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Quantitative exposure assessment for *Listeria monocytogenes* in cooked ham

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Abstract: The objectives of this study were predicting the transmission and survival of *L. monocytogenes* in cooked ham during supply chain. Cooked ham are frequently contaminated with *L. monocytogenes* during post-processing steps through contact on surface of processing, handling, packaging equipment. Transfer rate of *L. monocytogenes* on static and dynamic condition in various surface type was investigated. The prevalence and level of *L. monocytogenes* in cooked ham at plant as well as the prevalence of unsatisfactory processing at retail were studied. A Monte Carlo simulation model was created by using @risk. The simulation predicted that the prevalence was 11.76 % with 90% confidence interval of 2% to 25% and estimated level was -4.02 log CFU/ cm^2 . It was estimated to be occurred on slicing step at plant. Our results suggest that, the prevalence and level of *L. monocytogenes* can be reduced by Good Handling Process application and/or HACCP application.

Keywords: Quantitative assessment, *Listeria monocytogenes*, cooked ham

Introduction

L. monocytogenes are pathogenic for humans and animals (Vazquez-Boland *et al*., 2001) and grow in a wide temperature range, from -1.5 to 45ºC (Gray *et al.*, 1966; Junttila *et al*., 1988; Hudson *et al*., 1994). The growth of the organism at -1.5ºC is very slow, with a lag time of 174 h (Hudson *et al*., 1994). *Listeria* can move with flagella and polymerizing actin comet tails with a protein called ActA. Some studies suggest that 1 to 10% of humans may carry *L. monocytogenes* in their intestines (EMLab, 2009).

Listeriosis, caused by *L. monocytogenes,* is a food borne infection of great public health concern due to its clinical severity and high case fatality. Mostly affected by severe disease are people who are elderly or immunocompromised, pregnant women and neonates (younger than four weeks). *L. monocytogenes* can cause meningoencephalitis and/or septicemia in newborns, elderly, immunocompromised patients and abortion in pregnant women (Marchant, 2003). The infective dose of *L. monocytogenes* is unknown but is believed to vary with the strain and susceptibility of the victim.

The mortality rate of *L. monocytogenes* (20–30% in some epidemic cases) is greater than that of other pathogens, such as *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* (Farber, 1991; Norrung, 2000; Öktem, 2006). The reported yearly incidence of human listeriosis ranges from 0.1 to 11.3 cases per million persons (Notermans *et al.,* 1998), 0.3 to 7.5 cases per million people in Europe (Anonymous, 1999), 4.4 cases per million people in the United States of America (Mead et al., 1999) and 3 cases per million people in Australia. Most listeriosis cases are associated with a restricted number of serotypes: 1/2a (15–25%); 1/2b (10–35%); 1/2c (0–4%); 3 (1–2%); 4b (37–64%); and 4 not b (0–6%) (McLauchlin, 1990; Farber and Peterkin, 1991).

Outbreaks of listeriosis have been associated with the consumption of many kinds of food products, mainly ready-to-eat (RTE) food (Coillie, 2004). Roughly about 80% death and listeriosis cases were caused by RTE-deli meat (Galagher *et al.*, 2003). RTE cooked meats are frequently contaminated with *L. monocytogenes* during post-processing steps (Beresford *et al.*, 2001). *L. monocytogenes* can be present in product's ingredients due to a processing error. Even this product has successfully undergone a lethality treatment, *Listeria* contamination is still

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possible through several means such as contact with biofilms on surfaces of processing, handling, packaging equipment that can harbor pathogens, exposure to environmental contamination or cross-contamination in the processing environment encountered after the lethality treatment. (Saulo, 2005). A survey of *L. monocytogenes* contamination in the products was conducted at retail markets. The overall prevalence was 1.82%, with prevalence ranging from 0.17% to 4.7% among the product categories, with in-store-packaged foods significantly higher than manufacturer packaged foods (Gombas *et al.*, 2003).

L. monocytogenes can be spread easily by direct contact of food with a contaminated surface, growth at refrigeration temperatures as low as 2°C or under low oxygen tension such as found in vacuum-packaged RTE meats (Samelis *et al.*, 2002). The processing steps after cooking such as peeling, sorting, loading, slicing, packaging, etc., are potential sources of recontaminations for pathogens such as *L. monocytogenes*. A USDA-FSIS survey published in 2001 showed that 1-10% of retail RTE meat products were contaminated with *L. monocytogenes* (Levine *et al.*, 2001). So, the evaluation of the transfer rate of *L. monocytogenes* on cooked ham due to contact surface is useful to inform the regulator, industry and retailer setting appropriate handling process. From this point of view, the purpose of this article was to provide the analysis of *L. monocytogenes* contamination on cooked ham on plant to retail. We propose in this study to examine the transfer rate of *L. monocytogenes* on different surface type (stainless steel, normal belt, antimicrobial belt) in static and dynamic condition. Pressure and moisture level condition on surface were investigated as well.

The objectives of this study were predicting the transmission and survival of *L. monocytogenes* in cooked ham during supply chain. This analysis is essential to provide information of exposure assessment for *L. monocytogenes* in cooked ham.

Material and Methods

L. monocytogenes ATCC 35152 (Microbiologics, MediMark,Europe) were inoculated in brain-heart infusion (Oxoid) and incubated overnight at 37°C. Approximately 700 µl of this strain were mixed with 300 µl of glycerol (R and M) in the 0.1 ml cryo vials (Greiner Bio-one, Frickenhausen, Germany) and 5 glass beads (Ø 2 mm, Emergo, Landsmeer, The Netherlands). The stock cultures were maintained at -80°C in cryo vials (Greiner Bio-one, Frickenhausen, Germany).

Strains were cultured by transferring one glass bead onto Tryptic Soy Agar (Oxoid) for 24 h at 30°C. One formed colony is transferred onto 10 ml of BHI broth followed by incubation for 24 h at 37°C. The test suspensions were prepared by making serial dilutions of the micro organisms in Neutralized Bacteriological Peptone (Oxoid). Selective agar media were used for the enumeration of pathogens: PALCAM (Merck) for *L. monocytogenes* ATCC 35152 incubated for 24-48 h at 37°C. The viable counts from surfaces were carried out using contact plates with RODAC Plate. Cooked ham (approximately 2 kg) was purchased in chub form from a local retailer held at 4°C, and used within 7 days.

Transfer rate of L. monocytogenes *from surface to cooked ham and vice versa (static condition)*

About 1 ml of bacterial cell suspension of range from 1 to 6 Log CFU/cm^2 respectively was dropped and spread to the stainless steel surface (20 by 20 cm^2) with the pipette. The uncontaminated cooked ham and RODAC plate was placed as samples of about 4 and 5 pieces respectively.

Prior to inoculation, stainless steel trays were sterilized in an autoclave 121°C for 25 min. Antimicrobial conveyor belt (Habasit) and normal conveyor belt (Habasit), were sprayed in a alcohol 70% ethanol for 30 minutes, and then air dried in a laminar hood.

The transfer rates of *L. monocytogenes* ATCC 35152 from cooked ham to surfaces were collected by laboratory experiments. For this, 2 ml of bacterial cell suspension of approximately 5 Log CFU/cm^2 was spread evenly with a pipette onto cooked ham and held at room temperature for 15 min to facilitate attachment. The level of micro organisms on this artificially contaminated cooked ham was determined by sampling a 2 by 2 cm^2 area with cotton swab (on the surface). Thereafter, the swab was suspended in 1: 10 of peptone saline solution and subsequently enumerated on selective media PALCAM.

The cooked ham was then put on a surface of stainless steel (20 by 20 cm^2), antimicrobial conveyor belt (20 by 10 cm^2) or normal conveyor belt (20 by 10 cm^2). After 10 seconds, the cooked ham portion was removed, and the surface area where the cooked ham portion had been placed was sampled using RODAC plates for 10 seconds. The measurements were done on wet and dry condition of surface. Wet condition samples were taken directly after artificial contamination. The dry condition samples were taken 15 minutes after artificial contamination.

Pressure and non pressure condition were applied to the product in order to press the product on the

surface. The pressure ~25 gram/cm^2 was applied on the contaminated product and continued on the uncontaminated cooked ham as well as RODAC plate. The methods were shown in Figure 1. The transfer of *L. monocytogenes* ATCC 35152 from surfaces to sliced cooked ham were collected and from additional experiments that were carried out using different procedures.

About 1 ml of cocktail was put onto surface stainless steel (20 by 20 cm^2), antimicrobial conveyor belt (20 by 10 cm^2) and normal conveyor belt (20 by 10 cm^2). The cooked ham was put onto contaminated surface on wet and dry condition of surface. Pressure and non pressure were applied at about 25 gram/cm^2, as well. The cooked ham slices were sampled by suspending them onto sterile peptone saline solution (1: 10) and subsequently homogenizing them in a stomacher for 60 s. The levels of pathogens were then determined using spiral plate methods.

Transfer rate of L. monocytogenes *from contaminated cooked ham to uncontaminated cooked ham through slicer blade (dynamic condition)*

Transfer of *L. monocytogenes* ATCC 35152 from inoculated cooked ham product to the slicer blade was replicated three times for each product. The cooked ham products were inoculated with *L. monocytogenes* ATCC 35152 cocktail to obtain approximately 5 Log CFU/cm^2, as determined by spiral plating. After 15 min at room temperature to allow the inoculum to absorb, three slices were cut from each product to artificially contaminate the blade. Measurement of possible contaminated area of slicer machine were done with sponge of 3M and were diluted in 1:5 PPS and enumerated with spiral plate methods onto PALCAM medium. Plates were incubated for 48 h at 37^{o}C.

Cooked ham debris was prepared for inoculating the blade of slicer machine by diluting 25 g of RTE-cooked meat product 1:10 in sterile water and blended in stomacher. Thereafter, 9 ml of cooked ham debris was mixed with 1 ml of pure culture. It was used to inoculate the blade with a cotton bud. The difference between the amount of bacteria in 1 ml of inoculums and on the cotton bud will remain on the blade.

This slicer blade was used to obtain 1 slice of cooked ham and each experiment was replicated three times. The slice was diluted 1:5 (wt/vol) in PPS and homogenized in a stomacher for 2 min, and the homogenate was spread on PALCAM media that were incubated at 37^{o}C for 48 h. It was determined as the number of cells per cm^2.

Exposure assessment model (scenario)

The pathway of recontamination were used several type of surface. In this case, some scenarios were made as:

1. Antimicrobial belt → stainless steel (table) → slicing machine → antimicrobial belt → packaging (recontamination occurred at post-heating process step on plant)
2. Antimicrobial belt → stainless steel (table) → slicing machine → normal belt → packaging (recontamination occurred at post-heating process step on plant)
3. Antimicrobial belt → packaging loaf form → slicing machine → retail (recontamination occurred at packaging step on plant)
4. Normal belt → stainless steel (table) → slicing machine → antimicrobial belt → packaging (recontamination occurred at packaging step on plant)
5. Normal belt → stainless steel (table) → slicing machine → normal belt → packaging (recontamination occurred at post-heating process step on plant)
6. Normal belt → packaging in loaf form → slicing machine-retail (recontamination occurred at packaging step on plant)
7. Slicing machine → antimicrobial belt (recontamination occurred at slicing step on plant)
8. Slicing machine → normal belt (recontamination occurred at slicing step on plant)
9. Slicing machine (recontamination occurred on retail)
10. Antimicrobial belt (recontamination occurred at slicing step on plant)
11. Normal belt (recontamination occurred at slicing step on plant)

The scenarios were combined with the prevalence and level of *L. monocytogenes* contamination at plant and the prevalence of unsatisfactory processing at retail from literature study (Table 1 to 3). These model structures were based on the possible contamination sites at plant (Table 4). Input distributions for respectable

Table 1. Prevalence of *L. monocytogenes* on cooked ham at plant with *Listeria* spp. positive.

Source	No of samples (n_i)	No. of positives	Prevalence	F(x)
Samelis *et al.*, 1999	10	1[a]	0.1	0.476
Samelis *et al.*, 1999	6	1[b]	0.167	0.762
Samelis *et al.*, 1999	4	1[c]	0.25	0.952
Total	20	3		

[a]Unsliced vacuum pack cooked meat
[b]Sliced product like ham
[c]Sliced cooked ham
[d]F(x) is the cumulative probability, with $\sum [(n_i)/(n + 1)]$, where n_i is the number of the samples taken in each reference, and n is the total number of samples taken for all references.

Table 2. Prevalence of unsatisfactory processing at retail

Source	No of samples (n_i)	No. unsatisfactory	Prevalence	F(x)[a]	Remark
Elson, 2004	159	35	0.22	0.062	Cleaning frequency not recorded
Elson, 2004	1997	450	0.23	0.842	Not cleaned periodically
Elson, 2004	403	120	0.3	0.999	Cleaning at the end of trading
Total	2559	605			

[a]F(x) is the cumulative probability, with $\sum [(n_i)/(n + 1)]$, where n_i is the number of the samples taken in each reference, and n is the total number of samples taken for all references.

Table 3. Level of *L. monocytogenes* on cooked ham at plant with *Listeria* spp. positive.

Level (CFU/g)	Calculated levels (CFU/cm2)[b]	n	F(x)[a]	Source
0.001 - 0.04	0.000162 – 0.0065	2901	0.96	USDA, 2003
0.041 – 0.1	0.0066-0.01621	125	0.99	USDA, 2003
Total		3026		

[a]F(x) is the cumulative probability, with $\sum [(n_i)/(n + 1)]$, where n_i is the number of the samples taken in each reference, and n is the total number of samples taken for all references.
[b]Calculated with formula = CFU / (width area / weight)
= CFU / (132.786/21.525)
= CFU/6.169 cm^2.
Width area of 1 side cooked ham (with diameter 13 cm and thickness 0.2 cm) was 132.786 cm^2

Table 4. *L. monocytogenes* contamination sites in meat plant

Contamination site	Reference
Processing environment	Nesbakken *et al.*, 1996
Tumbling machine	Samelis *et al.*, 1998; Samelis *et al.,* 1999
Slicing machine	Suihko *et al.*, 2002
Conveyor belt	Salvat *et al.*, 1995; Giovannacci *et al.*, 1999; Chasseignaux *et al*., 2001; Suihko *et al*., 2002
Skinning machine	Suihko *et al.*, 2002
Mould	Salvat *et al.*, 1995

Table 5. Input distribution

Variable	Distribution	Rank[b]
At plant P[c]	Risk Cumul (0.01, 0.30{0.1,0.167,0.25},{0.476,0.762,0.952})[a]	
P (unclean properties in retail)	Risk Cumul (0.22,0.30, {0.22, 0.23, 0.3},{0.062,0.842,0.999})[a]	
At plant N[d] (log)	Risk Cumul (-3,-1, {-2.988, -1.985},{0.96,0.99})[a]	
T1[e] (log %)		
Stainless steel		
Pre[f] = 1, M[g] = 1	Risk Normal(2.027[h], 0.003[i])	2
Pre = 0 , M = 0	Risk Normal(-1.90, 0.174)	2
Antimicrobial belt		
Pre = 1, M = 1	Risk Normal(0.854, 0.346)	2
Pre= 0 , M = 0	Risk Normal(0.268, 0.346)	2
Normal belt		
Pre= 1, M = 1	Risk Normal(1.583, 0.057)	2
Pre= 0 , M = 0	Risk Normal(1.025, 0.03)	2
Slicer machine		
Pre= 1, M = 0	Risk Normal(-0.51, 0.631)	2
T2[j] (log %)		
Stainless Steel		
Pre= 1, M = 1	Risk Normal(1.67, 0.025)	2
Pre= 0 , M = 0	Risk Normal(0.556, 0.10)	2
Antimicrobial belt		
Pre= 1, M = 1	Risk Normal(1.561, 0.199)	2
Pre= 0 , M = 0	Risk Normal(0.163, 0.04)	2
Normal belt		
Pre= 1, M = 1	Risk Normal(1.338,0.012)	2
Pre= 0 , M = 0	Risk Normal(1.29,0.087)	2
Slicer machine		
Pre= 1, M = 0	Risk Normal(0.286, 0.185)	2

[a]Using the mean of the range of log value
[b]Normal distribution rank by the Anderson-Darling test
[c]Prevalence
[d]Level of contamination
[e]Transfer rate of *L. monocytogenes* from contaminated ham onto uncontaminated surface
[f]Pressure which consist of 1 (with pressure application) and 0 (without pressure application)
[g]Moisture which consist of 1 (wet surface) and 0 (dry surface)
[h]Mean data which was obtained from experiment
[i]Standard deviation data which was obtained from experiment
[j]Transfer rate of *L. monocytogenes* from contaminated surface onto uncontaminated ham

Table 6. Prevalence of contaminated cooked ham and levels of contamination on cooked ham (estimated by mean Monte Carlo simulation using log-transformed data)

INPUT		Unit/ Formula	Monte Carlo Simulation									
			Sce. 1	Sce. 2	Sce. 3	Sce. 4	Sce. 5	Sce. 6	Sce. 7	Sce. 8	Sce. 9	Sce. 10	Sce. 11
P	Prevalence of contaminated ham	P	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117
P (unsatisfactory)	Prevalence of unsatisfactory at retail	Pu			0.231			0.231			0.231		
N (at plant)	Contamination levels on ham	Log CFU/cm²	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942	-2.942
T1	Transfer rate rates, ham onto surface	Log (%)											
	SS-(P=1, Mois=1)		2.03	2.03		2.03	2.03						
	SS-(P=0, Moist=0)		-1.90	-1.90		-1.90	-1.90						
	AB (P=1, Moist=1)		0.85	0.85	0.85	0.85			0.85			0.85	
	AB (P=0, Moist=0)		0.268	0.268	0.268	0.268			0.268			0.268	
	NB (P=1, Moist=1)			1.58		1.58	1.58	1.58		1.58			1.58
	NB (P=0, Moist=0)			1.05		1.05	1.05	1.05		1.05			1.05
	SLICING (P=1, Moist=0)		-0.51	-0.51	-0.51		-0.51	-0.51	-0.51	-0.51	-0.51	-0.51	
T2	Transfer rate rates, surface to ham	Log(%)											
	SS-(P=1, Moist=1)		1.67	1.67		1.67	1.67						
	SS-(P=0, Moist=0)		0.55	0.55		0.55	0.55						
	AB (P=1,Moist=1)		1.561	1.561	1.561	1.561			1.54817			1.58428	
	AB (P=0,Moist=0)		0.163	0.163	0.163	0.163			0.16533			0.16604	
	NB-High (P=1,Moist=1)			1.338		1.338	1.338	1.338		1.338			1.338
	NB-Low (P=0,Moist=0)			1.29		1.29	1.29	1.29		1.29			1.29
	SLICING (P=1, Moist=0)		0.286	0.286	0.286		0.286	0.286	0.286	0.286	0.286	0.286	
OUTPUT													
Pv	Prevalence of contaminated ham (at sales point)		0.1176	0.1176	0.0272[a]	0.1176	0.1176	0.0272[a]	0.1176	0.1176	0.0272[a]	0.1176	0.1176
N (at P=1, Moist=1)	Estimated levels on contaminated ham (at sales point) (Log CFU/cm²)	$Log(10^{n}.10^{T1/100}.10^{T2/100})$	-10.639	-10.13	-8.75[b]	-10.13	-9.63	-8.25	-8.75	-8.25	-7.16	-4.53	-4.02
Percentile 5%			-12.21	-11.38	-9.98	-11.42	-10.74	-9.35	-9.98	-9.35	-8.27	-5.12	-4.15
Percentile 95%			-9.01	-8.84	-7.48	-8.86	-8.49	-7.14	-7.48	-7.11	-6.03	-3.91	-3.92
N (at P=0, Moist=0)	Estimated levels on contaminated ham (at sales point) (Log CFU/cm²)	$Log(10^{n}.10^{T1/100}.10^{T2/100})$	-19.648	-17.76	-10.74[b]	-17.104	-15.88	-8.85	-10.74	-9.96		-6.51	-4.63
Percentile 5%			-21.26	-19.07	-11.96	-19.07	-17.07	-9.96	-11.98	-9.96		-7.11	-4.81
Percentile 95%			-18.05	-16.44	-9.47	-16.45	-14.68	-7.74	-9.47	-7.7		-5.88	-4.46

Scenario 1 : Antimicrobial belt → Stainless steel → Slicing machine → Antimicrobial belt
Scenario 2 : Antimicrobial belt → Stainless steel → Slicing machine → Normal belt
Scenario 3 : Antimicrobial belt → Slicing
Scenario 4 : Normal belt → Stainless steel → Slicing machine → Antimicrobial Belt
Scenario 5: Normal belt → Stainless steel → Slicing machine → normal belt
Scenario 6 : Normal belt → Slicing
Scenario 7 : Slicing → antimicrobial belt
Scenario 8 : Slicing → normal belt
Sceanario 9 : Slicing
Scenario 10 : Antimicrobial belt
Scenario 11 : Normal belt

a. Calculation of prevalence of *L. monocytogenes* calculation (in scenario 3,6 and 9) = $P_{retail} = P * P_{unsatisfactory}$

b. Calculation of estimated level of *L. monocytogenes* (in scenario 3) = $N_t = Log(10^{n}.10^{T1/100}.10^{T2/100})$

At P=1, M=1 → $N_t = Log(10^{n}.10^{T1 \text{ antimicrobial belt at pressure and wet } /100}.10^{T2 \text{ antimicrobial belt at pressure and wet } /100}.10^{T1 \text{slicing machine } /100}.10^{T2 \text{ slicing machine } /100})$

At P=0, M=0 → $N_t = Log(10^{n}.10^{T1 \text{ antimicrobial belt at non pressure and dry } /100}.10^{T2 \text{ antimicrobial belt at non pressure and dry } /100}.10^{T1 \text{slicing machine } /100}.10^{T2 \text{ slicing machine } /100})$

Table 7. Prevalence of contaminated cooked ham and levels of contamination on cooked ham (estimated by worst case using log-transformed data)

INPUT		Unit/ Formula	Worse Case										
			Sce. 1	Sce. 2	Sce. 3	Sce. 4	Sce. 5	Sce. 6	Sce. 7	Sce. 8	Sce. 9	Sce. 10	Sce. 11
P	Prevalence of contaminated ham	P	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
P (unsatisfactory)	Prevalence of unsatisfactory at retail	Pu			0.3			0.3			0.3		
N (at plant)	Contamination levels on ham	Log CFU/cm²	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79	-1.79
T1	Transfer rate rates, ham onto surface	Log (%)											
	SS-(P=1, Mois=1)		2.03	2.03	2.03	2.03	2.03	2.03	2.03	2.03	2.03	2.03	2.03
	SS-(P=0, Moist=0)		-1.699	-1.699	-1.699	-1.699	-1.699	-1.699	-1.699	-1.699	-1.699	-1.699	-1.699
	AB(P=1, Moist=1)		1.145	1.145	1.145	1.145	1.145	1.145	1.145	1.145	1.145	1.145	1.145
	AB (P=0,Moist=0)		0.666	0.666	0.666	0.666	0.666	0.666	0.666	0.666	0.666	0.666	0.666
	NB (P=1, Moist=1)		1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63
	NB (P=0,Moist=0)		1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046
	SLICING (P=1, Moist=0)		-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
T2	Transfer rate rates, surface to ham	Log(%)											
	SS-(P=1, Moist=1)		1.698	1.698	1.698	1.698	1.698	1.698	1.698	1.698	1.698	1.698	1.698
	SS-(P=0, Moist=0)		0.671	0.671	0.671	0.671	0.671	0.671	0.671	0.671	0.671	0.671	0.671
	AB (P=1, Moist=1)		1.576	1.576	1.576	1.576	1.576	1.576	1.576	1.576	1.576	1.576	1.576
	AB (P=0, Moist=0)		0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204
	NB (P=1, Moist=1)		1.351	1.351	1.351	1.351	1.351	1.351	1.351	1.351	1.351	1.351	1.351
	NB (P=0, Moist=0)		1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39
	SLICING (P=1, Moist=0)		0.487	0.487	0.487	0.487	0.487	0.487	0.487	0.487	0.487	0.487	0.487
OUTPUT													
Pv	Prevalence of contaminated ham (at sales point)		0.25	0.25	0.075[a]	0.25	0.25	0.075[a]	0.25	0.25	0.075[a]	0.25	0.25
N (at P=1, Moist=1)	Estimated levels on contaminated ham (at sales point) (Log FU/cm²)	Log($10^{a}.10^{T1/100}.10^{T2/100}$)	-8.153	-7.893	-6.602[b]	-7.893	-7.633	-6.342	-6.602	-6.342	-5.323	-3.069	-2.809
N (at P=0, Moist=0)	Estimated levels on contaminated ham (at sales point) (Log CFU/cm²)	Log($10^{a}.10^{T1/100}.10^{T2/100}$)	-16.611	-15.045	-8.453[b]	-15.045	-13.479	-6.887	-8.453	-6.887		-4.92	-3.354

Scenario 1 : Antimicrobial belt → Stainless steel → Slicing machine → Antimicrobial belt
Scenario 2 : Antimicrobial belt → Stainless steel → Slicing machine → Normal belt
Scenario 3 : Antimicrobial belt → Slicing
Scenario 4 : Normal belt → Stainless steel → Slicing machine → Antimicrobial Belt
Scenario 5: Normal belt → Stainless steel → Slicing machine → normal belt
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Scenario 10 : Antimicrobial belt
Scenario 11 : Normal belt

a. **Calculation of prevalence of** *L. monocytogenes* **calculation (in scenario 3,6 and 9) =** $P_{retail} = P * P_{unsatisfactory}$

b. **Calculation of estimated level of** *L. monocytogenes* **(in scenario 3) =** $N_t = \text{Log}(10^{a}.10^{T1/100}.10^{T2/100})$

At P=1, M=1 → $N_t = \text{Log}(10^{a}.10^{T1}$antimicrobial belt at pressure and wet /100 $.10^{T2}$ antimicrobial belt at pressure and wet /100 $.10^{T3}$slicing machine /100 $.10^{T2}$ slicing machine /100)

At P=0, M=0 → $N_t = \text{Log}(10^{a}.10^{T1}$antimicrobial belt at non pressure and dry /100 $.10^{T2}$ antimicrobial belt at non pressure and dry /100 $.10^{T3}$slicing machine /100 $.10^{T2}$ slicing machine /100)

parameter were generated from data sets in Table 5.

Transfer rate model of L. monocytogenes *from one surface to another surface*

The percent transfer can be described as below:

$$\frac{\text{CFU on target}}{\text{CFU on source}} * 100 = \text{percent transfer rate} \quad (1)$$

Based on equation 1 the estimated level of bacteria on the surface due to transfer rate from contaminated source can be described as below:

$$N_s = N_0 * T_1/100 \quad (2)$$

Where:

N_0 = the initial concentration of microorganism for the contamination source [cooked ham (cfu/cm^2)]

N_s = the number of bacteria transferred for the contaminated destination [surface (cfu/cm^2)]

T_1 = the transfer rate from contaminated source to surface (cfu/cm^2 of surface * cfu/cm^2 of cooked ham) * 100%

If contaminated surface was attached with uncontaminated product, the estimated level of bacteria will be:

$$N_t = N_s * T_2/100 \quad (3)$$

Where:

N_s = the initial concentration of microorganism for the contamination source [surface(cfu/cm^2)]

N_t = the number of bacteria transferred for the contaminated destination cooked ham(cfu/cm^2)]

T_2 = the transfer rate from contaminated source to cooked ham (cfu/cm^2 of cooked ham * cfu/cm^2 of surface) * 100%

In general equation 2 and 3 can be made in 1 formulation with substitution of the number of bacteria transferred on the surface (N_s) that is:

$$N_t = N_s * T_2/100$$

$$= N_0 * T_1/100 * T_2/100 \quad (4)$$

Each of these simple examples would require a series of calculations, using models that generally do not yet exist. At the same time, it should be realized that the real situation is considerably more complex than these simple examples showed, and that there may be literally dozens of cross-contamination possibilities in even a simple food process or meal preparation. Kusumaningrum *et al.* (2003) revealed that the contamination level on the product can be calculated as the equation below:

$$N_t = N_0 \text{ x } T_1/100 \text{ x } T_2/100 \quad (5)$$

Where:

N_0 = the initial concentration of microorganism for the contamination source [cooked ham (cfu/cm^2)]

N_t = the number of bacteria transferred for the contaminated destination[cooked ham (cfu/cm^2)]

T_1 = the transfer rate from contaminated source to surface (cfu/cm^2 of surface * cfu/cm^2 of cooked ham) * 100%

T_2 = the transfer rate from contaminated surface to uncontaminated product (cfu/cm^2 of cooked ham * cfu/cm^2 of surface) * 100%

Statistical analysis

For the statistical analysis, all results were primarily submitted to the normality test and variance homogeneity. The treatments were analyzed by ANOVA, considering significant for significant difference at P values ≤ 0.05 by using SPSS for Windows 98/NT/2000 release 12. This data will be combined with previous studied in order to get the prevalence and level contamination of cooked ham at sales point with scenario to describe recontamination

on direct contact between food and surface. Data sets for T_1 (transfer rate from contaminated ham onto uncontaminated surface) and T_2 (transfer rate from contaminated surface onto uncontaminated ham) were fitted using Bestfit (@Risk software version 4, Palisade, New York). The accuracy of fit of a distribution was ranked using Anderson-Darling test. A Monte-Carlo simulation with Latin-Hypercube sampling was used for simulation in 10,000 iterations. This simulation was done in order to get the distribution of probability and level contamination of *L. monocytogenes* on cooked ham from food contact surface.

Results

The transfer rate of *L.monocytogenes* from cooked ham onto stainless steel in static condition was higher than dynamic condition. It was 5.23% ± 1.13% and 4.29% ± 2.15 %. In the opposite direction, it was 2.057% ± 0.903% and 0.51% ± 0.45 %, respectively.

By using antimicrobial belt, we found the transfer rate of *L. monocytogenes* from cooked ham to surface (1st direction) and surface to cooked ham (2nd direction) were 7.15% ± 2.22% and 36.39% ± 1.58%, respectively. These were studied on P1M1 (with pressure, wet surface). It was higher than P0M0 (without pressure, dry surface). The results of P0M0 were 1.85% ± 2.22% and 1.46% ± 1.10% for 1st and 2nd direction.

By using normal belt, the study showed the transfer rates were 38.28% ± 1.14% and 21.78% ± 1.03% on P1M1. It was observed on 1st and 2nd direction. P0M0 showed to be lower for 10.59% ± 1.07% and 19.50% ± 1.22%, respectively.

By using stainless steel, the transfer rates on P1M1 were observed 106.41% ± 1.01% and 46.77% ± 1.06%. This result was shown by cooked ham to surface and the opposite direction. As comparison, the transfer rates on P0M0 were 0.01% ± 1.49% and 3.60% ± 1.26%, respectively.

The presence of *L. monocytogenes* on cooked ham on plant quantitatively was observed in 10% to 25%. These data was found on unsliced vacuum pack cooked meat, sliced product like ham and sliced cooked ham. These findings indicated that *listeriae* was associated with product handled after cooking. Post process contamination was likely to occur in the cutting room. Level of contamination *L. monocytogenes* on cooked ham was assumed from -3 to -1 log CFU/cm^2. The prevalence of unsatisfactory processing at retail was estimated from 1% to 30%.

The probabilities of cooked ham contaminated with *L. monocytogenes* as the result of recontamination were shown in Figure 1 and 2. The mean value of the probability of contamination with *L. monocytogenes* was 11.76% with 90% confidence interval from 2% to 25%. The highest level of *L. monocytogenes* on cooked ham due to recontamination was estimated in scenario 11 (recontamination occurred at slicing step on plant via normal belt) with the mean -4.02 log CFU/ cm^2 in a 90% confidence interval of -4.15 to -3.92 log CFU/cm^2. It means that 5% of the cooked ham may be contaminated with *L. monocytogenes* at a level 7.08/10^5 cm^2 or less but also 5% of cooked ham may be contaminated with 1.20 CFU / 10^4 cm^2 or more.

The lowest levels was found in scenario 1 (recontamination occurred at post-heating step on plant) in the dry condition and no pressure application (P0M0). The mean value was -19.65 log CFU/cm^2 with 90% confidence interval of -21.26 to -18.05 log CFU/cm^2.

The scenario of cooked ham processing calculated the highest and lowest estimation level. Scenario 11 (recontamination occurred at slicing step on plant via normal belt) showed the highest estimated level of *L. monocytogenes* on cooked ham. It was followed by scenario 10 (recontamination occurred at slicing step on plant via antimicrobial belt). It means conveyor belt is a favor site for *L. monocytogenes* to be attached on its surface and can make recontamination on food product. The scenarios showed bacteria can still transfer in dry condition. In worst case, it was calculated for 0.001550454 CFU/ cm^2 or 155 CFU/10^5 cm^2.

The highest level of *L. monocytogenes* on cooked ham due to recontamination was estimated in scenario 11 (recontamination occurred at slicing step on plant via normal belt) with the mean -4.02 log CFU/ cm^2 in a 90% confidence interval of -4.15 to -3.92 log CFU/cm^2. It means that 5% of the cooked ham may be contaminated with *L. monocytogenes* not only at a level 7.08/10^5 cm^2 or less but also at a level 1.20 CFU / 10^4 cm^2 or more. The lowest levels was found in scenario 1 (recontamination occurred at post-heating process step on plant) in the dry condition and without pressure. The mean value was -19.65 log CFU/cm^2 in a 90% confidence interval of -21.26 to -18.05 log CFU/cm^2.

Prevalence of *L. monocytogenes* at the retail level was shown at figure 5. The mean value of the probability of contamination with *L. monocytogenes* was 2.71% with 90% confidence interval of 0% to 6%. The highest level of *L. monocytogenes* contamination on cooked ham due to recontamination was estimated in scenario 9 (recontamination at slicing step) with the mean -7.17 log CFU/ cm^2 in a 90% confidence

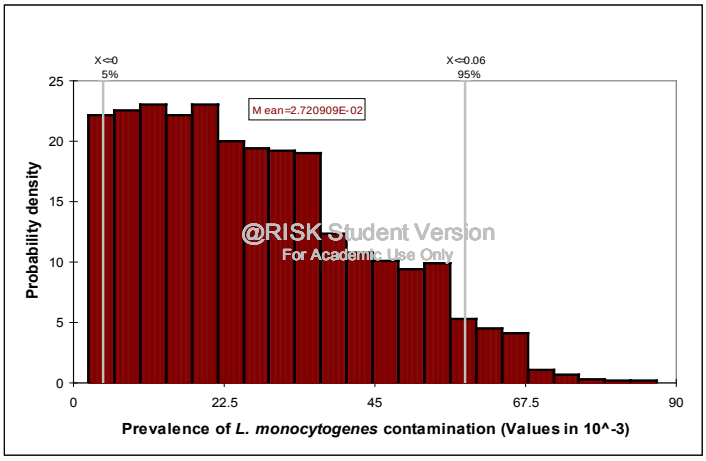

Figure 1. Prevalence of *L. monocytogenes* contamination (Scenario 11 – Pressure, wet)

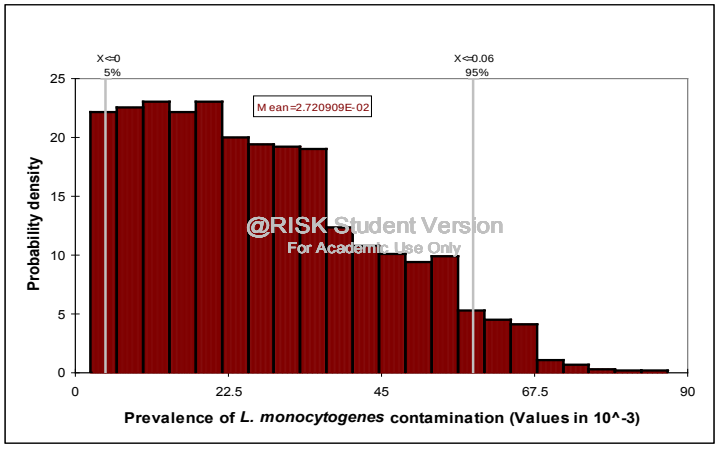

Figure 2. Prevalence of *L. monocytogenes* contamination (Scenario 1 – Non pressure,dry)

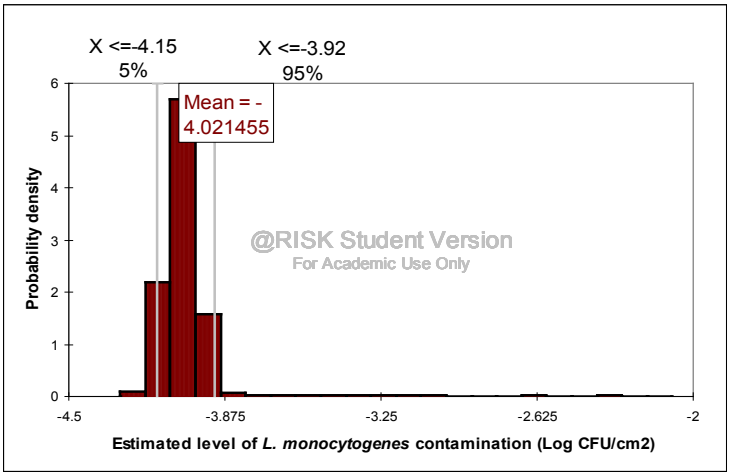

Figure 3. The highest estimated levels of *L. monocytogenes* (Scenario 11–Pressure, wet)

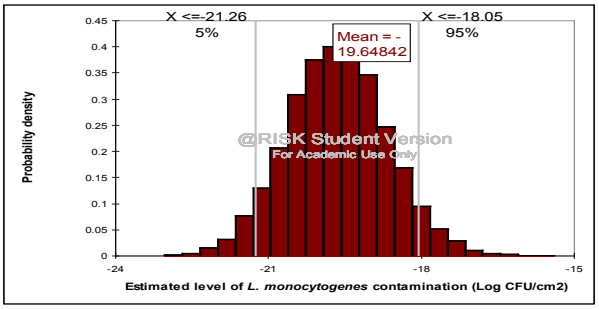

Figure 4. The lowest estimated levels of *L. monocytogenes* (Scenario 1– Non pressure,dry)

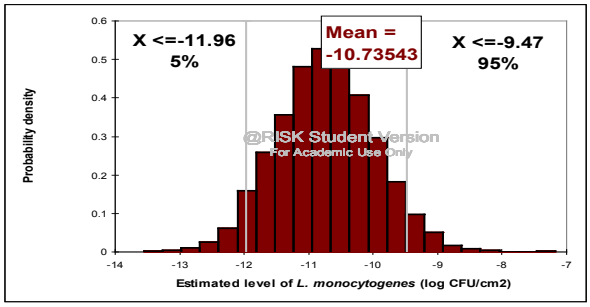

Figure 6. Lowest level of *L. monocytogenes* contamination on cooked ham at sales point (found on scenario 3 – P0M0) (antimicrobial belt - slicing at retail)

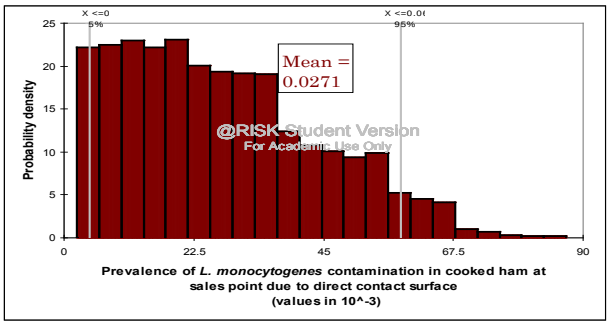

Figure 5. Prevalence of *L. monocytogenes* contamination at retail

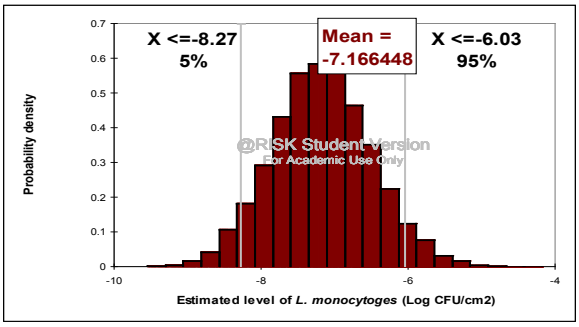

Figure 7. Highest level of *L. monocytogenes* contamination on cooked ham at sales point due to direct contact surface with slicer machine at the retail (found on scenario 9)

interval of -8.27 to -6.03 log CFU/cm^2. It means that 5% of the cooked ham may be contaminated with *L. monocytogenes* at a level 6.76 CFU /10^8 cm^2 or less but also 5% of cooked ham may be contaminated with 9.33 CFU/ 10^7 cm^2 or more. The lowest levels were found in scenario 3 (antimicrobial belt - slicing at retail). The mean value was -10.73 log CFU/cm^2 in a 90% confidence interval of -11.96 to -9.47 log CFU/cm^2. It was observed that prevalence of *L. monocytogenes* on ham at sales point of retail's processing was 11.76% with 90% confidence interval 2% to 25% (Monte Carlo simulation) and 7.5% (worst case). These results agreed with the studies of Elson (2004) and Wong (2005) studies which were 0.00035 and 0.333. The prevalence of *L. monocytogenes* at sales point without retail's processing was 25% (worst case). In Monte Carlo simulation, it was found 2.72% with 90% confidence interval 0% to 6%.

Discussion

Scenarios were calculated and the consideration was based on application of pressure and moisture level due to its significance difference (P<0.05) on transfer rate. Sattar *et al.* (2001) found that the bacterial transfer from moist donor fabrics was always higher than that to and from dry ones. Moreover the transfer rate would be higher if pressure was applied (Vorst, 2006).

Bloomfield and Scott (1997) proposed that the risk of food borne illness associated with cross-contamination depend on two factors: the level of contamination on the surface and the probability of its transfer to the foods being consumed. The probability of bacterial transfer between surfaces or between surface and food is poorly characterized. Kusumaningrum *et al.* (2003) studied that *Salmonella enteridis, Staphilococcus aureus* and *Campylobacter jejuni* were still viable on dry stainless steel surfaces for hours (*C. jejuni*) or days after contamination (*S. enteridis* and *S. aureus*) depended on their initial number.

Other factor can influence the transfer rate of bacteria has been studied, such as surface type (Sinde and Carballo, 2000). *L. monocytogenes* was stronger to attach polyurethane than stainless steel (Sinde *et al.*, 2000). Thus, *L. monocytogenes* can contaminate food processing plants for extended periods of time, and the contamination is often caused by a few dominating strains or persistent strains (Autio

et al., 1999; Lunden, 2002). This happening make opportunity for the bacteria to attach for a long time in following process due to inadequate cleaning and can persist. It may make a recontamination. The sliced product would be a risk product due to a larger area of the surface and can contact directly with equipment surface. Lunden (2002) revealed that conveyor is generally contaminated after slicing process. This means slicing process could be a critical point for *L. monocytogenes* to contaminate cooked ham product. The probabilities of cooked ham contaminated with *L. monocytogenes* as the result of recontamination from plant were 11.76 % with 90% confidence interval of 2% to 25%.

The recontamination on several scenarios indicated that the highest was found at slicing step via normal belt in wet condition-pressure application for 0.00033572 CFU/cm^2. Midelet and Carpentier (2002) reported that *L. monocytogenes* attached more strongly to polymer than stainless steel. Conveyor belt materials are polyvinyl chloride and polyurethane. Some microorganism, including *L. monocytogenes* strains, adheres in higher numbers to more hydrophobic materials (Cunliffe *et al*., 1999; Sinde and Carballo 2000; Donlan 2002). Rubber and plastic are hydrophobic materials; meanwhile glass and stainless steel are hydrophilic materials (Sinde *et al*., 2000; Donlan, 2002).

In worst case, it was 0.001550454 CFU/cm^2. It can be described that persistence of *L. monocytogenes* in equipment of cooked ham production becomes important factor. The scenario showed that recontamination in dry condition can occur in longer time to do recontamination than in wet condition.

The lowest levels was found in scenario 1 (recontamination occurred at post-heating process step on plant via antimicrobial belt) in the dry condition and without pressure. The mean value was -19.65 log CFU/cm^2 in a 90% confidence interval of -21.26 to -18.05 log CFU/cm^2.

Antimicrobial belt was impregnated by silver ion which can prevent the attachment of bacteria. Silver ions are used to coat surface of food processing properties. Silver ion is an inorganic anti-microbial. It controls a wide variety of pathogens in several ways such as destroying the cell walls of microbes, disrupting the microbe growth by interrupting RNA replication, interfered the respiration of bacteria, inhibition the production of enzymes in assimilation of nutrients. Moreover silver ion is an inorganic biocide which can be useful in managing a wide range of bacteria and moulds (Anonymous, 2001).

Level of contamination of cooked ham in the retail (9th scenario) was 5.025x10^{-6} CFU/cm^2 for worst case. It was very low when compare with Elson (2003) about $10^4 – 10^5$ CFU/gram in 1 sample of 2894 and Wong (2005) about 50 CFU / gram in 1 sample of 3. The high contamination was observed due to food contact surface and abuse temperature. In this study the recontamination mentioned on food contact surface only. Thus, it will be interesting if the future study can study further more on the temperature abuse and can include this factor into model's input.

Elson (2005) revealed that poor microbiological quality was associated with pre-sliced meats, infrequent cleaning of slicing equipment and poor control of practices that may lead to cross contamination. In order to reduce level of *L. monocytogenes* from plant and retail, some action should be done.

1. Adequate cleaning on the susceptible equipment

2. Applying the proper methods by using ham Good Handling Process and/or HACCP system.

Conclusions

Factors that had effect on transfer rate of *L. monocytogenes* were application of pressure, moisture level of surface, source and destination of transfer (include type of surface), inoculums size, and static-dynamic condition. Inadequate cleaning can cause recontamination on the cooked ham from plant to at sales point in retail. Some actions that can be done are adequate cleaning of equipment cooked ham production at retail and plant and Good Handling Process application and/or HACCP application. Other product should be studied in order to get more data of transfer rate and can be implied with the real condition in the market. Deli meat has a high risk of listeriosis. It is therefore type of deli meat should be studied as a different character of its tissue. These data can be put in the scenario in order to describe with the real condition.

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