Growth was observed under microaerobic conditions at 42°C. Catalase and oxidase production were also analyzed. Identification to the species level could be done by testing for the hydrolysis of hippurate and indoxyl acetate. *Campylobacter jejuni* exhibited positive results with both hydrolysis tests (*Campylobacter coli* and *Campylobacter lari* are hippurate hydrolysis negative; OIE, 2008). The resulting percentage after being adjusted with the confirmation ratio (percentage of positive from the confirmation) was used to determine the actual counts of *C. jejuni* from the plates. For the fresh products and cooked chicken meat products, 25 g of each sample was mixed with 225 mL of phosphate buffer. Ten-fold serial dilutions were made, and the samples were analyzed for *C. jejuni* by mCCDA plating as described above.

Enumeration by MPN Method for *Campylobacter*

The SimPlate method for *C. jejuni* was also used for the enumeration of *C. jejuni* and other *Campylobacter* (BioControl Systems, 2010). The culture medium (1 package) was dissolved in 100 mL of sterile distilled water and distributed in sterile test tubes (9.0 mL/tube). One milliliter of the sample or the dilution was added to each tube and the tube was transferred to the SimPlate device. The plates were incubated in the dark for 48 to 52 h at 42°C under microaerobic conditions. The red wells were presumed positive for *C. jejuni*. This procedure involved counting the number of red wells that did not fluoresce blue by holding them up to a UV light (366 nm wavelength) approximately 5 cm (2 in.) above the SimPlate device to confirm the quantification of *Campylobacter*. The MPN of colony-forming

units was determined using the chart supplied by the manufacturer.

The colonies grown on mCCDA plates originating from negative and positive wells when using the Simplate method for *Campylobacter* were picked up for *C. jejuni* identification. The isolates recovered were then confirmed as *C. jejuni* by biochemical tests, as mentioned above. The results obtained from mCCDA and SimPlate MPN were compared and selected for use in further calculation and model development.

Analysis of Process Parameters

To analyze the process parameters, the temperatures (°C) of the scald water and the chill water were monitored intermittently (every 5 min) by using an infrared thermometer (model DP-88, Digicon, Kukot, Thailand) and continuously by using a thermometer data logger (CMC 281, Ellab A/S, Copenhagen, Denmark). The pH of the chill water samples was also analyzed (every 5 min) by using a waterproof portable pH meter (HI 98128, Hanna Instruments, Woonsocket, RI). The free chlorine concentration of the chill water was measured (every 5 min) by using a colorimetric comparison technique (semiquantitative method; Lovibond Checkit Comparater Tintometer, Tintometer Ltd., Salisbury, UK). This test for residual chlorine is commonly used in poultry slaughterhouses in Thailand.

Data Analysis and Probability Distribution

The probabilistic risk assessment (quantitative model) was created to describe the propagation of *C. jejuni* through the various stages of the chicken slaughterhouse and processing plant, and stochastic models of *C. jejuni* contamination were developed at each step. Regression analysis was carried out to estimate parameter values and uncertainty. The schematic diagram of model construction is shown in Figure 1.

The experimental data [*C. jejuni* on chicken meat (log cfu), *C. jejuni* in water samples (log cfu), and pro-

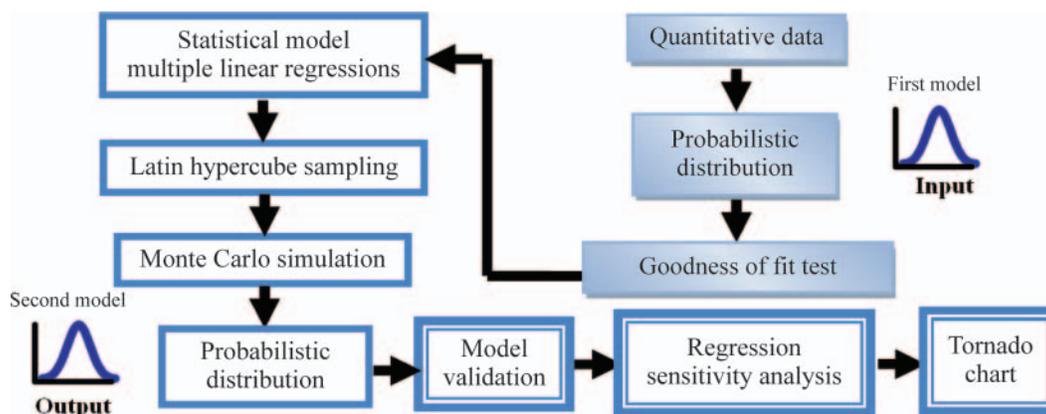


Figure 1. Schematic diagram of probabilistic quantitative microbial risk assessment modeling. Color version available in the online PDF.

cessing parameters] were analyzed for their distribution using the Best Fit function of @Risk software (Palisade Corporation, 2003). The probability density function or cumulative density function was fitted to the observed concentration data and processing parameter data (input and output data). During the fitting process, the goodness of fit with chi-square, Kolmogorov-Smirnov, and Anderson-Darling statistics was used to identify the types of distributions. The exponential, normal, log-normal, uniform, and triangular distributions as suggested by Vose (2008) were tested in this study. The most likely distribution was then chosen for each parameter (input data). The output was a statistical distribution of concentrations of *C. jejuni* in chicken carcasses. After the distributions were fit, to deal with the variability and uncertainty of the input, 10,000 iterations of the Monte Carlo simulation were run using a Latin hypercube sampling technique to ensure convergence for the tails of the output distribution.

Model Development and Simulation

In this study, the distributions of the experimental data at each step were evaluated and incorporated into a multiple linear regression model (equation 1). The model was then simulated to obtain the outputs (concentration of *C. jejuni* on broiler carcasses). Regression analysis was used based on the probabilistic analysis technique demonstrated by Iman et al. (1985) and Frey et al. (2003). The advantage of using regression methods in our research was the convenience of applying them with the @Risk software package (Vose, 2008). This typically involves fitting the relationship between inputs and outputs, such as in this linear equation (Frey et al., 2003):

$$Y_i = b_0 + b_1X_{1,i} + b_2X_{2,i} + \dots + b_mX_{m,i} + e_i \quad [1]$$

where Y_i is the i th output data point for the i th input data point; $X_{j,i}$ is the i th input data point for the j th input; b_j is the regression coefficient for the j th input; and e_i is the error for the i th data point. For a linear model, the regression coefficient b_j can be interpreted as the change in output Y_i when the input $X_{j,i}$ for a given value of j increases by 1 unit and the values of all other inputs remain fixed (Devore and Peck, 1996; Frey et al., 2003).

In this study, the multiple linear regression model was tested by adding input data and output data to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, CA). The microbiological analysis results were converted into \log_{10} units and submitted to the regression analysis by using the analysis tool program in Microsoft Excel. The fitted distributions of input data were then used in the multiple linear regression model so that the output of contamination levels of *C. jejuni* in chicken carcasses could be estimated based on values of the input parameters.

A Monte Carlo simulation (10,000 iterations) was used to generate input and output values in the exposure assessment model. Variable factors were randomly taken from each parameter distribution in a series of iterations to obtain results with the distributions, usually in the form of a probability density function or cumulative density function. This simulation represented the intrinsic variability and uncertainty in *C. jejuni*-contaminated broiler meat production. Latin hypercube sampling of parameters, representing uncertainty, was conducted once only for each simulation. The parameters were held constant during multiple iterations in the simulation.

Model Validation

To validate the model of regression analysis, the distribution of the number of *C. jejuni* on fresh breast meat was fitted with the data sets for model validation (number of *C. jejuni* on fresh wings and fresh thighs). Model validation was carried out using summed square residuals or sums of squared error (SSE). This statistic measured the total deviation of the observed values from the predicted values (Anonymous, 2009).

$$\text{SSE} = \text{sum}(i = 1 \text{ to } n)[w_i(y_i - f_i)^2], \quad [2]$$

where y_i is the observed value and f_i is the predicted value from the fit. The variable w_i is the weighting applied to each data point, usually $w_i = 1$. A value of SSE close to 0 indicated that the model had a small random error component and that the fit would be more useful for prediction (Anonymous, 2009).

Sensitivity Analysis

To obtain the effects of variations in operating conditions on process performance, a sensitivity analysis was carried out using the rank order correlation analysis features in @Risk (Anonymous, 2009). Generally, the analysis could use either a mathematical, statistical, or graphical method. In this study, the sensitivity analysis of *C. jejuni* in the broiler slaughterhouse process used a combination of statistical and graphical methods. The regression sensitivity analysis used rank order correlations based on Spearman's rank correlation calculations, a nonparametric statistic for quantifying the correlation relationship between 2 means (Frey et al., 2003). The analysis involved running simulations in which inputs were assigned probability distributions and assessing the effect of variance in inputs on the output distribution (Frey et al., 2003). Tornado graphs could then be generated. Horizontal bars of the graph represented each input variable, with the length of the bars representing the degree of correlation with the mean numbers (\log cfu) of *C. jejuni* on fresh chicken meat (output variables). The signs (+, -) indicated in-

creasing or decreasing effects of the input variables (x) on the number of *C. jejuni* in chicken meat (outputs, y).

RESULTS

Contamination of *C. jejuni* in the Commercial Poultry Processing Plant (Data Set for Model Development and Simulation)

The prevalence of *C. jejuni* in all 240 samples ($n = 20$ from each step) was as follows: 100% (scalded carcass), 100% (scald water), 100% (plucked carcass), 100% (plucked feathers), 100% (eviscerated carcass), 20% (chilled carcass), and 15% (fresh breast samples). *Campylobacter jejuni* was not detectable in the chilling water, fresh leg, fresh thigh, fresh wing, and karaage samples (Table 1). However, the mean concentrations of *C. jejuni* for the steps were 2.93 ± 0.31 (scalded carcass), 1.39 ± 0.70 (scald water), 2.98 ± 0.38 (plucked carcass), 3.28 ± 0.52 (plucked feather), 2.88 ± 0.31 (eviscerated carcass), 0.85 ± 0.95 (chilled carcass), not detectable (chill water), and 0.5 ± 1.22 log cfu (fresh breast). The pathogen was not detectable in the fresh leg, fresh thigh, or fresh wing portions or in the karaage product. High prevalence and concentrations of *C. jejuni* were evident despite the fact that the mean value of the scalding temperature in this study was rather high, 59 to 62°C (mean value = $61.08 \pm 0.05^\circ\text{C}$). Mean concentrations of *C. jejuni* for the scalded and plucked carcass and feathers were among the highest mean values. We found that the normal rate of punctured intestines was approximately 5% by manual evisceration and 20% by machine evisceration (P. Youeaimyut, QA manager, B. Food International, Thailand; personal communication).

The poultry carcasses were cooled rapidly to prevent bacterial or pathogen growth. Chilling appeared to be a very important process because it was the last process in the slaughterhouse that could reduce microbial

contamination or growth. Chlorination of immersion water and a poultry residence time of approximately 60 min were effective in reducing the microbial loads on carcasses. The mean chilling water temperature ($7.16 \pm 0.42^\circ\text{C}$), residual chlorine (1.66 ± 0.15 ppm), and pH (8.42 ± 0.04) are shown in Table 1. *Campylobacter jejuni* in the chill water may be injured and decreased in number by the effects of oxidizing agents such as sodium hypochlorite. However, *C. jejuni* was still found in broiler carcass samples at a low level (0.85 ± 0.2 log cfu), whereas the bacterium was undetectable in the chill water. The target pathogen was also not detected in most carcass portions and products except the fresh breast. This may be the result of cross-contamination.

Prevalence and Concentration of *C. jejuni* in Chicken Meat (Data Set for Model Validation)

Of the 90 samples obtained from this visit, *C. jejuni* was found on the scalded carcass, chilled carcass, fresh wing, and fresh thigh samples, but it was undetectable in the fresh breast and steamed breast product samples. In most cases, except for the chilling step, the numbers of organisms obtained from mCCDA plating and SimPlate MPN were not significantly different ($P < 0.05$). However, mCCDA failed to detect the injured pathogens in the chill water, whereas SimPlate MPN was more sensitive to injured cells after exposure to the sodium hypochlorite treatment in the chill water. Therefore, the SimPlate MPN results were used to develop the model. Midway through the production time (1000 to 1400 h), mean concentrations of *C. jejuni* on scalded carcasses were reduced along the production steps. Mean values for the scalded carcass, chilled carcass, fresh wing, and fresh thigh samples were 1.86 ± 0.75 , 0.16 ± 0.43 , 0.34 ± 0.68 , and 0.08 ± 0.15 log cfu, respectively (Table 1). The concentrations of *C. jejuni* in the carcasses ranged from undetectable to 2.87, 1.45,

Table 1. Data sets used to estimate the prevalence and concentration of *Campylobacter jejuni* in the slaughterhouse and processing plant (model development)

Process	Sample	Number of samples	Number of positive samples (%)	Number of <i>C. jejuni</i> ¹ (log cfu) ¹	Temperature (°C)	Free chlorine (ppm)	pH
Scalding	Carcass rinse sample	20	20 (100)	2.93 ± 0.31	—	—	—
	Scald tank water sample	20	20 (100)	1.39 ± 0.70	61.08 ± 0.05	—	—
Plucking	Carcass rinse sample	20	20 (100)	2.98 ± 0.38	—	—	—
	Feather sample	20	20 (100)	3.28 ± 0.52	—	—	—
Evisceration and washing	Carcass rinse sample	20	20 (100)	2.88 ± 0.31	—	—	—
Chilling	Carcass rinse sample	20	20 (100)	0.85 ± 0.95	—	—	—
	Chiller tank water	20	0 (0)	0	7.16 ± 0.42	1.66 ± 0.15	8.42 ± 0.04
Carcass portions	Fresh breast	20	3 (15)	0.50 ± 1.22	—	—	—
	Fresh leg	20	0 (0)	0	—	—	—
	Fresh thigh	20	0 (0)	0	—	—	—
	Fresh wing	20	0 (0)	0	—	—	—
Processing plant	Chicken karaage ²	20	0 (0)	0	—	—	—
Total		240	123				

¹Mean \pm SD.

²Restructured fried chicken.

Table 2. Data sets used to estimate the prevalence and concentration of *Campylobacter jejuni* in the slaughterhouse and processing plant (model validation and simulation)

Process step	Sample	Number of samples	Number of positive samples (%)	<i>C. jejuni</i> ¹ (log cfu)	Probability distribution of concentration
Scalding	Carcass rinse sample	30	30 (100)	1.86 ± 0.75	RiskNormal ² (1.89, 0.75)
Chilling	Carcass rinse sample	30	4 (13.3)	0.16 ± 0.43	RiskNormal (0.15, 0.42)
Carcass portions	Fresh wing	5	1 (20)	0.34 ± 0.68	RiskNormal (0.27, 0.61)
	Fresh thigh	5	1 (20)	0.08 ± 0.15	RiskNormal (0.06, 0.13)
	Fresh breast	5	0	0	—
Processing plant	Steamed breast	15	0	0	—
Total		90	36		

¹Mean ± SD.²See Vose (2008).

1.36, and 0.30 log cfu, respectively. Only positive values of log colony-forming units are shown in Table 1. Contamination was the highest in the scalding step. We found that birds were contaminated differently by feces, with birds from the top of the truck being cleaner and those from the bottom of the truck being heavily contaminated with feces on their feathers. The immersion of these birds into the scalding tank therefore introduced a tremendous amount of feces as well as pathogens such as *Campylobacter* and *Salmonella* into the tank.

The probability distributions of concentrations of *C. jejuni* were fitted by using the @RISK program and were simulated by a Monte Carlo simulation, as explained above. The distributions of the respective steps (Table 2) were all normal distributions. The relationship between the number of *C. jejuni* on chilled carcasses and cut-up products (Table 2) indicated that the distribution on the chilled carcasses was not different from that on the fresh wings, having numbers close to zero. The shapes of simulated distributions of *C. jejuni* concentrations at each step in the slaughterhouse were shown previously (Osiriphun, 2009). The presence of *C. jejuni* on fresh wing and fresh thigh samples might have been caused by cross-contamination during handling and processing at the plant.

Simulation Model of *C. jejuni* in the Broiler Slaughterhouse

The distributions of *C. jejuni* concentrations in the slaughterhouse processing steps were simulated by a Monte Carlo simulation method using the data shown in Table 2. Values of the input factors and one output factor in the simulation model are displayed in Table 3. Concentrations of *C. jejuni* in the scald water, scalded carcass, plucked feathers, plucked carcass, washed carcass, chilled carcass, and fresh breast were then defined as probability distributions and are tabulated in Table 3. Input parameters in the model were sampling time, scald tank water temperature, number of *C. jejuni* in the processing steps, scalding water, scalded carcass, plucked feathers, plucked carcass, washed carcass, chiller water temperature, residual or free chlorine concentration, and pH of the chill water. The output of the model was the number of *C. jejuni* on fresh chicken meat. We found that most parameters exhibited normal distribution behavior except time and scald tank water temperature, which were uniform and triangle distributions, respectively (Table 3).

The multiple regression model statistics are provided in Table 4, which gives the coefficient estimate, SE of each coefficient, *t*-statistic, *P*-value, and 95% CI for

Table 3. Summary of simulation model parameters affecting the prevalence and concentration of *Campylobacter jejuni* in chicken meat at each step in the slaughterhouse¹

Type of parameter	Model parameter	Model description	Probability distribution of concentration ²	
Input	Time	Time of sampling	RiskUniform (−5, 100)	
	SW temp	Temperature of the scald water (°C)	RiskTriangle (61, 61, 62.05)	
	SW	<i>C. jejuni</i> in the scald water (log cfu)	RiskNormal (1.39, 0.70)	
	SC	<i>C. jejuni</i> on the scalded carcass (log cfu)	RiskNormal (2.93, 0.31)	
	PF	<i>C. jejuni</i> on the plucked feathers (log cfu)	RiskNormal (3.28, 0.52)	
	PC	<i>C. jejuni</i> on the plucked carcass (log cfu)	RiskNormal (2.87, 0.38)	
	WC	<i>C. jejuni</i> on the washed carcass (log cfu)	RiskNormal (2.59, 0.31)	
	CW temp	Temperature of the chill water (°C)	RiskNormal (7.16, 1.86)	
	CW Cl	Residual chlorine in the chill water (ppm)	RiskNormal (1.66, 0.07)	
	CW pH	pH of the chill water	RiskNormal (8.42, 0.20)	
	CC	<i>C. jejuni</i> on the chilled carcass (log cfu)	RiskNormal (0.85, 0.96)	
	Output	BR	<i>C. jejuni</i> on the fresh breast (log cfu)	RiskNormal (0.50, 1.22)

¹For the data in Table 1.²For explanation of RiskUniform, RiskTriangle, and RiskNormal, see Vose (2008).

Table 4. Multiple regression model results¹ for *Campylobacter jejuni* contamination at the slaughterhouse²

Item	Coefficient	SE	t-Statistic	P-value	Lower 95% CI	Upper 95% CI
Intercept	-86.62	163.20	-0.53	0.61	-472.53	299.29
Time of sampling	0.017	0.04	0.43	0.68	-0.08	0.11
Temperature of the scald water (°C)	2.28	2.34	0.97	0.36	-3.26	7.82
<i>C. jejuni</i> in the scald water (log cfu)	-0.97	0.94	-1.03	0.34	-3.18	1.25
<i>C. jejuni</i> on the scalded carcass (log cfu)	0.034	1.69	0.02	0.99	-3.96	4.02
<i>C. jejuni</i> on the plucked feathers (log cfu)	0.99	1.02	0.98	0.36	-1.42	3.42
<i>C. jejuni</i> on the plucked carcass (log cfu)	0.077	1.06	0.07	0.95	-2.44	2.59
Temperature of the chill water (°C)	-0.19	0.22	-0.85	0.43	-0.73	0.34
Free chlorine in the chill water (ppm)	7.14	7.31	0.98	0.36	-10.15	24.43
pH of the chill water	-7.66	6.04	-1.27	0.25	-21.94	6.62
<i>C. jejuni</i> on the chilled carcass (log cfu)	0.18	0.57	0.32	0.76	-1.18	1.54

¹Multiple linear regression equation: BR = (-86.62) + (0.017 × time) + (2.28 × SW temp) - (0.966 × SW) + (0.034 × SC) + (0.999 × PF) + (0.077 × PC) - (0.63 × WC) - (0.192 × CW temp) + (7.139 × CW Cl) - (7.657 × CW pH) + (0.181 × CC), where BR is *C. jejuni* on fresh breast samples (log cfu), time is time of sampling, SW temp is temperature of the scald water (°C), SW is *C. jejuni* in the scald water (log cfu), SC is *C. jejuni* on the scalded carcass (log cfu), PF is *C. jejuni* on the plucked feathers (log cfu), PC is *C. jejuni* on the plucked carcass (log cfu), WC is *C. jejuni* on the washed carcass (log cfu), CW temp is temperature of the chill water (°C), CW Cl is residual chlorine in the chill water (ppm), CW pH is pH of the chill water, and CC is *C. jejuni* on the chilled carcass (log cfu).

²For data in Table 1. The output is the concentration of *C. jejuni* on fresh breast in log-transformed colony-forming units.

each independent variable. Through multiple regression analysis, 11 independent variables were found to affect the number of *C. jejuni* on fresh chicken breast. The regression analyses for sensitivity analysis of slaughterhouse was log₁₀ of *C. jejuni* on fresh chicken breast = -86.62 + 0.017 (time) + 2.28 (SW temp) - 0.97 (SW) + 0.034 (SC) + 0.99 (PF) + 0.077 (PC) - 0.63 (WC) - 0.19 (CW temp) + 7.14 (CW Cl) - 7.66 (CW pH) + 0.18 (CC), where time is sampling time, SW temp is the scald tank water temperature, SW is the scald water, SC is the scalded carcass, PF is the plucked feathers, PC is the plucked carcass, WC is the washed carcass, CW temp is the chiller water temperature, CW Cl is the residual or free chlorine concentration, and CW pH is the pH of the chill water.

Model Validation of *C. jejuni* at the Broiler Slaughterhouse

The model for *C. jejuni* contamination of fresh breast meat was tested by using the data set for model development. It was then verified with 2 data sets for model validation (fresh thigh and fresh wing samples) because *C. jejuni* was not found to have contaminated any fresh breast samples. This was based on the assumption that the contamination rates were not different. The SSE values of the 2 data sets calculated by equation 2 were small and close to zero (0 to 0.0095 for fresh wings, and 0 to 0.0105 for fresh thighs).

Statistical Sensitivity Analysis of Broiler Processing

Even though a sensitivity analysis can be conducted using either a multivariable stepwise rank regression or a rank order correlation in the @Risk program, the rank order correlation method was preferred because it

made no assumptions about the relationship between input and output variables. The stepwise regression method assumed that there was a linear relationship between the 2 variables. Sensitivity analyses relied on rank order correlations, which were based on Spearman's rank correlation calculations (a nonparametric statistic) for quantifying the correlation relationship between 2 nonparametric variables. The correlation statistic is not affected by the type of mathematical relationship between the variables (Vose, 2008). In our study, tornado chart horizontal bars were plotted for each input variable, with the length of the bars representing the degree of correlation with the output variable (Figure 2).

Results from the sensitivity analysis of the broiler slaughterhouse process (Figure 2), in rank order, were

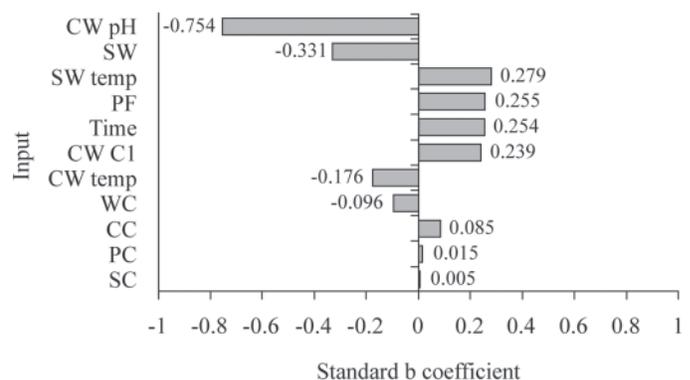


Figure 2. Sensitivity analysis of process parameters in the contamination of fresh breast samples with *Campylobacter jejuni* by using Spearman ranked correlations. CW pH = pH of the chill water; SW = *C. jejuni* in the scald water (log cfu); SW temp = temperature of the scald water (°C); PF = *C. jejuni* on the plucked feathers (log cfu); time = time of sampling; CW Cl = residual chlorine in the chill water (ppm); CW temp = temperature of the chill water (°C); WC = *C. jejuni* on the washed carcass (log cfu); CC = *C. jejuni* on the chilled carcass (log cfu); PC = *C. jejuni* on the plucked carcass (log cfu); SC = *C. jejuni* on the scalded carcass (log cfu).

pH of the chill water (-0.764), scald water (-0.335), scald tank water temperature (0.282), plucked feathers (0.258), sampling time (0.257), residual or free chlorine concentration (0.242), chiller water temperature (-0.178), washed carcass (-0.097), chilled carcass (0.086), plucked carcass (0.015), and scalded carcass (0.005). Negative and positive signs illustrated negative and positive effects of the parameters for the contamination of carcasses and chicken meat products with *C. jejuni*. The values indicated the degree of sensitivity. The higher the correlation between inputs and outputs, the more influence the input variable had on determining the value of the output variable; when the input and output variables were perfectly monotonically related, the rank correlation coefficient became positive (+).

The sensitivity analysis results (Figure 2) indicated that conditional parameters affecting the survival of the pathogen were pH of the chilling water, scald water temperature, residual chlorine in the chill water, and temperature of the chill water, respectively. The significant intervention steps that contributed control effects on *C. jejuni* were scalding, chilling, and cut-up. In this case, the hot scald water temperature was high, in the range of 61 to 62°C, which was unfavorable for the growth and survival of *Campylobacter*. In another study, the reduction in *Campylobacter* numbers in chicken meat at temperatures ranging from 55 to 65°C was 0.09 to 1.6 log cfu (ICMSF, 1996). Our unreported data implied that high solids (2.6 mg/L) and pH (8.0) in the scalding tank might have contributed to its resistance to heat.

In this study, the pathogen was detected in the scald water and carcass samples leaving the tank. *Campylobacter* that survived the process could cause contamination in the next steps, including cut-up. Our study found a rather high number of pathogens (1.39 ± 0.70 in the scald water and 2.93 ± 0.31 log cfu associated with carcasses after scalding) when using scald water at a pH of 8.0. It appeared that some *C. jejuni* survived the scalding conditions. Moreover, the residual chlorine in the chilling water was quite low, at 1.66 ± 0.15 ppm, and the pH values of the chill water were in the range of 8.27 to 9.20, which appeared to be out of the optimal range indicated by FAO/WHO (2009). Adjustments to a higher chlorine concentration and a more neutral pH were needed for this operation. Most free residual chlorine was available as hypochlorous acid at pH 6.0, compared with less than 10% at the higher pH of 8.42 reported in this study. This explains the high sensitivity analysis coefficient for pH in this study, indicating that lowering the pH of the chiller water could improve the performance.

From results of the sensitivity analysis, risk factors associated with broiler carcass contamination with *C. jejuni* were identified. Significant factors included pH of the chill water, number of *C. jejuni* in the scald tank water, and scald water temperature. Critical control

points in this slaughtering plant were scald water overflow rates, inside-outside washing, and chlorine concentration of the chill water. Therefore, these CCP could be revised to control the pathogens more effectively. Results of the sensitivity analysis suggested that the pH of the chill water should be reduced to enhance the effectiveness of the chlorine, and scald water should be included.

The scalding process is not only a process for reducing pathogenic bacterial populations on carcasses, but it is also one of the most important areas in the processing plant for cross-contamination with pathogenic bacteria (Russell, 2009). Because the scald tank was a common bath containing hot water, the birds were submerged in the bath to soften the feather follicles, which aids in feather removal. Bacterial cross-contamination from one carcass to other carcasses would occur if the rates of water flow were not high enough to thoroughly remove dirty material and bacteria from the chicken carcasses.

Another effective process for reducing pathogenic bacteria from carcasses was the chilling step, in which pathogen reduction can be accomplished more effectively in a properly balanced immersion chiller than anywhere else in the processing plant (Russell, 2009). Bacterial cross-contamination during the chilling step resembles that associated with the scalding process because one contaminated chicken carcass may contaminate others through the water or by direct contact with other carcasses (Yang et al., 2002). Immersion chilling is the last process to decrease the pathogen numbers on broiler carcasses at slaughter.

Scalding and chilling are operations in which the numbers of *C. jejuni* can be significantly reduced. The objective of this investigation was to gain a better understanding of the effects of operating conditions on the prevalence and concentration of *C. jejuni* in processed chickens by measuring the numbers of organisms associated with the scalding and chilling operations.

DISCUSSION

Contamination of *C. jejuni* in the Commercial Poultry Processing Plant (Data Set for Model Development and Simulation)

Our results indicated that *C. jejuni* levels on broiler carcass samples were higher than the 2.38 mean log cfu/carcass reported by Naonsawang (2005), who collected samples in another province in Thailand. The difference in results indicated the nature of variation that can be expected with different flocks, management styles, and sampling methods (Berrang et al., 2000). The high numbers of *C. jejuni* in feather samples at the plucking step indicated that the equipment, working surfaces, and process water were serious sources of con-

tamination of other carcasses (Oosterom et al., 1983; Jacob-Reitsma, 2000). Furthermore, evisceration and washing was a common process in which carcasses were washed with chlorinated water to remove contamination, such as with blood, tissue fragments, and feces (Keener et al., 2004). This was an important step in microbial cross-contamination because of the seepage or leakage of intestinal contents onto the carcasses (Baker et al., 1987; Saleha et al., 1998). Our study explained 100% prevalence (20/20) in carcasses after the evisceration process. The levels of *C. jejuni* on broiler carcasses during plucking and evisceration were much higher than those at the chilling step. Although contamination levels of *C. jejuni* on fresh breast samples were low (0.50 ± 0.27 mean log cfu), they were nevertheless potentially hazardous to human health, especially if the products might be mishandled, inadequately cooked, or allowed to cross-contaminate ready-to-eat foods (Saleha et al., 1998).

Prevalence and Concentration of *C. jejuni* in Chicken Meat (Data Set for Model Validation)

The highest prevalence, of 100% (20/20), was found in the scalding step based on these results. This high prevalence rate may be due to high loads of soil, dust, and fecal material from the feet, feathers and skin and intestinal leakage from carcasses released into the scalding water. Water from the scald tank is absorbed by the feathers and skin during scalding (ICMSF, 2005). In the chilling process, the efficacy of pathogen control by chlorine is apparently limited, depending on chlorine level of the water, temperature, and contact time (Lake et al., 2007). Therefore, *Campylobacter* may survive in chill water and spread from one carcass to other carcasses (Lake et al., 2007). It was observed that the mixing and flow of carcasses was not effective because of the relatively slow, rotating movement. It appeared that ineffective mixing caused some fluctuation of the residence time of carcasses in the chilling tank, and this contributed to variation in the contact times with chlorinated water. Results of *C. jejuni* contamination on the fresh wing and thigh samples in this study (about 20% prevalence, at 0.34 and 0.08 log cfu) were similar to those reported in other studies outside Thailand. A prevalence of *Campylobacter* in fresh chicken meat (breast and thighs) and chicken by-products (wings, livers, gizzards, and hearts) as high as 64.7% was reported on a retail level in Sapporo, Japan. Among the different products, chicken wings showed the highest prevalence (77.1%), followed by the chicken thighs (70%), whereas chicken gizzards and hearts showed the lowest incidence of contamination (45 and 40%, respectively; Sallam, 2006). Some 35.2% of frozen poultry carcasses were contaminated by *Campylobacter* in Bulgaria, with rather high percentages of 91.1 and 88.9% in the wing and thigh cuts, respectively (Stoyanchev et al., 2007).

Simulation Model of *C. jejuni* in the Broiler Slaughterhouse

The simulated concentration distributions of *C. jejuni* in the slaughterhouse processing steps are shown in Table 3. Table 3 exhibits the input parameters (abbreviations) used in model development as well as the sensitivity analysis. The distributions of *C. jejuni* for each model parameter demonstrated a normal distribution behavior except for sampling time and scalding water temperature, for which the distributions were uniform and triangular, respectively.

Model Validation of *C. jejuni* at the Broiler Slaughterhouse

The multiple regression model was acceptable for the prediction of *C. jejuni* contamination numbers on fresh chicken breast (model development) because the SSE values of the 2 data sets (fresh thigh and fresh wing data) for validation were small and near zero (Anonymous, 2009). The SSE values also showed that the overall shapes of the distributions of the fresh thigh and fresh wing samples were closely predicted by the model.

Statistical Sensitivity Analysis of the Broiler Process

The sensitivity analysis results (Figure 2) showed that the processes affecting the survival of *C. jejuni* were the scalding and chilling processes. Humphrey and Lanning (1987) suggested that at the scalding step, maintaining the scalding water at $\text{pH } 9.0 \pm 0.2$ would significantly increase the death rates of *Salmonella* and *C. jejuni* in the water. Moreover, the authors recommended that a more alkaline pH (9.0 ± 0.2) would be best for reducing *Salmonella* and *Campylobacter* in the scald water (Humphrey and Lanning, 1987). A more acidic pH (3 to 4) was also shown to be effective in decreasing the levels of *Salmonella* in chicken carcasses (Okrend, et al., 1986). Uric acid from poultry feces could reduce the pH from 8.4 to 6.0 in less than 2 h (Humphrey, 1981). Organic matter in the tank acted as a buffer to maintain a more neutral pH (6 to 7), where *Salmonella* were more heat resistant (Okrend et al., 1986). The USDA/FSIS (2009) has recommended that plants should monitor the pH in scald tanks as frequently as necessary to determine the pH highs and lows that occur during operation. For immersion chilling, the available chlorine should be maintained at 50 to 70 ppm, and available free chlorine should be maintained at 0.4 to 5.0 ppm, with a pH of 6.0 to 6.5 (FAO/WHO, 2009). An earlier study showed that 10 to 12 ppm of free chlorine in the water did not have much effect on *Campylobacter*, and this was probably due to the high bacterial loads and large amount of organic material in the chill water, which neutralized the effects of chlorine (Berndtson et al., 1992, 1996). A pH of 6.5 to 7.5 to optimize the disinfectant properties

of chlorine has been reported elsewhere (White, 1998; Northcutt et al., 2008). The USDA/FSIS (2010) has recommended maintaining the chill water pH between 6.0 and 6.5 at a temperature of less than 40°F. We could detect *C. jejuni* at very low levels (0.7 ± 0.5 log cfu). Hypochlorous acid is the active compound that kills *C. jejuni* (Keener et al., 2004). The activity of disinfectants was also affected by factors such as time of contact between the disinfectant and microorganisms, the concentration of the disinfectant, and temperature (Sincero and Sincero, 2003). The length of time that a disinfection process continues is a function of the underlying reaction between the microorganisms and the disinfectant; thus, it is also a function of pH and temperature. The USDA/FSIS (1996) has recommended a chilling temperature of less than 4.4°C, but the average temperature of all water samples in our study (7.16°C) was higher than the USDA recommendation. Significant cross-contamination of *C. jejuni* may occur during the plucking (plucked feathers and plucked carcass), scalding (scalded carcass), washing and evisceration (washed carcass), and chilling (chilled carcass) steps. The contamination of broiler carcasses at the slaughterhouse and during processing could be caused by the plant environment (Newell and Fearnley, 2003) and by cross-contamination between batches of chickens (Saleha et al., 1998). The concentration of *C. jejuni* can be reduced by washing the chickens before scalding to remove dirt and feces and after evisceration to remove blood and contamination associated with evisceration. Particulate matter protects organisms during scalding and during soaking in the chill water with chlorine. Preventing fecal contamination during transportation would significantly reduce the organic load entering the scald tank.

The interventions for the broiler slaughterhouse could be divided into 2 groups: hygienic measures and decontamination methods. Hygienic measures were aimed at reducing fecal contamination during the slaughter process, and decontamination methods were aimed at reducing the number of *Campylobacter* by means of physical and chemical decontamination (Rosenquist et al., 2008). To improve the control of hygienic measures, the process needs to be changed to reduce the fecal contamination of carcasses and equipment (Berndtson et al., 1996). In addition, chlorinated-water sprays could be used to limit microbial contamination on the equipment and working surfaces, and any unnecessary carcass contact surfaces in the processing plant could be removed (Mead et al., 1995). When focusing on decontamination, the cross-contamination of *Campylobacter* during the scalding process could be minimized by using a countercurrent flow, the addition of as much fresh water as possible, and a scald temperature as high as possible to reduce levels of *Campylobacter*. In addition, *Campylobacter* could penetrate the feather follicles, which become closed during the chilling process, resulting in protection of the bacteria in the subcutaneous skin. Thus, the bird washer used to rinse chickens

before the chiller might also press the *Campylobacter* deeper into the soft tissue of the chicken carcass (Berndtson et al., 1992, 1996).

Conclusions

The purpose of this experiment was to assess the exposure of raw chicken meat and cooked chicken products to *C. jejuni*. A process and operational sensitivity analysis was conducted to determine the steps that were influential in contamination and the concentration of *C. jejuni* numbers in chicken meat during the scalding and chilling steps. Results of the sensitivity analysis were used to determine validity of the HACCP program and to prioritize the CCP of the company. Interventions were also suggested to the steps that were the most sensitive to the contamination of chicken carcasses and chicken products with pathogens. The model fitted well with the actual data. We found that pH of the chill water was the most sensitive to the contamination of chicken carcasses with *C. jejuni*, followed by the scald water temperature, *C. jejuni* on plucked feathers, and the concentration of free chlorine in the chill water (FAO/WHO, 2009). The suggested interventions included preventing fecal contamination of chicken feathers during transportation, increasing the scalding temperature as high as possible without physical damage to the chicken skin, reducing *C. jejuni* in the scald water by reducing the solids and organic matter in the scalding tank, reducing the contamination at plucking (by spraying water), monitoring pH of the chill water and maintaining it at 6.0 to 6.5 (USDA, 2010), and increasing the residual chlorine in the chill water. The sensitivity analysis indicated that some of the current CCP (chlorine concentration and water temperature) did not effectively control contamination. We suggest that the HACCP plan should include controlling the pH of the chill water and reducing solids and organic matter in the scalding tank, such as by having a higher countercurrent flow rate of the water.

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