Table 2. MIC distribution and resistance rates of *C. jejuni* isolated from humans and poultry in Thailand

Source (No. of strains)	Antimicrobial drugs[a]	No. of isolates for each of the following MIC values:											Breakpoint of MIC concentration	MIC_{50}/MIC_{90} (mg/liter)	No.(%) of resistant isolates
		0.25	0.5	1	2	4	8	16	32	64	128	≧128	(mg/liter)		
Humans (70 strains)	NA	0	0	0	0	0	1	2	24	25	14	4	≧32	32/128	67(96)[b]
	CPFX	0	3	0	0	0	28	17	16	6	0	0	≧4	16/32	67(96)[b]
	ABPC	0	0	0	10	10	14	16	10	10	0	0	≧32	16/64	20(29)[c]
	TC	0	12	7	11	0	0	6	28	6	0	0	≧16	2/32	40(57)[b]
	EM	0	1	5	37	17	5	3	2	0	0	0	≧8	2/8	10(14)[c]
Poultry (69 strains)	NA	0	1	1	1	3	4	6	16	18	16	3	≧32	32/128	53(77)[b]
	CPFX	10	3	3	0	0	10	33	8	1	1	0	≧4	16/32	53(77)[b]
	ABPC	0	0	1	2	11	33	7	8	5	2	0	≧32	8/64	15(22)[c]
	TC	1	1	4	9	18	18	7	8	2	1	0	≧16	8/32	18(26)[b]
	EM	1	5	9	27	15	4	4	2	1	1	0	≧8	2/16	12(17)[c]

[a] NA: nalidixic acid, CPFX: ciprofloxacin, ABPC: ampicillin, TC: tetracycline, and EM: erythromycin.

[b] Significant differences ($P<0.01$) between human and poultry isolates.

[c] No significant differences ($P<0.01$) between human and poultry isolates.

Table 3. Counts of *C. jejuni* with different resistance profiles

Profile	No. of isolates	
	Humans	Poultry
No resistance demonstrated		11
Resistance to one agent[a]		
TC only	3	5
Resistance to two agents[a]		
NA-CPFX	20	26
Resistance to three agents[a,b]		
NA-CPFX-ABPC	5	4
NA-CPFX-TC	22	4
NA-CPFX-EM	5	8
Resistance to four agents[a,b]		
NA-CPFX-ABPC-TC	10	7
NA-CPFX-ABPC-EM		2
Resistance to five agents[a,b]		
NA-CPFX-ABPC-TC-EM	5	2
Total	70	69

[a] NA: nalidixic acid, CPFX: ciprofloxacin, ABPC: ampicillin, TC: tetracycline, and EM: erythromycin.

[b] Multiresistant isolate because combined resistance to NA-CPFX in an isolate does not count.

those of NA-CPFX-EM (8 strains), NA-CPFX-ABPC-TC (7 strains), TC only (5 strains), NA-CPFX-ABPC (4 strains), NA-CPFX-TC (4 strains), NA-CPFX-ABPC-EM (2 strains), and NA-CPFX-ABPC-TC-EM (2 strains). When combined resistance to NA-CPFX in an isolate did not count as multiresistance, a larger number of multiresistant isolates were found in human samples than in poultry samples because human isolates (47/70) (67%) and poultry isolates (27/69) (39%) were found to be multiresistant. The use of antimicrobial agents in animals in Thailand was regulated by the Thai Department of Livestock Development only in 2003, and not all farms in the country have met the new standards for antimicrobial drug use. In addition, the use of antimicrobial agents is unrestricted in Thailand, and many people self-treat diarrhea with antimicrobial drugs. In Thailand, previous reports regarding antimicrobial susceptibility have shown that the prevalence of fluoroquinolone-resistant human and poultry *Campylobacter* isolates was relatively high as compared to those reported in the studies conducted in the U.S., Europe, and Japan (1, 3, 10, 13, 14, 26). In addition, Padungtod et al. (26) suggested that the rate of prevalence of multiresistant and CPFX- and TC-resistant *Campylobacter* isolates from hospitalized humans and chicken meat in the market was higher than that in the farm, and the reasons for this were presumed to be that the organism entered the market directly from the chicken meat, cross-contamination at the marketplace, and consumption of the contaminated food. Our result was the same: for example, the rate of prevalence of multiresistant and CPFX- and TC-resistant *C. jejuni* isolates from humans was found to be higher than that of the isolates from chickens in the poultry farms.

Figure 1 shows the RAPD-PCR profiles of the 8 isolates having Penner serotype B and NA-CPFX-resistant *C. jejuni* and 7 isolates having Penner serotype C and NA-CPFX-TC-resistant *C. jejuni*. DNA band patterns of all strains were different. Our results obtained by RAPD-PCR analysis suggest that human and poultry

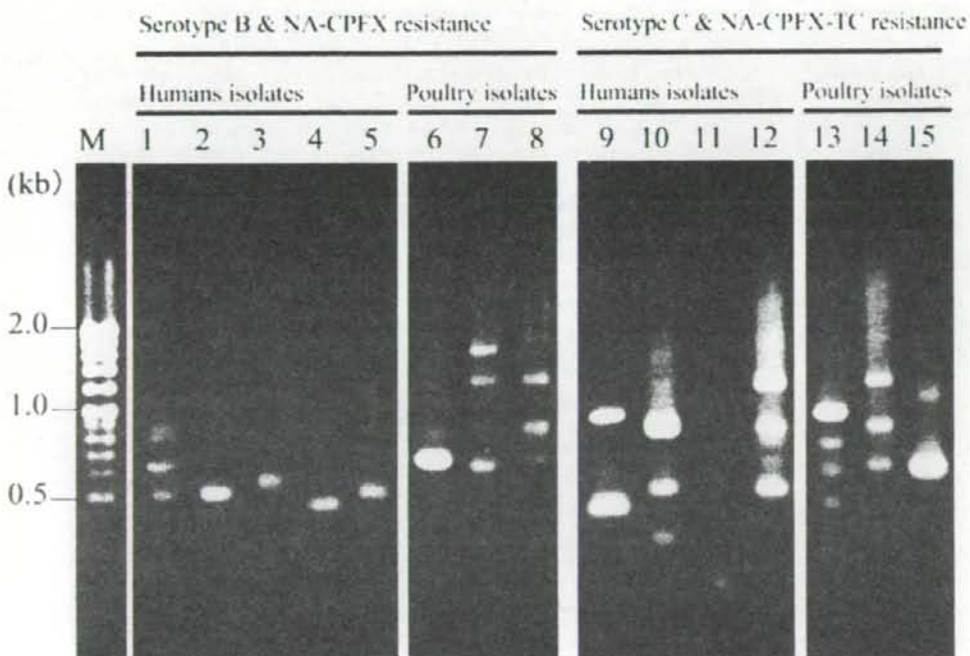

Fig. 1. Ethidium bromide-stained 1.5% (w/v) agarose gel showing RAPD-PCR products. Lanes: (M) DNA molecular mass marker (100-bp ladder), (1–8) RAPD-PCR profiles of 8 isolates having Penner serotype B and NA-CPFX-resistant *C. jejuni* strain, and (9–15) 7 isolates having Penner serotype C and NA-CPFX-TC-resistant *C. jejuni* strain. DNA band patterns of all the strains were different.

isolates were genetically different.

A total of 139 isolates show 4 types of nucleotide sequences in *gyr A* such as H_{81}(CAC)-T_{86}(ACA), H_{81}(CAT)-T_{86}(ACA), H_{81}(CAC)-I_{86}(ATA), and H_{81}(CAT)-I_{86}(ATA). Our strains had a point mutation in the Thr-86 codon (wherein T_{86} was replaced with I_{86}) that is linked to quinolone resistance. In 67 (96%) of the 70 human isolates and 53 (77%) of the 69 poultry isolates possessing the point mutation in the Thr-86 codon, T_{86} had been replaced with I_{86} and all the strains showed resistance to both NA (MIC concentration: ≧32 mg/liter) and CPFX (≧8 mg/liter). The results were found to be similar to those previously reported using molecular studies (7, 9, 17, 18, 32, 38). No point mutation was found in the Ala-70 and Asp-90 codons. In addition, a nucleotide substitution was found [CAT and CAC found in the His-81 codon (H_{81})], but this mutation was synonymous. The strain NCTC11168 that has been listed complete genome in GenBank (Accession no. AL111168) shows the H_{81}(CAC)-T_{86}(ACA) mutation type of *gyr A*, and only one human strain and 3 poultry strains had the same type to the strain NCTC11168 in this study. There were significant differences ($P<0.01$) in the prevalence of mutation in the Thr-86 codon in the isolates between human and poultry.

The chickens used in this study were administered coccidiostats (amprolium) and antimicrobial agents (including sulfamethoxazole and enrofloxacin) during the raising period. In the United States, from September 2005, the Food and Drug Administration (FDA) has decided to prohibit the use of the antibiotic enrofloxacin for poultry because this entails the risk of the promotion of fluoroquinolone-resistant bacteria that can be harmful to humans (http://www.fda.gov/oc/antimicrobial/baytril.html). The use of enrofloxacin as a feed additive in chicken farms may be one of the causative factors for the appearance of several fluoroquinolone-resistant *C. jejuni* strains with a point mutation.

Case control studies on *C. jejuni* frequently pointed toward chicken or poultry as the most important source of *Campylobacter* infections (20). However, studies performed in several countries have also pointed toward pets in the household, contaminated drinking water, and cattle liver as the source of infection in humans (8, 20, 33). In our studies carried out in Thailand, we found

that the predominant serotypes in humans were B (9 strains) and C (8 strains), of which the highest percentage (96%) of isolates possessed a point mutation in the Thr-86 codon of the *gyr A* gene and showed resistance to NA and CPFX. On the other hand, in poultry, the predominant serotype was A (16 strains) and C (7 strains), of which the highest percentage (77%) of isolates showed T_{86} to I_{86} replacement and showed resistance to NA and CPFX. In Thailand, the strains with substitution in the *gyr A* gene ($T_{86} \rightarrow I_{86}$) were already widespread.

The rate of antimicrobial drug resistance in isolates obtained from human and poultry appears to be slightly different. In addition, by using the RAPD-PCR analysis, human and poultry isolates were found to be genetically different. Previously, a report in Thailand suggested that contamination after carcasses are shipped off from the slaughterhouse is an important factor contributing to the spread of resistant bacteria in the human food chain (26). Poultry has been considered a potential vehicle for the transmission of *Campylobacter* food poisoning; however, other factors may also be responsible for the spread. To have a better understanding of the epidemiology of *Campylobacter* in Thailand, studies on the prevalence of *Campylobacter* present in humans, animals, retail meat, and environment need to be carried out.

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講　座

公衆衛生上問題となる動物由来感染症
—特に家畜が感染源となる感染症について—

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「動物由来感染症」は，「人畜共通感染症」，「人獣共通感染症」，「ズーノーシス」と同義語である。近年は人の健康の問題という観点にたって，「動物由来感染症」という言葉が使用されている。World Health Organization（WHO：世界保健機構）では動物由来感染症は「脊椎動物と人の間で自然に移行するすべての病気または感染」と定義している。

現在，WHOのZoonoses and veterinary public healthのホームページ（http：//www.who.int/zoonoses/en/）では，動物由来感染症として150種以上の起因病原物質が紹介されている。また，わが国で平成16年度に編さんされた「臨床獣医師のための共通感染症」（(社) 日本獣医師会発行）では起因病現物質としてウイルスが24種，プリンオンが1種，細菌・リケッチア等が26種，真菌が2種，原虫が4種，蠕虫が19種，節足動物が3種の合計79種の感染症が掲載されている。

起因病原物質の本来の病原巣（reservoir）は動物であることが多く，その起因病原物質によってreservoirが感染しても，症状を起こさないか軽く，致死性の臨床症状に移行しない。しかし，人がその起因病原物質に感染した場合，人は重篤な症状を示すことが多い。

伝播様式は，感染源である動物から直接人にうつる「直接伝播」と，感染源動物と人間との間に何らかの媒介動物（vector：ベクター）や環境・動物性食品など存在する「間接伝播」に大別される。「直接伝播」する感染症としては狂犬病（咬傷），猫引っかき病（ひっかき傷），レプトスピラ（排泄物：尿），結核やインフルエンザ（咳やくしゃみ）等がある。「間接伝播」でベクターが介在する感染症としては日本脳炎（蚊），ペスト（ノミ），日本住血吸虫（貝）等，環境が介在するものはクリプトスポリジウム（水系），炭疽（土壌）等，動物性食品が介在するものは腸管出血性大腸菌157，サルモネラやカンピロバクター（食肉）等である。

食中毒菌である腸管出血性大腸菌O157，サルモネラやカンピロバクター等も動物由来感染症であり，家畜はそれらの病原体を腸管内に保菌しており，人はその動物との接触による病原体の暴露や，病原体に汚染された食肉の喫食等により感染することがある。平成19年（2007年）に発生した1,289件の食中毒のうちカンピロバクターは第1位（32％：416件），サルモネラは第3位（10％：126件）であり，動物由

来感染症起因菌であるこれらの細菌は食品衛生上きわめて重要な起因病原物質である。

近年，新型インフルエンザへの変異が危惧されている高病原性鳥インフルエンザも，動物由来感染症であるが，今回，特に食品の安全・安心の確保を基本として，日本で存在し，または，これから注目されると予想され，そして，その原因が家畜由来となる場合がある動物由来感染症の発生状況や病原物質の家畜の保有状況等について紹介する。

腸管出血性大腸菌

腸管出血性大腸菌（Enterohemorrhagic *Escherichia coli*：EHEC）は，人に重篤な症状を起こし，公衆衛生上問題となる感染症起因菌である。人の腸管出血性大腸菌感染症から分離されるEHECの血清型はO157が最も多く，次いでO26，O111の順になっている[50]。EHECは，反芻動物，特に牛の消化管内に保菌されていることが報告されていることから[11, 16, 18, 23, 25, 31, 38, 52]，牛や牛肉は人のEHEC感染症に関わりが深いと考えられている。

本感染症の発生の多くは，本菌に汚染された食品の喫食によるものであるが，牧場を訪問した多くの幼児がO157感染症に罹患することもあり[9]，本菌に感受性の高い小児や老人が牛と接触したり，牧場で遊ぶ場合は注意が必要である。

人の症状：100個程度の少量の菌で感染が成立する。本菌の摂取後，2～9日（多くは2～5日）の潜伏期間の後に腹痛，下痢（血便）などを主症状とする腸管感染症を起こす。典型的な症状は，激しい腹痛を伴う頻回の水様便があり，続いて血便が認められる（血便は出血に近い場合もあり）。発症者の約5％は，溶血性尿毒症症候群（HUS）や脳症（けいれんや意識障害）などの合併症を起こし，時には死亡することもある。同じものを喫食しても，無症状の者から重篤の症状を起こす者まで様々である。

家畜の症状・保菌状況：牛は通常無症状で，本菌は，糞便や胃内容から容易に分離される。本菌の検出状況について表1に示す。肥育牛の保菌率は，繁殖牛や搾乳牛のそれより高率であることがうかがえる。わが国の肥育牛の飼育形態は穀物肥育(grain fed)で独特であり，第一胃内が酸性になる（アシドーシス）傾向がある。腸管出血性大腸菌O157は酸性に耐性を持っていることから酸性条件下では選択的に本菌が生育すること[12, 34]等が，肥育牛の保菌率が高い理由と考えられている。

牛の糞便中のEHEC菌量は＜10^2～10^9 cfu/gまで様々であり[12]，EHECを10 cfu/gになるように添加した牛糞便をビニール袋で5℃と25℃で保存した場合，EHECは4週目まで，15℃で保存した場合は8週目まで生存すること，10^3や10^5 cfu/gになるように添加した牛糞便を25℃保存した場合は12週目まで，15℃保存した場合は18週目まで，5℃で保存した場合は14週目まで生存する[13]。牛糞便中のEHECは，長期間環境中でも生存するので注意が必要である。

予防策：特に本菌の感受性の高い小児や高齢者が牛と接触した後には，石けんと流水でよく手を洗うことが重要である。経済動物である牛に乾草のみを給餌することは難しいと思われるが，牛に乾草を給餌するとO157の定着が抑制され[1]，さらに実験的にO157を保菌した牛に乾草を給餌するとO157の排菌量が減少すること[33]が報告されている。

クリプトスポリジウム

クリプトスポリジウム症は，クリプトスポリジウム属の寄生により腸炎症状を示す寄生虫病で，糞口(fecal-oral)感染の伝播様式をとる。人では*C. parvum*（主な宿主：牛，羊，山羊，人）が主要な病原体で，*C. parvam*は遺伝子学的に人に由来するヒト型（Genotype 1あるいは*C. hominis*：主な宿主は人，サル）と人を含む広く哺乳動物に感染するウシ型（Genotype 2あるいはbovin genotype）に分類される。その他，*C. canis*（主な宿主：犬），*C. felis*（猫），*C. meleagridis*（七面鳥，人），*C. muris*（げっ歯類，フタ

表1　腸管出血性大腸菌(EHEC)の検出状況

動物・品種等	調査頭数	陽性頭数(%)[a]	血清型	検出頭数(%)	内訳			調査年等	文献
					第一胃内容	盲腸内容	直腸便		
牛・黒毛和種	120	3(2.5)	O157	3(2.5)	-[b]	·	3	2008	23)
牛・黒毛和種	46	4(8.7)	O157	3(6.5)	1	2	·	2004	16)
			O111	1(2.2)	0	1	·		
牛・交雑種	217	12(5.5)	O157	6(2.8)	1	5	·		
			O26	5(2.3)	1	4	·		
			O111	1(0.5)	0	1	·		
繁殖牛・黒毛和種	14	0(0)	O157	0(0)	·	0	·	1998-2001	38)
搾乳牛・ホルスタイン種	82	0(0)	O157	0(0)	·	0	·		
肥育牛・黒毛和種	186	12(6.5)	O157	12(6.5)	·	12	·		
肥育牛・交雑種	237	14(5.9)	O157	14(5.9)	·	14	·		
肥育牛・ホルスタイン	156	9(5.8)	O157	9(5.8)	·	9	·		
搾乳牛・ホルスタイン種	112	2(1.8)	O157	2(1.8)	·	·	2	1998	25)
肥育牛・ホルスタイン	16	0(0)	O157	0(0)	·	·	0		
肥育牛・交雑種	313	20(6.4)	O157	20(6.4)	·	·	20		
肥育牛・黒毛和種	95	13(13.7)	O157	13(13.7)	·	·	13		
肥育牛	90	38(42.2)	その他[c]	—[d]	·	—	38[e]	1993-1994	31)
		3(3.3)	その他[c]	—		3[e]			
成牛	2,507	8(0.3)	O157	5(0.2)	·		5	1993 夏	18)
			O26	3(0.1)	·		3		
成牛	2,407	3(0.1)	O26	2(0.1)	·		2	1993-1994 冬	
			O157	1(0.04)	·		1		
牛・交雑種	59	6(10.2)	O157	5(8.5)	·	5	·	1993	11)
			O146	1(1.7)	·	1	·		
牛・ホルスタイン種	66	1(1.5)	O:157	1(1.5)	·	1	·		

a）EHECが検出された頭数
b）実施せず
c）O157は検出されず。血清型ではO45:NMが最も多く、ついでO2:H29、O22:NM、O45:H25、O45:H8、O109:NM等が検出
d）記載なし
e）盲腸と直腸を同時に検索した50頭のうち直腸は21頭(42%)、盲腸は2頭(4%)からSTECが分離

コプラクダ)等も人にクリプトスポリジウム症を引き起こす[34, 35]。

1976年に初めて人の感染が報告された原虫疾患[34]で，それまで確認されていなかったが，現在最も注目される水系感染症のひとつであり，発展途上国のみならず先進国を含む世界中で確認されている[8, 36]。人への感染は，感染動物から便とともに排出されたオーシストが環境(主に水)を介して人の口に入ることで成立する。オーシストは塩素系薬剤にも高い抵抗性を示すことから，通常の水道法の塩素消毒法である，「給水栓における水が遊離残留塩素を0.1 mg/L(結合残留塩素の場合は，0.4 mg/L)以上保持するように塩素消毒すること」では死滅しない。よって，生水のみならず，水道水やプールのようなレクリエーション水域がオーシストによって汚染され，それを摂取してしまうと感染が成立する。

人の症状：オーシストは1個でも感染が成立する可能性があり[10]，オーシスト摂取後，約4～10日で，水様性下痢，胃痛，悪心，嘔吐，倦怠感，まれに発熱が認められる。下痢は軽度のものから，1日に20

回以上のものまであり，約２週間程度続く。この下痢便中にはオーシストが含まれており，下痢のピーク時には１日に約10億個排出する[17]。人によっては不顕性感染を示し，症状が無くても，オーシストを排出することもある。

家畜の症状・保有状況：動物により症状は異なるが，一般的に若齢の家畜で発症する。牛や馬は水溶性下痢，脱水，食欲低下などがおこるが，豚・猫はほとんど無症状で経過する。水様性下痢は通常１～２週間で徐々に回復する。

牛における感染は，北海道から九州まで広く流行しており，３カ月齢以下の子牛の*C. parvum*感染率は25.5％（147頭/567頭）で，成牛では*C. parvum*は確認されていないが，*C. muris*が3.7％（８/217）から確認されている[56]。

とちく場搬入牛の糞便検査では0.2％（１頭/582頭）から*C. parvum*を，1.5％（９頭/528頭）から*C. muris*を検出し，さらに，クリプロスポリジウム陽性牛が存在している農場の牛の19.0％（22頭/116頭）から*C. muris*または*C. parvum*を検出した報告[37]もあることから，本症は特定の農場で保有していると思われる。

予防策等：人や家畜の治療法は対症療法のみを行うにすぎない。オーシストには消毒剤がほとんど効かない。糞便に排泄されたオーシストを経口的に摂取することで感染が成立するので，予防対策は衛生的な生活習慣の徹底にほかならない。

発症牛の下痢便中にはオーシストが含まれており，１日に約200億個排出する[17]。また，牛糞からの堆肥製造過程においてもオーシストが検出されなくなるまで44日間を要するという報告[14]もある。よって，家畜，特に子牛の糞便中にオーシストが存在する可能性を考慮にいれ，本原虫の感受性の高い小児が家畜の糞便と接触した後には，石けんと流水でよく手を洗い，オーシストが口に入らない措置を講じることが重要である。また，人が本症に罹患すると感染力が非常に強く，容易にヒトーヒト感染が成立してしまうので，患者の介護等を行う場合は，感染症の標準予防策（stanndard precautions）を確実に実施しなければならない。

ストレプトコッカス　スイス

2005年７月中旬から８月下旬にかけて，中国四川省で215名が*S. suis*感染症に罹患し，39名が死亡した事例[58]は記憶に新しい。豚のレンサ球菌症は，養豚を産業としている主要な国では発生が認められ，日本においても本菌は豚から分離されている[3, 20, 21, 22]。本菌は健康な豚の口蓋扁桃や上部気道に保菌されており，容易に他の豚に伝播する。本菌は35の血清型があり，人の感染症報告が多い血清型は２型であるが，他の血清型による感染例も確認されている[4]。中国四川省の事例は，人および豚から血清型２型菌が分離されている。

人では養豚業従事者，獣医師，食肉処理業従事者など，職業上豚や豚肉と接する機会の多い人に感染することがある。LUTTICKENら[27]は，44例の*S. suis*感染症（髄膜炎は39例，敗血症は５例）のうち40例は発症前に豚や生の豚肉との接触があると報告している。日本国内では養豚業者の化膿性髄膜炎の発症例[30]の他，いくつかの報告がある[6, 15]。群馬県衛生環境研究所においても，細菌性髄膜炎と診断された豚肉加工処理業従事者の髄液より*S. suis*血清型２型を分離しており，*S. suis*による人の感染症は意外と多いかもしれない。

人の症状：詳細は不明であるが，*S. suis*に感染した豚やその豚の生肉との接触した際に，皮膚の外傷を介して感染すると推定されている。ヒトーヒト感染はない。潜伏期間は数時間から数日で，細菌性髄膜炎になることが多く，発熱，頭痛，聴覚障害，運動失調等の症状を認める。中国の事例[58]においても，ヒトーヒト感染は証明されておらず，豚から人が感染する。この事例では，*S. suis*（血清型２型）に感染した215名のうち髄膜炎は102名で１名が死亡，毒素性ショック症候群（TSS：toxic shock syndrome）は61名で38名死亡，敗血症は52名で死亡者は０名で，死亡例の多くはTSSであった。TSS患者の潜伏期間は

1.6日（9時間～9日），TSSを除く患者の潜伏期間は2.5日（6時間～14日）であった。

家畜の症状・保菌状況等：*S. suis*は主に日和見感染症をおこし，感染豚は敗血症，髄膜炎，心内膜炎，肺炎，関節炎など様々な病態を示す。ストレプトコッカス属菌は扁桃から容易に分離される。また，豚心内膜炎病変部の25.7%（127/495）から*S. suis*が分離されている[22]。

予防策等：本感染症は創傷感染といわれているので，養豚業従事者，獣医師，食肉処理業従事者など，職業上，豚や豚肉と接する機会の多い人は，傷口があればそれを覆う措置を講ずる（手袋をする）ことである。また，難聴や化膿性脳炎の疑いとなった場合は，医師に「職業上，豚や豚肉と接する機会が多い」という情報を伝えて頂きたい。一般的な注意点として，①手指などに外傷のある人は，生の豚肉を扱う際に手袋を着用する。②豚肉を調理した後に，手洗いと器具の洗浄を徹底する。③豚肉は表面のみならず内部まで火を通した上で食べる。などに留意する必要がある（http://www.niah.affrc.go.jp/disease/S_suis/S_suis.html）。

カンピロバクター

カンピロクバターのうち，*C. jejuni/coli*は主に食中毒起因菌として食品衛生上重要で，本菌による食中毒はわが国だけでなく多くの国々で頻発している[7,35,48]。*C. fetus*は牛の流産菌として家畜衛生上重要であるが，散発的に人に敗血症を起こすことが報告されている[51]。*C. jejuni/coli*は牛，鶏，豚などの家畜や不衛生に取り扱われた動物性食品から分離することができる。特に食肉や汚染された食品の喫食，牛肝臓の生食は人のカンピロバクター食中毒の主な原因となっている[19,36]。農場を訪問した人や農場で働く人が家畜から直接感染したという報告はみあたらないが，家畜糞便中に本菌は存在している。

人の症状：本菌（100個程度）の摂取後，約2～7日の潜伏期間の後に，発熱（38℃以下），下痢，腹痛を主症状とする腸管感染症を起こす。腹痛は下痢よりも長期間継続する。カンピロバクター腸炎の回復後（約10日後）に，まれ（0.1%）にギランバレー症候群を併発することがあるので，腸炎症状回復後も注意が必要である。ギランバレー症候群とは運動障害を起こす末梢神経疾患で，筋力低下による歩行困難，顔面の神経麻痺，手足のしびれ等が長期間続き，生活に支障を起こす疾患である。

家畜の症状・保菌状況：家畜は通常無症状である。本菌は腸管内容物，胆汁や肝臓から容易に分離することができる。本菌の検出状況について表2に示す。牛と鶏から検出されるカンピロバクターのほとんどは*C. jejuni*，豚から検出されるものは*C. coli*である。牛の肝臓の汚染調査[26]によると，本菌の胆汁からの本属菌の検出率は25.4%（60/236検体），肝臓は11.4%（25/236検体）である。肝臓の部位別では，尾状葉<方形葉<左葉の順で検出率が高くなる。本菌が検出された検体の菌量は，肝管内の胆汁で6.2×10^4 cfu/10 mL，胆のう内の胆汁で2.7×10^4 cfu/10 mL，肝臓左葉で5.5×10^2cfu/10 g，方形葉で2.2×10^2 cfu/10 g，尾状葉で100 cfu/10 gである。よって，牛の肝臓の生食は食品衛生上きわめて危険である。

予防策：農場を訪問した人や農場で働く人が家畜から直接感染したという報告はみあたらないが，家畜糞便中に本菌は存在するので，本菌の感受性の高い小児や高齢者が家畜の糞便と接触した後には，石けんと流水でよく手を洗うことが重要であろう。食肉については十分な加熱調理と肉類に触れた器具や手指の洗浄，生食する野菜と肉類の接触防止といった二次汚染の防止処置を行えば簡単に防ぐことが出来る。しかし，本菌による食中毒の多くが生肉や牛レバーの生食等に起因するため，特に若齢者，高齢者，低免疫状態の人はこれらを食べないよう厚生労働省は啓発している（http://www.mhlw.go.jp/qa/syokuhin/campylo/index.html）。

表2　カンピロバクターの検出状況

動物・品種等	調査頭数	陽性頭数 (%)[a]	菌種等	検出頭数 (%)	内訳			調査年	文献
					胆汁	盲腸内容	直腸便		
牛・黒毛和種	120	44 (36.6)	*C. jejuni*	43 (35.8)	・[b]	・	43	2008	23)
			C. coli	1 (0.8)	・	・	1		
牛・交雑種	75	57 (76.0)	*C. jejuni*	57 (76.0)	・	57	・	2002	32)
豚	105	67 (63.8)	*C. jejuni*	2 (1.5)	・	2	・		
			C. coli	65 (1.5)	・	65	・		
鶏・ブロイラー	32	16 (50.0)	*C. jejuni*	16 (50.0)	・	16	・		
牛・交雑種	47	24 (51.1)	*C. jejuni*	20 (42.6)	20	1	・	2000	41)
			C. coli	1 (2.1)	1	0	・		
			C. lali	1 (2.1)	1	0	・		
			C. coli +*C. lari*	1 (2.1)	1	0	・		
			C. fetus	1 (2.1)	1	1	・		
繁殖牛[c]	59	10 (16.9)			10	・	・	2002-2003	26)
搾乳牛[d]	68	17 (25.0)			17	・	・		
肥育牛[e]	109	33 (30.3)			33	・	・		

a）カンピロバクターが検出された頭数
b）実施せず
c）黒毛和種，雌，60カ月以上
d）乳用種，雌，36カ月以上
e）肥育牛（c，dを除く牛）

表3　サルモネラの検出状況

血清型	肥育豚				ブロイラー		採卵鶏	肥育牛	
	2000-2003[a]	2005		2002	2000-2003	2002	2000-2003	2000-2003	2008
	便[b]	直腸便	胆汁	盲腸内容	便	盲腸内容	便	便	直腸便
	20/527[c] (3.8)	8/110 (7.3)	3/110 (2.7)	4/105 (3.8)	57/283 (20.1)	17/32 (53.1)	15/444 (3.4)	16/650 (2.5)	0/110 (0)
	39株[d]	8頭	3頭	4頭	91株	17羽	28株	25株	0株
S. Typhimurium	17	5						19	
S. Agona	4				4		2		
S. Anatum	4								
S. Derby		3	3	1					
S. Infantis				3	65	11	2		
S. Virchow					4		1		
S. Enteritidis					3		2		
S. Hader					3	6			
S. Thompson					2		4		
S. Dublin								4	
その他	14	0	0	0	10	0	17	2	0
文献	2)	43)	43)	32)	2)	32)	2)	2)	23)

a）調査年
b）検体
c）サルモネラが検出された頭数/調査頭数（%）
d）血清型別を実施した菌株数または頭（羽）数

サルモネラ

サルモネラは家畜衛生および食品衛生上きわめて重要な感染症起因菌で，家畜伝染病予防法では*S.* Gallinarum-Pullorum biovar Pullorumと biovar Gallinarumによる鶏・あひる・七面鳥・うずらの感染症は家畜伝染病（家禽サルモネラ感染症）に*S.* Dublin, *S.* Enteritidis, *S.* Typhimurium, *S.* Choleraesuisの４血清型による家畜・家きん（牛，水牛，しか，豚，いのしし，鶏，あひる，七面鳥，うずら）の感染症は届出伝染病（サルモネラ症）に指定されている。

これらのうち，豚からは*S.* Typhimuriumと*S.* Choleraesuis，鶏からは，*S.* Enteritidisと*S.* Typhimurium，牛からは*S.* Typhimuriumと*S.* Dublinが多く分離される。本菌は腸管内に生存するが，胆汁中でも生育できることから，本菌が胆のうに移行している動物は難治療性で長期間保菌し続ける[43, 53]。

S. Enteritidisならびに*S.* Typhimuriumは人の食中毒から分離されることが多く，また，両血清型ともに多剤耐性菌の出現が問題となっている[2, 40, 43]。人のサルモネラ感染症の多くは食汚染食品の摂取に起因する。農場を訪問した人や農場で働く人が家畜から直接感染したという報告はみあたらないが，家畜糞便中に本菌は存在している。

人の症状：本菌の摂取後，通常８～48時間の潜伏期を経て急性胃腸炎症状を示す。しかし，最近の*S.*Enteritidis感染等では少量の菌（100個程度）でも感染が成立し，その場合は潜伏期間が３～４日と長くなることもある。発熱，悪心，嘔吐で始まり，数時間後に腹痛および下痢を起こす。下痢は１日数回から十数回で，３～４日持続するが，１週間以上に及ぶこともある。小児では意識障害，痙攣および菌血症，高齢者では急性脱水症および菌血症を起こす等，重症化しやすく，回復も遅れる傾向がある。

家畜の症状・保菌状況：農場で家畜伝染病の「家禽サルモネラ感染症」および届出伝染病の「サルモネラ症」が発生した場合は，家畜は重篤なチフス様疾患・急性胃腸炎症状を示し致死性な感染症となることがある。しかしながら，「サルモネラ症」に該当する血清型のみならず，さまざまな血清型が無症状の家畜の糞便や胆汁から分離されている（表３）。

予防策：本菌が農場に侵入したか否かについて，定期的な検査を実施し，保菌動物の摘発，隔離，汚染環境の徹底した消毒等の措置を実施することや，保菌動物の導入防止，飼育環境・使用器具の消毒等，衛生管理の徹底が必須である。個別に抗生物質等の治療を実施する場合，すでに多くの多剤耐性サルモネラがわが国には存在するので[2, 40, 46]，分離菌の薬剤感受性試験を実施したうえで，使用する抗生物質を選択し投与することが重要である。農場を訪問した人や農場で働く人が家畜から直接感染したという報告は見当たらないが，家畜糞便中に本菌は存在することを考慮にいれ，本菌の感受性の高い小児や高齢者が家畜の糞便と接触した後には，石けんと流水でよく手を洗うことが重要であろう。

その他

1）E型肝炎

E型肝炎は*Hepevirus*属のE型肝炎ウイルス（HEV）を原因ウイルスとする感染症で，本感染症例の多くは外国の不衛生な環境下で過ごした人が日本に帰国後に発症する，輸入感染症であった。しかし，渡航歴が無くとも日本国内で感染したと推定される例[44]や，シカ生肉の喫食による感染症事例[49]，イノシシの生レバーや肉の喫食による感染症事例[28, 47]等，国内感染例が相次ぎ，わが国にもHEVの感染源があることが示された。豚のHEV平均抗体保有率が約60%（２カ月齢が７%，３カ月齢が40%，４カ月齢が87%，５カ月齢と６カ月齢が90%）であること[45]，豚のHEV抗体保有率は養豚場によって異なること[39]，市販豚肝臓の1.9%（７/363）からHEV遺伝子が検出されること[39]，と畜された豚の血清の1.8%（３/169）からHEV遺伝子が検出されること[39]，獣医師のHEV抗体価は通常の献血者よりも高いこと[29]等から，人のHEV感染症における豚の関与を示唆する報

告が多数認められる。しかし，養豚業者や獣医師がHEVに感染したという報告はみあたらない。HEVについては未だ感染経路等，不明なことが多く，今後の研究が待たれている。

2）コリネバクテリウム　ウルセランス

ジフテリア毒素を産生する*Corynebacterium ulcerans*がジフテリア様患者から分離されている。海外での*C. ulcerans*感染症患者の環境調査では，牛等の家畜との接触や生の乳製品の摂取による場合[5,42]，または愛玩用動物であるイヌ，ネコからの感染等が報告されている。本感染症についても未だ感染経路等，不明なことが多く，今後の研究が待たれる。

まとめ

動物由来感染症，特に家畜が関与するものは，伝染性海綿状脳症(牛)，日本脳炎(豚)，インフルエンザ(豚，鶏)，牛結核(牛)等さまざま存在する。また，未だ家畜の役割等も明確に解明されていない疾病も認められる。家畜から直接人に感染する疾病は，衛生状態の向上しているわが国は，きわめて少ないと思われる。カロリーベースで6割を海外にたよっているわが国にとって，日本国内で生産される家畜や畜産物は，安心・安全な食品の象徴と思われる。しかし，腸管出血性大腸菌感染症やクリプトスポリジウム感染症等，身近な家畜から人が感染する危険性のある疾病が存在することを考慮に入れなければならない。そして，衛生的な取扱いを実施するだけでも，これらの疾病の多くは防止することが可能である。

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NOTE *Public Health*

Hazard Analysis of *Listeria monocytogenes* Contaminations in Processing of Salted Roe from Walleye Pollock (*Theragra chalcogramma*) in Hokkaido, Japan

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ABSTRACT. Hazard analysis of *Listeria monocytogenes* contamination during processing of salted walleye pollock (*Theragra chalcogramma*) roe was performed for a seafood plant in Japan from December 2005 to February 2006. As a result, *L. monocytogenes* number was detected on the pallet used for transport of barrels in the salting process and one of the rollers of the roller conveyor, which rotates while in contact with the bottoms of the barrels, but was not detected in any raw materials, interim products or final products. Thus, we believe that the pallet contamination initially occurred because of insufficient washing, that it was passed on to the bottoms of the barrels and that it was then passed on the roller of the roller conveyor by cross-contamination. Therefore, it is possible that interim and final products may become contaminated by processing devices and machinery. In addition, we conducted an inoculation study designed at the 1/20 actual factory scale using interim products with or without artificial color and seeded with *L. monocytogenes* to observe changes in its growth. In the inoculation study, multiplication of *L. monocytogenes* during the salting process was not confirmed in the samples with artificial color.

KEY WORDS: food hygiene *Listeria monocytogenes*, salted walleye pollock roe.

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L. monocytogenes is known to be the causative agent of listeriosis in humans and animals [6, 8]. Its clinical signs include influenza-like symptoms, headache and nausea, together with acute gastroenteritis-like symptoms, meningitis and bacteremia [3, 15, 17]. Neonates, pregnant women and immunocompromised patients have high likelifoods of developing symptoms [4, 14].

Listeriosis outbreaks resulting from contamination of milk, cheese, vegetables and meat have been reported worldwide [5, 9, 16, 18, 20]. In the U.S.A., approximately 2,500 people develop serious listeriosis, and 500 people die as a result of listeriosis each year [11]. This proportion is equivalent to approximately 10% of the total number of deaths due to food-borne infection. In Japan, there have been no reports of outbreaks documented so far, but a single case of food-borne listeriosis due to natural cheese in 2001 was reported for the first time in 2005 [10]. Since the level of food contamination in Japan is similar to that in Europe and the US [13], there is a high chance of food-borne listeriosis outbreaks in Japan in the near future. Salted walleye pollock (*Theragra chalcogramma*) roe is a common, raw, ready-to-eat seafood product in Japan, but the prevalence and rate of contamination for *L monocytogenes* in food has not been sufficiently investigated. The salting process is one of the most important steps during processing therefore, we performed a hazard analysis of *L. monocytogenes* contamination of walleye pollock roe during processing and conducted an inoculation study using interim products seeded with *L. monocytogenes* to observe changes in its growth in the salting process in order to investigate the cause of contamination in a seafood plant in Hokkaido, Japan.

The processing plant used frozen raw walleye pollock roe imported as raw material for production of a ready-to-eat product (Tara-ko). In the processing plant, the raw material was thawed, salted using a commercial tilting machine for 7 hr at 20°C and then washed, drained, formed, chilled, boxed and frozen as shown in Fig. 1. At the end of each day, a cleanup crew sanitized the equipment according to a company sanitation procedure based on the Current Good Manufacturing Practices of the U.S. Food and Drug Administration (FDA) [1].

A total of 80 samples were collected from the processing environment (surfaces and drains), machines and employees' gloves. During processing of Tara-ko, the above items were swabbed around a 10.0 cm by 10.0 cm area using sterile cotton plugs (Eiken-Kizai Co., Ltd., Tokyo, Japan) premoistened with sterile saline. Two plugs were used for each sample. Samples from the gloves of the handlers in each step of processing were taken by swabbing. At each step, 25-g portions of semi-processed and final roe products and

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Fig. 1. Process for producing salted Tara-ko. The processing plant used frozen raw walleye pollock roe imported as raw material for production of a ready-to-eat product (Tara-ko). In the processing plant, the raw material was thawed, salted with a commercial tilting machine for 7 hr at 20°C, washed, drained, formed, chilled, boxed and then frozen.

of salting solution were collected.

Examinations for *L. monocytogenes* and other *Listeria* spp. were carried out according to the ISO method 11290 part 2 [19]. Each sample was incubated in half-Fraser broth (primary *Listeria* enrichment broth; Oxoid, Hampshire, UK) at 30°C for 24 hr; 1-m*l* portions of the culture were then transferred to Fraser broth (secondary *Listeria* enrichment broth; Oxoid) and incubated at 30°C for 24 hr. An aliquot of secondary enrichment culture from each sample was streaked with a sterile loop onto both Palcam agar (Merck, Darmstadt, Germany) and CHROMagar Listeria (CHROMagar *Microbiology*, Paris, France). Five suspected colonies isolated from each agar plate were streaked onto blood agar, and then the beta-hemolytic colonies were identified by the methods described in the Bacteriological Analytical Manual of the U. S. FDA [7]. Total aerobic bacteria and coliform bacteria were also enumerated. For these analyses, each sample from the processing plant was plated onto Standard Plate Count Agar (Oxoid) and CHROMagar ECC (CHROMagar *Microbiology*), respectively, and incubated at 35°C for 24 to 48 hr before counting the numbers of colonies.

Sample pH was measured using a pH meter (F23, Horiba Seisakusho, Kyoto, Japan). The salt content and water activity of the samples were determined as follows. A 10 g sample was weighed, homogenized with 50 m*l* of distilled

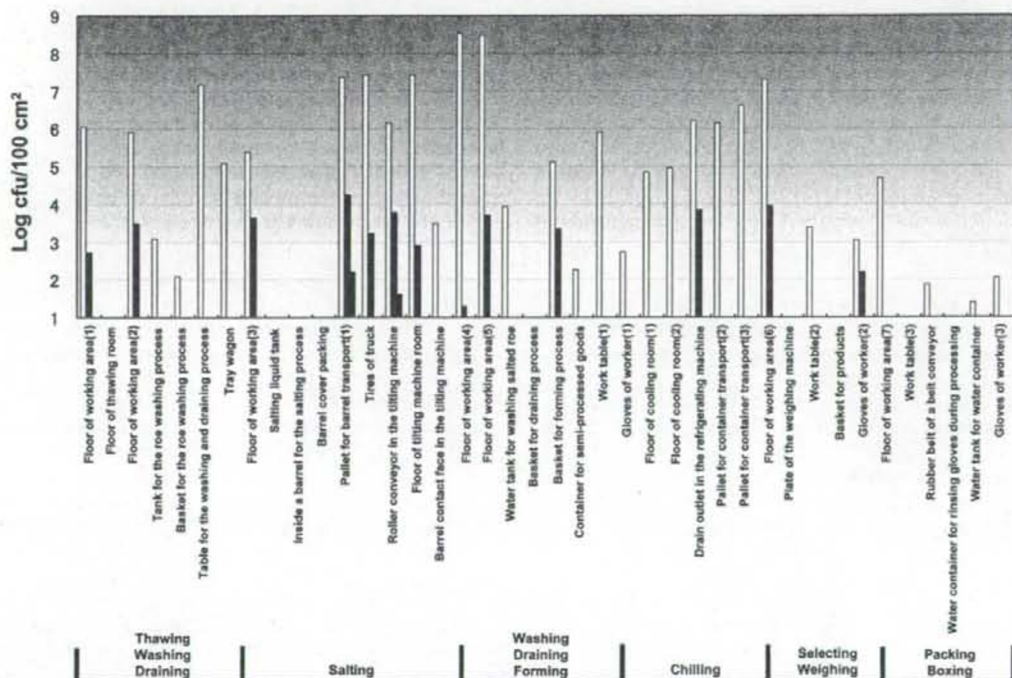

Fig. 2. Results of microbiological tests on samples collected from processing stages. □: Total aerobic bacteria. ■: Coliform bacteria. ■: *L. monocytogenes*.

Table 1. Microbiological and physiological characteristics of raw material, interim products, salting solution and final products

Samples tested	pH	Salt content (%)	Aw	Aerobic count (CFU/g)	Coliform (CFU/g)	Listeria spp. (/25 g)
Thawed, washed and drained roe	6.08	0.8	0.99	30	Negative	Negative
Salting solution (before use)	6.97	Not determined	Not determined	35	Negative	Negative
Salting solution (after use)	6.17	Not determined	Not determined	1.3×10^3	Negative	Negative
Roe after salting	6.18	5.3	0.94	4.9×10^2	Negative	Negative
Washed and drained roe	6.19	5.2	0.94	6.3×10^2	Negative	Negative
Chilled and stocked roe	6.22	5.4	0.94	2.1×10^2	Negative	Negative
Final products	6.19	5.6	0.94	70	Negative	Negative

Table 2. Changes of pH in raw roe, salted roe and during strage at different temperatures

Batch Number	Temperature (°C)	Samples tested			
		Raw roe	Salted roe	Incubation time 1*	Incubation time 2**
1	4	ND	ND	6.25	6.25
2	4	ND	ND	6.31	6.31
3	4	ND	ND	6.3	6.29
4	10	ND	ND	6.25	6.25
5	10	ND	ND	6.31	6.3
6	10	ND	ND	6.3	6.27
7	20	6.6	6.24	6.26	6.2
8	20	6.16	6.28	6.31	6.26
9	20	6.06	6.26	6.29	6.25

ND: Not determined.
* Samples incubated for 20 hr and ** 40 hr after salting process were finished.

water and then diluted to 100 m*l* for determination of salt content. A 5-m*l* aliquot of this solution was titrated with 0.1 N $AgNO_3$, and the salt content was then calculated. For water activity, one gram of each sample in duplicate was placed into a pre-weighed aluminum foil plate (30 mm) that was then placed into a Conway water activity test apparatus (Sibata Scientific Technology Ltd., Tokyo, Japan) containing a saturated salt solution at a relative humidity of 75.8% to 100%. The samples were incubated for 2 hr at 25°C after sealing the unit with Vaseline. The samples were then weighed, and the water activities of the samples were calculated.

We conducted an inoculation study designed at 1/20 of the actual factory scale using interim products seeded with *L. monocytogenes* in order to observe changes in growth directly in the walleye pollock roe. *L. monocytogenes* strain ATCC 7644 and smoked salmon isolated strain SS02 [10] were combined in this study to serve as an inoculum. Stock cultures were transferred to Tryptone Soya Broth (Oxoid) and incubated for 24 hr at 30°C under aerobic conditions. After cultivation, the cells were concentrated by centrifugation, washed twice with phosphate-buffered saline (0.1 M, pH 7.0) and then suspended in the same buffer. Suitable amounts of this working culture were dispersed into 50 m*l* of the buffer, and 1 m*l* portions from each cell suspension were used as inocula for survival studies of *L. monocytogenes* in interim Tara-ko products as described below.

The ovary membrans were aseptically removed from 18 kg of raw walleye pollock roe from the processing plant to prepare homogeneous starting inocular samples, and the roe was then divided into 18 × 1-kg batches along with the salting solution containing 10% NaCl, $NaNO_2$ and a few kinds of seasonings provided by the plant. Half of these batches contained artificial color (Food Color Red 102 and Food Color Yellow 5, Hodogaya Chemical Co., Ltd., Kawasaki, Japan). Details about the contents, concentrations and application methods of the additives (except NaCl) in the solution were unknown because they are proprietary information. After inoculation of these samples with *L. monocytogenes*, 9 batches were incubated at 20°C for 7 hr, divided again into three groups (three replicates each) according to incubation temperatures of 4°C, 10°C and 20°C and then incubated for 20 hr and 40 hr to monitor survival and growth. The colony forming units (CFU) were counted in duplicate on Palcam agar and CHROMagar Listeria plates after cultivation at 30°C for 48 hr. In addition, all samples were screened for total aerobic bacteria as well as coliform bacteria using Standard Plate Count Agar (Oxoid) and CHROMagar ECC (CHROMagar *Microbiology*), respectively.

The microbiological and physiological characteristics of the interim products in each processing step, of the salting solution (before and after being used) and of the final products are summarized in Table 1. Neither *L. monocytogenes* nor coliform bacteria was detected in any samples. Total bacterial counts were within normal limits. Figure 2 shows the microbiological test results for samples collected from the processing environment (surfaces and drains), machines and employees' gloves at each processing stage. *L. monocytogenes* was detected only on the pallet used for transport

Table 3. Salt concentration and changes of water activity (Aw) in raw roe, salted roe and during storage at different temperature

Batch Number	Temperature (°C)	Salt content (%)				Aw***
		Raw roe	Salted roe	Incubation time 1*	Incubation time 2**	
1	4	ND	ND	6.25	6.25	0.94
2	4	ND	ND	6.31	6.31	0.94
3	4	ND	ND	6.3	6.29	0.94
4	10	ND	ND	6.25	6.25	0.94
5	10	ND	ND	6.31	6.3	0.94
6	10	ND	ND	6.3	6.27	0.94
7	20	0.7	6.24	6.26	6.2	0.94
8	20	0.7	6.28	6.31	6.26	0.94
9	20	0.7	6.26	6.29	6.25	0.94

ND: Not determined.
* Samples incubated for 20 hr and **40 hr after salting process were finished.
*** Samples incubated for 40 hr after salting process were used for measuring of Aw.

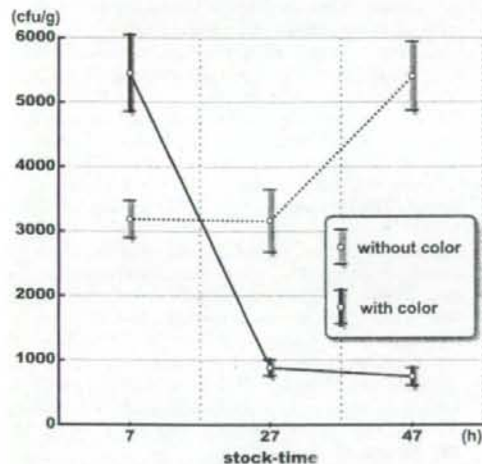

Fig. 3. *L. monocytogenes* counts (mean and 95% CI) with and without artificial color at each incubation time. An inoculation study was designed at 1/20 of the actual factory scale using interim products seeded with *L. monocytogenes* to observe changes in growth directly in the walleye pollock roe. *L. monocytogenes* strain ATCC 7644 and smoked salmon isolate strain SS02 were combined in the present study to serve as an inoculum. - - - - :without artificial color. —— : with artificial color.

and one of the rollers of the conveyor, which rotates while in contact with the bottoms of the barrels, in the salting process. Tables 2 and 3 showed the pH and salt concentrations of raw roe, respectively, before and after salting and chill storage at different temperatures. We found no significant change at any temperature. The difference in total aerobic bacterial counts was great: 30 to 35 CFU/g in raw-roe and 1.0×10^3 CFU/g after storage for 47 hr following the salting procedure. No coliform bacteria were isolated from any samples (Table 1).

During these investigations, *L. monocytogenes* was detected on the pallet used to transport barrels in the salting process and on a conveyor roller, which rotates when in contact with the bottoms of the barrels, but not in any raw materials, interim products, or final products. We believe that contamination of the pallet first occurred because of insufficient washing, that it was passed on to the bottoms of the barrels and then, that it was potentially passed to the roller by cross-contamination, leading to diffusion to downstream processing sections by spatter of water from floors, hands, gloves and the ventilation system. Therefore, it is possible that interim and final products may be contaminated with *L. monocytogenes* indirectly via processing devices and machinery.

At the beginning of the inoculation study, the *L. monocytogenes* counts were 3.0×10^4 CFU/g for samples with and without artificial color (Fig. 3). Since there were no statistical differences in the averaged *Listeria* counts among the three batches at each temperature (4°C, 10°C and 20°C), the nine Listeria counts were averaged separately (with and without artificial color) for the 7, 27 and 47 hr stock times and are shown in Fig. 3 [as means of the 95% Confidence Interval (CI)]. The average counts significantly decreased after 7 hr with and without artificial color, but the counts of the samples with color were higher than those without color. However, the average counts of the samples without color were significantly higher at later time points (27 and 47 hr). At 47 hr, the counts differed by a factor of approximately 8.

Interim products in the salting process had average values for pH (6.28), salt concentration (5.4%) and Aw (0.941). The minimum Aw level for *Listeria* spp. growth requirement is 0.93, but some *Listeria* strains can grow at Aw 0.90 [2], and during the salting process, Aw was close to the minimum Aw level. The *Listeria* counts of samples exposed to this salting solution first decreased and then increased after 47 hr in samples without artificial color. This observation coincides with our finding that after the total aerobic bacterial numbers and coliform bacteria counts decreased, they again increased with longer incubation time in the processing plant samples (data not shown). In our inoculation study, multiplication of *L. monocytogenes* was not confirmed in the samples of roe during the salting process with

and without artificial color. However, artificial color seems to have some kind of bacteriostatic effect as a chelating compound because the *L. monocytogenes* counts became much lower in the artificially colored samples over time. It is generally known that current artificial colors do not have a very strong effect on bacterial growth [12]; therefore, our conclusion is that the inhibition of *L. monocytogenes* growth was caused by the mutual influences of addition of artificial color and a low Aw.

Salted walleye pollock roe is a raw, ready-to-eat seafood product that the Japanese commonly consume, but it is sporadically contaminated with *L. monocytogenes*. As multiplication of *L. monocytogenes* during the salting process was not confirmed in the present inoculation study, we recommended that more exhaustive sterilization, washing and routine bacteriological examinations be performed for production lines in plants, that shorter shelf-lives be used for these products and that they should be stored more often below 5°C in the retail setting in order to avoid the occurrence of food-borne listeriosis due to contaminated salted walleye pollock roe.

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NOTE *Public Health*

Detection of *Salmonella* spp. Isolates from Specimens due to Pork Production Chains in Hue City, Vietnam

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ABSTRACT. From August 2007 until March 2008, we perfomed a detection and epidemiological analysis for *Salmonella* spp. in specimens collected from pork production chains to improve the quality of meat hygiene conditions in Hue, Vietnam. A total of 306 specimens were examined for *Salmonella* spp., aerobic bacterial counts and coliform. Seven serovars of *Salmonella* spp. were detected in retail pork, slaughterhouse carcasses and environmental specimens with the following detection rates: 32.8% of retail pork, 15.5% of slaughterhouse carcasses, 47.4% of floors, 38.1% of weighing bowls, 28.6% of cooking boards and 16.7% of tank water samples. Based on these results, we recommend that exhaustive sterilization, washing, routine bacteriological examinations and treatments at low temperature are performed in slaughterhouses, transportation facilities and retail stores.

KEY WORDS: pork hygiene, *Salmonella* spp., Vietnam.

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The demand for improved food safety has induced changes in methods used for meat production that rely on scientific hazard analysis and prevention methods through use of foodborne pathogen control testing in order to improve food safety and the shelf life of meat products in the meat industry [19–21]. Currently, much attention has been focused on determining the prevalence of foodborne pathogens at different stages in the pork production chain, including at the slaughter, cutting, transportation and retail levels in open markets in the northern and southern regions of Vietnam [5, 25], but the prevalence and rate of contamination for pathogens in pork production chains have not been sufficiently investigated, especially in the central part of the country. Bacterial pathogen controls in these areas involve control of various hazards, and one of the most important pathogens is *Salmonella* spp., which live in the enteric systems of animals and which can easily be introduced into the production chain via animals and contaminated pork [17, 22]. *Salmonella* spp. are known to be the causative agents of salmonellosis in humans and animals [1, 3, 4], and most can cause gastroenteritis when ingested by eating contaminated foods. It is generally considered that the risk due to this pathogen is growing because of the lack of good hygienic slaughtering practices and implementation of cold chains. Therefore, investigation and epidemiologic analysis of pork production chains first is important for improvement of the quality of meat hygiene conditions and to ease the economic burden associated with adverse impacts upon human health, especially in the central Vietnam.

In the Program of Founding Research Centers for Emerging and Reemerging Infectious Disease, sampling and bacterial isolation were carried out at two slaughterhouses, one open market and the Institute for Resources Environment and Biotechnology of Hue University in Hue City, Vietnam, with the cooperation of Vietnamese and Japanese experts. A total of 306 specimens (180 from slaughter carcasses, 55 from retail pork and 71 from various other environments) were investigated for the presence of *Salmonella* spp., aerobic bacteria and coliform [9, 13]. Carcasses were swabbed on two sides, a location on the back that touched the floor and a location on the abdomen that did not touch the floor in a slaughterhouse. Swabbing was performed in 10.0 cm square by using sterile cotton plugs (Eiken Kizai Co., Ltd., Tokyo, Japan) pre-moistened with sterile saline and transferred to 10 m*l* of sterile saline. Environmental surfaces, including floors, measuring bowls and cooking boards were swabbed in the same manner as carcasses. Samples of retail pork (100 g) were collected from the open market. Tank water samples (100 m*l* each) were collected in sterile bottles. All samples were collected aseptically and transported to the laboratory at 10°C for testing. The pork samples and swabs were examined for *Salmonella* spp. [2] as follows: 25 g of retail pork samples or 1-m*l* portions of swab suspensions were transferred to inoculated in Buffered Peptone Water (primary *Salmonella* pre-enrichment broth; Oxoid, Hampshire, UK) and incubated at 35°C for 18 hr; 0.1-m*l*

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portions of the culture were then transferred to Rappaport-Vassiliadis broth (secondary *Salmonella* enrichment broth; Oxoid) and incubated at 43°C for 18 hr. Tank water was tested by the most probable number method [13] using Rappaport-Vassiliadis broth. An aliquot of secondary enrichment culture from each sample was streaked with a sterile loop onto DHL agar (Eiken Kagaku Co., Ltd., Tokyo, Japan). Suspected colonies isolated from the agar plate were screened as *Salmonella* spp. using TSI medium (Eiken Kagaku Co., Ltd.) and LIM medium (Eiken Kagaku Co., Ltd.), and then identification of serovar was performed by slide and tube agglutination tests using anti-sera (Denka Seiken Co., Ltd., Niigata, Japan) with the latest versions of the Kauffmann-White scheme [16]. The total numbers of aerobic bacteria and coliform bacteria were enumerated by plating a sample from each specimen onto Standard Plate Count Agar (Oxoid) and DHL agar, respectively, and then incubating them at 35°C for 24 to 48 hr before counting the numbers of colonies. Colonies on DHL agar that were positive for lactose and/or sucrose fermentation and cytochrome oxidase reaction using Oxidase Identification Sticks (Oxoid) were considered to be coliform bacteria. Prevalence estimates are given for each sample with the corresponding absolute precision at a confidence interval (CI) of 95%.

High level contaminations with aerobic bacteria and coliforms were detected, and significant differences were demonstrated between the abodomen and back location of swabs obtained from slaughterhouses as shown in Table 1. Seven *Salmonella* serovars were isolated from the pork, carcass, and environmental specimens with the following detection rates: 32.8% of retail pork, 15.5% of carcasses, 47.4% of floors, 38.1% of weighing bowls, 28.6% of cooking boards and 16.7% of tank water samples (the detection rate is 3.6 MPN/100 m*l*). Among the 69 isolates found, 6 were *S.* Derby (8.7%), 4 were *S.* Brunei (5.8%), 27 were *S.* Newport (39.2%), 10 were *S.* Bareilly (14.5%), 9 were *S.* Coeln (13.1%), 1 was *S.* Larochelle (1.5%) and 1 was *S.* Weltevreden (1.4%). The differences in the detecting rates of *Salmonella* spp. between the back and abdomen location of carcass swabs are also shown in Table 1. The odds ratio 3.61, including that there was a significant difference in *Salmonella* spp. detection between the abdomen and back swab locations. Slaughtering practices may have allowed cross-contamination before transportation to the market.

To investigate the antimicrobial susceptibility of 40 strains of the 69 *Salmonella* spp. isolates, disk diffusion susceptibility tests [15] using Sensi-Disc Susceptibility Test Discs (Japan Becton Dickinson, Co., Ltd. Tokyo, Japan) were performed according to the supplier's instructions using nine antibiotics, 10 μg ampicillin (Am), 30 μg chloramphenicol (Cp), 30 μg kanamycin (Km), 30 μg nalidixic acid (Nal), 10 μg streptomycin (Sm), 250 μg sulfisoxazole (Sul), 30 μg tetracycline (Tc), 10 μg gentamicin (Gm) and 15 μg ciprofloxacin (Cip). Most *Salmonella* spp. isolates were sensitive to all nine antimicrobials, but 23 strains were resistant to sulfisoxazole, and three of them were also resistant to ampicillin. It is known that antimicrobial use in the human and veterinary medical fields and in food animals promotes the appearance of antimicrobial-resistant pathogens, and this phenomenon is important for appropriate antimicrobial therapy [18].

A wide variety of serovars, seven in total was found in the isolates from these specimens, and these seven serovars are common potential hazards in meat hygiene. Since *S.* Typhimurium is a serovar predominantly isolated from humans and food-producing animals throughout the world [8, 11, 12, 14, 23, 24], pig and pork products must be one of the most important potential hazards of *Salmonella* infections in humans, however *S.* Typhimurium was not isolated in the present study. The prevalence rates of *Salmonella* spp. for the retail pork and carcass samples were much higher than those previously reported [7, 26], and this leads to a high contamination of slaughtering environments and, as a result, contamination with *Salmonella* spp. diffused downstream in the pork production chain. In fact, high levels of contamination pressure from the enteric systems of the animals, evisceration, cutting and rinsing with contaminated tank water for carcasses lying on the floor is probably reflected in the differences in the detection rates of *Salmonella* spp. for the back and abdomen locations. The slaughtering practices observed in the present study allowed cross-contamination before transportation to the market. Therefore, we recommend exhaustive sterilization and washing of floors, instrument and worker's hands; reduction of total aerobic bacteria and coliforms using water rinses with sanitizing sprays [6,

Table 1. Detections of aerobic bacteria, coliform and *Salmonella* spp. among the specimens tested

Specimens	Sample numbers	Detection (log counts; mean ± SD)		Positive for
		Aerobic bacteria[a]	Coliform[a]	Salmonella spp. (%)[b]
Carcass (total)	180	4.57 ± 0.58	2.96 ± 0.86	28/180 (15.6)
(Abdomen site)	90	4.39 ± 0.55	2.66 ± 0.88	7/90 (7.8)
(Back site)	90	4.75 ± 0.55	3.26 ± 0.72	21/90 (23.4)
Floor	19	4.46 ± 0.84	3.09 ± 0.98	9/19 (47.4)
Bowl	21	4.90 ± 0.48	3.74 ± 1.06	8/21 (38.1)
Tank water	24	3.56 ± 1.40	2.29 ± 1.14	4/24 (16.7)
Pork	55	4.99 ± 0.58	3.81 ± 1.19	18/55 (32.8)
Cooking board	7	4.73 ± 0.83	3.71 ± 1.02	2/7 (28.6)

Significant differences between abdomen and back locations of swabs in detection of aerobic bacteria (CI; 95%)[a], colifon (CI; 95%)[a] and Salmonella spp. (odds ratio: 3.61)[b].