Kasetsart J. (Nat. Sci.) 44 : 401 - 410 (2010)

Amplified Fragment Length Polymorphism Analysis of *Campylobacter jejuni* and *Campylobacter coli* from Broiler Farms and Different Processing Stages in Poultry Slaughterhouses in the Central Region of Thailand

Chalermkiat Saengthongpinit[1,2]*, Sasitorn Kanarat[3], Theerapol Sirinarumitr[4], Patamaporn Amavisit[5] and Thavajchai Sakpuaram[2]

ABSTRACT

The aim of this study was to use the high-resolution genotyping method of amplified fragment length polymorphism (AFLP) to investigate the genetic diversity of *Campylobacter jejuni* and *C. coli* isolates obtained from broiler farms and different stages of slaughterhouse processing. Of 490 samples, 328 isolates of *Campylobacter* spp. were found (328/490, 66.9%) and the isolates were identified as *C. jejuni* (24.1%, 118/490) and *C. coli* (42.8%, 210/490). The genetic fingerprint of the 314 *Campylobacter* isolates, as determinated by AFLP, revealed 48 AFLP strains of *C. jejuni* and 95 AFLP strains of *C. coli*. In most flocks, a broad diversity of *C. jejuni* and *C. coli* strains was found and the distribution of AFLP types changed during slaughter line processing. Some genotypes were found to be the contamination source of both species in chicken intestines and by direct contamination of chicken meat during the slaughtering and cutting processes in the slaughterhouse. AFLP fingerprinting was an effective method to discriminate between *C. jejuni* and *C. coli* strains, in which the interlinkage homology of the AFLP pattern was only 35-42%. In addition, AFLP fingerprinting could distinguish between strains that were genetically unrelated or related. Therefore, AFLP analysis was considered a suitable epidemiological tool for investigation of *Campylobacter*.

Keywords: AFLP analysis, *Campylobacter*, chicken, genotyping

INTRODUCTION

In the last 20 years, the infection rate of *Campylobacter* has increased and has recently exceeded that of *Salmonella* in many developed countries (Tauxe, 2000; EFSA, 2006). *Campylobacter*, the most common food-borne pathogen worldwide, causes gastroenteritis characterized by watery and/or bloody diarrhea (FSAI, 2002). In the European Union, approximately 0.2

1 Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
2 Department of Veterinary Public Health and Diagnostic Services, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
3 Veterinary Public Health Laboratory, Bureau of Quality Control of Livestock Products, Department of Livestock Development, Pathum Thani 12000, Thailand.
4 Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
5 Department of Microbiology and Immunology, Faculty, of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fvetcksn@ku.ac.th

Received date : 19/08/09 Accepted date : 02/12/09

million human cases of campylobacteriosis have been reported annually (EFSA, 2006). Wild birds and domestic animals are reservoirs for *Campylobacter* and shed bacteria to the environment. Poultry is an importance source of human *Campylobacter* infection. Similar strains were reported with samples obtained from chickens and humans (Coker, 2000). *Campylobacter* colonizes chicken intestines without any apparent harm to the birds. In addition, *Campylobacter* can also colonize their feathers and skin. Colonized birds, thus, are potential sources of *Campylobacter* contamination in the slaughtering facility, as well as in chicken products (Jacobs-Reitsma, 2000; Stern and Line, 2000). It has been reported that three out of four live broilers and more than 80% of retail poultry meat were contaminated with bacteria (Jacobs-Reitsma, 2000; Hiett *et al*., 2002). Case-controlled studies have revealed that handling and consumption of contaminated chicken are important risk factors for human campylobacteriosis (Newell and Wagenaar, 2000).

Genotyping of *Campylobacter* spp. has been a useful tool for epidemiological investigations. It facilitates the quick detection of the source and route of transmission of the bacteria, and thus, assists in disease control and surveillance. Several genomic typing methods e.g. ribotyping, pulse-field gel electrophoresis (PFGE) and flagellin typing (*fla* typing) have been used to differentiate *Campylobacter* strains and to show the great diversity of genotypes and the potential for genetic instability in *Campylobacter* (Wassenaar and Newell, 2000).

Amplified Fragment Length Polymorphism (AFLP) is one genotype method. The advantage of the AFLP technique is that a random portion of the whole genome is sampled. AFLP is the most promising method for providing reproducible profiles and appears to be insensitive to genetic instability, which makes it less complicated than other molecular methods. Although major capital investment may restrict the use of this method in research laboratories, it seems that it will be used more widely for global epidemiological studies (Newell *et al*., 2000; Wassennaar and Newell, 2000). The aims of the present study were to investigate the presence of *Campylobacter jejuni* and *C. coli* in samples obtained from broiler farms and different stages of slaughterhouse processing in the central region of Thailand and to examine the genetic diversity of those *Campylobacter* isolates using AFLP.

MATERIALS AND METHODS

Bacterial samples

Seven commercial broiler farms in one province of the central region of Thailand were selected and subjected to fecal sampling, with 10 samples per farm. In each sample, one pooled sample of ten fresh chicken droppings was collected from the litter on each farm. Two slaughterhouses were also visited in order to collect specimens of slaughtered chickens raised on the seven farms sampled. Samples from specimens during slaughter were obtained using a standard process. Ten carcasses were collected from each stage of six different slaughtering processes (pre-evisceration, cecum, post-evisceration, post-chiller, chilling and freezing). Cecal samples were collected aseptically during evisceration. In the pre-evisceration, post-evisceration and post-chiller stages, 25 g of neck skin from each carcass was taken. In the chilling and freezing stages, 25 g of chicken meat from each carcass was taken. Overall, 70 chicken samples were sampled at each of the seven broiler farms. Sample collection occurred from March to July 2006. Within 24 h of collection, all samples were transported on ice to the laboratory for analysis.

Isolation of *Campylobacter* and preparation of DNA

Isolation and identification of

Campylobacter spp. were based on the method described by the International Organization for Standardization (ISO 10272-1:2006(E), 2006). In brief, 25 g of each pooled fecal sample was taken or 25 g of chicken meat was aseptically removed using sterile scissors and forceps. Samples were placed in 225 ml of Bolton broth. The samples in Bolton broth were homogenized in peristaltic bags for two minutes in a stomacher. The bags were incubated microaerophilically in anaerobic jars with gas-generating kits at 37°C for 4 h and then at 41.5°C for 44 h. The culture was streaked onto CCDA plates and Karmali agar plates. The inoculated plates were incubated at 41.5°C for 48 h under a microaerophilic environment.

For the isolation of chromosomal DNA, freshly grown cells were scraped from plates and washed with 100 µL TE (10 mM Tris/HCl, pH 8.0, 1 mM EDTA). DNA was isolated using the phenol-chloroform extraction method (Sambrook and Russell, 2001). The DNA was eluted with 50 µL TE pH 8.0 and stored at -20°C until processed.

Identification by multiplex PCR (mPCR)

Preliminary identification of *Campylobacter* was based on mPCR using genus-specific and species-specific primers previously described by Denis *et al*. (1999). Briefly, the genus-specific primers selected to amplify only the *Campylobacter 16S* rRNA gene were 5′-ATC TAA TGG CTT AAC CAT TAA AC-3′ (MD16S1) and 5′-GGA CGG TAA CTA GTT TAG TAT T-3′ (MD16S2). In order to subtype *C. jejuni*, the primer pair, 5′-CTA TTT TAT TTT TGA GTG CTT GTG-3′ (MDmapA1) and 5-GCT TTA TTT GCC ATT TGT TTT ATT A-3′ (MDmapA2) was used to amplify its *mapA* gene. A third pair of primers, 5′-AAT TGA AAA TTG CTC CAA CTA TG-3′ (COL3) and 5′-TGA TTT TAT TAT TTG TAG CAG CG-3′ (MDCOL2), was used to identify the *C. coli* subtype based on the amplification of its *ceuE* gene. All oligonucleotide primers were synthesized commercially by Isogen Bioscience (Maarsen, The Netherlands).

All mPCR reactions were performed as described by Denis *et al*. (1999) with minor modifications. The reaction were performed in 50 µL mixture containing 30 µL extracted DNA, 200 µM dNTPs (Fermentas, MD, USA), 1.5 mM $MgCl_2$, 1X PCR reaction buffer, 1.2 U *Taq* DNA polymerase (Invitrogen, USA), 0.5 µM of each MD16S1 and MD16S2 primers, 0.42 µM of each MDmapA1, MDmapA2, COL3 and MDCOL2 primers. The amplification reactions were carried out in a Primus 96 plus thermocycler (MWG-Biotech, England) programmed with a cycle of heating at 95°C for 10 min, followed by 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 59°C for 1 min 30 s and extension at 72°C for 1 min. The PCR reaction was completed with a final extension stage at 72°C for 10 min. Amplification generated 857 bp, 589 bp and 462 bp DNA fragments corresponding to the genus *Campylobacter*, and the species *C. jejuni* and *C. coli*, respectively. The *C. jejuni* strain ATCC 33291 and the *C. coli* strain ATCC 33559 (Microbiologics, MN, USA) were used as positive controls. The amplified products were detected by electrophoresis using 5 µL PCR product loaded onto a 1% (w/v) agarose gel (Seakem LE agarose; BMA, ME, USA) containing 0.05 µg/mL ethidium bromide (Amresco; OH, USA) run at 100 V for 30 min and photographed under UV light using Biorad Gel Doc 1000 (Biorad, USA).

Genotyping by AFLP

The AFLP analysis was performed using a protocol adapted from Duim *et al*. (1999). Briefly, isolated chromosomal DNA was digested with *Hin*dIII (New England Biolabs [NEB], Hertfordshire, England) and *Hha*I (NEB). In the simultaneous reaction, the fragments were ligated to the *Hin*dIII adapter and the *Hha*I adapter (National Science and Technology Development Agency, Bangkok [NSTDA], Thailand). The forward primer (*Hin*dIII adapter specific), labeled

with the D4 WellRED™ dye, contained an extra selective base at the 3′ end (*Hin*dIII +A, 5′-GAC TGC GTA CCA GCT TA-3′ [Proligo, CO, USA]). The reverse primer (*Hha*I adapter specific) also contained an extra selective base at the 3′ end (*Hha*I +A, 5′-GAT GAG TCC TGA TCG CA-3′ (NSTDA).

PCRs were performed in a Biometra T-Gradient thermocycler (Whatman Biometra, Gottingen, Germany) as described by Duim *et al.* (1999). The final products were separated by capillary electrophoresis using the CEQ 8000 genetic analysis system (Automated sequencer) (Beckman Coulter, CA, USA). For sample preparation, 25 μL of loading buffer solution (Beckman Coulter) was combined with 0.25 μL of CEQ DNA size standard-600 base pair ladder (Beckman Coulter) and 1 μL of amplification product. Fragment data were analyzed using the CEQ 8000 software with the analytical parameters calibrated to detect peaks with a slope of 10% and 10% of the height of the second highest peak. The maximum bin width was adjusted to 2. Each sample was scored as "1" for each bin, if a fragment of that size was present, and as "0" if not. Only AFLP profiles in the molecular size range from 60 to 640 bp were analyzed. A table containing this binary information was applied to calculate the in-lane similarity by Dice coefficient correlation. Cluster analysis of the AFLP banding pattern was carried out using the unweighted pair-group method with arithmetic averages (UPGMA) using the NTSYS-pc software package, version 2.2 (Exeter software, NY, USA).

Correlation levels were expressed as a percentage of similarity. Repeated analysis of the same isolates revealed a similarity value of at least 90%, and therefore the cut-off for highly related strains was set at 90%, a value that was in accordance with the findings of previous reports (Duim *et al.*, 1999; On and Harrington, 2000). Each member of the 90% similarity group was called a phenon. All strains within a window of similarity between 95% and 100% homology were considered as identical and thus belonging to the same AFLP strain (Lindstedt *et al.*, 2000). Individual phenons were designated by letters of the Roman alphabet. Roman numerals were added when strains appeared clonal.

RESULTS AND DISCUSSION

Isolation rates of *C. jejuni* and *C. coli* at farms and slaughterhouses

The isolation rates of *Campylobacter* by mPCR recovered from fecal samples and chicken carcasses during the slaughtering process are presented in Table 1. Of 490 samples, 328 isolates of *Campylobacter* spp. were found (328/490, 66.9%) and the isolates were identified as *C. jejuni* (24.1%, 118/490) and *C. coli* (42.8%, 210/490). At the farm level, only 8.6% (6/70) of all fecal

Table 1 Isolation rates of *Campylobacter* by mPCR recovered from fecal samples and chicken carcasses during the slaughtering process.

Species	Sampling stage							
	Feces (n = 70)	Cecum (n = 70)	Pre-evisceration (n = 70)	Post-evisceration (n = 70)	Post-chiller (n = 70)	Chilling (n = 70)	Freezing (n = 70)	Total (n = 490)
Campylobacter spp.	8.6[a]	62.9	65.8	70.0	81.4	92.9	87.2	66.9
C. jejuni	4.3	8.6	22.9	28.6	27.1	42.9	34.3	24.1
C. coli	4.3	54.3	42.9	41.4	54.3	50.0	52.9	42.8

[a] Number of positive samples/number of samples examined (%)

samples tested positive for *Campylobacter* spp., but more cecal content samples tested positive (62.9%, 44/70). The percentage of positive fecal samples was less than for cecum samples because only a portion of the *Campylobacter* passed through the feces due to environmental conditions in the intestinal tract and broiler houses. At the beginning of the slaughter process, 65.8% of the chicken samples tested positive for *Campylobacter* spp. at the pre-evisceration stage. The isolation rate at the pre-evisceration stage was high due to fecal contamination of feathers and the skin from transportation through the defeathering stage. Then, at the post-evisceration stage, the percentage of positive testing for *Campylobacter* spp. increased to 70%, as a result of possible fecal recontamination by intestinal content leakage. Isolation rates of samples at the post-chiller and chilling stages were 81.4 and 92.9%, respectively, with the increase resulting from cross contamination between carcasses. However, at the final freezing stage, the *Campylobacter* spp. isolation rate was reduced to 87.2%, due to the temperature decreasing to -18°C.

A high prevalence of *Campylobacter* has been reported on chicken carcasses and their environment during processing at the slaughterhouse (Klein *et al*., 2006; Takahashi *et al*., 2006). According to Takahashi *et al*. (2006), the prevalence of *C. jejuni* in chicken transport crates was 50%, in wing surface swabs after defeathering was 52.5% and chicken wing meat after mantling had a rate of 37.5%. However, the prevalence of *C. coli* was lower at 6.7% and 12.5% in the transport crates and chicken wing meat, respectively. Klein *et al*. (2006) reported positive testing of *Campylobacter*, with rates of 73.3% in cloacal swabs, 77.8% in scalding water, 53.3% in carcasses after scalding and defeathering, 66.7% in carcasses after evisceration, 40.0% in carcasses after cooling, 33.3% in breast meat with skin and 26.7% in fillets.

The reduction of bacterial load by slaughter-specific stressors, such as scalding bath temperature, presence of oxygen, drying of chicken skin or chilling at 0-4°C could not completely eliminate contamination in each processing stage and somehow the bacterial load increased due to cross contamination between carcasses. Results from the current study showed there was a continued increase in the rate of positive testing for *Campylobacter* on chicken carcasses from the pre-evisceration stage to the chilling stage, with a small decrease from 92.9 to 87.2% after the final freezing stage. Consequently, the high isolation rate in the chicken meat in the last stage still remains a high risk factor for human infection.

AFLP analysis of polymorphism within *C. jejuni* and *C. coli* isolates

Using AFLP, 109 isolates of *C. jejuni* and 205 isolates of *C. coli* presented 48 and 95 distinct patterns, respectively. The fingerprints generated by the *Hin*dIII and *Hha*I restriction enzymes gave sharp and distinguishable peaks at 50 to 80 DNA fragments for *C. jejuni* and 80 to 120 DNA fragments for *C. coli* ranging from 60 to 640 bp. AFLP of *C. jejuni* strains had 18 common amplified fragments, while AFLP of *C. coli* strains had 30 common amplified fragments.

AFLP analysis was performed of *C. jejuni* and *C. coli* strains from farm numbers 1 to 7 and used to construct dendograms (not shown). The intralinkage homology of the AFLP patterns among *C. jejuni* isolates in each farm ranged from 68.5 to 96% and among all *C. jejuni* isolates was 65%. The intralinkage homology of the AFLP patterns among *C. coli* strains in each farm ranged from 73 to 87% and among all *C. coli* isolates was 73%. Results from the current study showed that *C. jejuni* strains and *C. coli* strains produced very distinct AFLP banding patterns with the interlinkage homology of the AFLP patterns ranging from 35 to 42%.

AFLP banding patterns of *C. coli* strains

showed more common fragments and were more homologous than the patterns for *C. jejuni* strains. In addition, the results showed that *C. coli* strains from the broilers and slaughtering process were more clonal than *C. jejuni* strains. The AFLP banding patterns of *C. coli* strains contained many closely distributed bands, which were more homologous than the patterns for *C. jejuni*, which was similar to the findings of other authors (Duim *et al*., 1999; 2001). Duim *et al*. (2001) found that *C. coli* strains from multiple sources, such as human feces, pig feces and chicken feces, seemed to be more closely related to each other than *C. jejuni* strains, and this finding suggests that *C. coli* strains are more clonal than *C. jejuni* strains.

Distinguishing between these two species based on biochemical tests is often uncertain. *C. jejuni* and *C. coli* were identified as separate species that exhibit 25 to 49% homology on the basis of hybridization testing and multilocus enzyme electrophoresis typing (Duim *et al*., 1999). Results from the current study showed that the interlinkage homology of the AFLP patterns ranged from 35 to 42%. Duim *et al*. (1999) and Keller *et al*. (2007) found that the genetic homology value of *C. jejuni* and *C. coli* determined by AFLP analysis was 22% and less than 20%, respectively. The use of AFLP analysis resulted in a high degree of discrimination and thus seems to be useful and practical for the identification of *C. jejuni* and *C. coli* strains.

Strain diversity and AFLP analysis of *C. jejuni* and *C. coli*

From the seven farms, the 109 isolates of *C. jejuni* from fecal samples and chicken carcasses during the slaughtering process were distributed into 27 different AFLP phenons (a to z and aa) and 48 different AFLP strains (Table 2). In most farms, the distribution of AFLP types changed during processing and a broad diversity of *C. jejuni* strains was found, except for farms 1, 2 and 3. In farm 1, AFLP types were found to have only three phenons and six strains. The dominating genotypes were found at the cecum, pre-evisceration, post-evisceration, post-chiller, chilling and freezing stages (i1, i2, i3 and k1). The closely related strains of phenon i (i1, i2 and i3) and phenon k (k1 and k2) were isolated along the processing line of farm 1. In farm 2, genotype t1 was found only at the pre-evisceration and post-evisceration stages and genotype u1 was found at the post-evisceration, chilling and freezing stages. The only closely related strain of phenon a (a1, a2, a3 and a5) was found along the processing line of farm 3.

Table 2 AFLP types of *C. jejuni* strains recovered from fecal samples and chicken carcasses during the slaughtering process.

Sources	Sampling stage							Number of isolates studies
	Feces	Cecum	Pre-evisceration	Post-evisceration	Post-chiller	Chilling	Freezing	
Farm 1 (A) [a]		i1[c], j1, k1	2i1, 2i2, 3k1	i2, 4k1, k2	4i2, 5k1	4i2, i3, 3k1, 2k2	4i2, 3i3	42
Farm 2 (B) [b]			2t1	5t1, 5u1		5u1, u2, v1, v2	2u1, v3, v4	24
Farm 3 (B)			a1	a2	a2, a5	a3		5
Farm 4 (A)	b1	c1						2
Farm 5 (A)					a4, w1	l1	aa1	4
Farm 6 (A)			4p1, q1	r1, z1		x1, x2, y1	d1, s1, s2	13
Farm 7 (A)		a6, e1			m1, n1, n2, n6, o1	3f1, h1, 2n3	e1, f2, g1, g2, n4, n5	19

[a]A = Slaughterhouse A; [b]B = Slaughterhouse B

[c] Individual phenons are designated by letters from the Roman alphabet and Roman numerals added when strains appeared clonal.

For farms 2 to 7, the 205 isolates of *C. coli* from fecal samples and chicken carcasses during the slaughtering process were distributed into 33 different AFLP phenons (A to Z and AA to AG) and 95 different AFLP strains (Table 3). *C. coli* could not be detected from samples taken at farm 1. In most farms, the distribution of AFLP types changed during processing and a broad diversity of *C. coli* strains was found, except for farm 2. Dominating genotypes (A1, A3, A4 and Y1) were found along the processing line from at least two farms. This was different from the diversity recorded for the *C. jejuni* strains, where a dominating genotype was found only on one farm. Genotype A1 was found in the cecum of the broilers, at the pre-evisceration stage and at the post-evisceration stage of farm 3. This dominating genotype was also found at the post chiller stage on farm 4 and at the chilling stage on farm 2. Genotype A3 that was closely related with genotype A1 was found in the cecum of the broilers on farm 3 and also at the pre-evisceration, post-evisceration, chilling and freezing stages on farm 4. Genotype A4 was found in the cecum of the broilers on farm 3 and at the chilling stage on farm 2. Genotype Y1 was observed in many samples of the cecum, pre-evisceration, post-evisceration and freezing stages of farm 3 and also found in samples from the cecum, pre-evisceration, post-evisceration, post-chiller and chilling stages of farm 4. The closely related strains of phenon A (A1 to A13) were isolated along the processing line in farms 3 and 4 and at some processing stages on farms 2 and 6. In most phenon Y (Y1 to Y11) also found along the line in farm 2, 3, and 4.

Different genotypes of *C. jejuni* and *C. coli* not only exhibited a different potential for colonization, but also a different potential to survive environmental stressors, such as the scalding, chilling and freezing period in the processing line. However, only some of the *Campylobacter* subpopulations are dominating genotypes and can remain in the food chain having resisted such environmental stressors. The local

Table 3 AFLP type of *C. coli* strains recovered from fecal samples and chicken carcasses during the slaughtering process.

Sources	Sampling stage							Number of isolates studies
	Feces	Cecum	Pre-evisceration	Post-evisceration	Post-chiller	Chilling	Freezing	
Farm 1 (A) [a]								0
Farm 2 (B) [b]		5Y7, Y8 [c]	2Y7, Y8		5Y7	A1, A4		16
Farm 3 (B)		2A1, 2A3, A4, A5, A6, 2Y1, AG1	A1, 5Y1, 2Y9	A1, 2Y1, 3Y9, Y10	L1, 6AA1, AA5	A10, L2, L3, L4, 2L5, L7, W1	L6, 2Y1, Y2, 4AA1, AA2, AA4	51
Farm 4 (A)		8Y1	A3, 7Y1	A3, A11, 9Y1	A1, 3A11, A12, A13, Y1, Y3, Y4	6A3, 2Y1, AC1, Y11	3A3, 2A11, F1, Y5, Y6, Z1, Z2	56
Farm 5 (A)			2J2, J4, M1, M7, M10	M1, M3, M8, N1	J2, M3, M4	I1, J4, M5, M9, M11, O1, AA3, AB1	J1, J2, J3, K1, M2, M6, M11	28
Farm 6 (A)	A9, C1	A2, 2A8, B1, 3D1	A2, A7, D3, Q1	D2, D8, 2Q2, R1	D5, D7, E1, E2, G1, 2Q2, Q3, R2, S1	D4, D6, Q2, Q4, Q6, R3	D1, D4, D6, H1, 3Q5, T1	42
Farm 7 (A)	U1	U2, AD1, AE1	X1, AE1		AE1, AF1	V1	P1, 2AE1	12

[a]A = Slaughterhouse A; [b]B = Slaughterhouse B

[c] Individual phenons are designated by letters from the Roman alphabet and Roman numerals added when strains appeared clonal.

predominant genotypes in the same clonal groupings shared specific characteristics, which were advantageous for colonization in poultry, pathogenicity in humans and survival in the environment (Alter *et al*., 2005).

Many genotypes, such as e1, i1 and k1 of *C. jejuni* and genotypes A1, A2, A3, Y1, Y7, Y8 and AE1 of *C. coli*, were found in the cecum and were visible in other stages of processing. These genotypes could be proved as the source of *C. jejuni* and *C. coli* contamination in chicken intestines and of the transmission to chicken meat by the processing method in slaughterhouse (Tables 2 and 3). In most farms, many genotypes (i2, k1, t1, A1, A3, M1 and Y1) were isolated in the pre-evisceration and post-evisceration stages. From this fact, it could be concluded that both species of *Campylobacter* were capable of contamination, not only by leakage of intestinal contents in the evisceration stage, but also by feces or environmental contamination from the transportation stage and the scalding process. Some AFLP strains of *Campylobacter* were not detected in the early processes of the slaughter line, but later appeared on the carcasses, possibly as a result of cross contamination between carcasses or more than one strain of bacteria contaminating individual carcasses. In the current study, one colony of bacteria was collected from each sample, so it was possible to find only one bacterial strain isolate from one sample or carcass.

An automated sequencer is an important advantage of AFLP fingerprinting method because a database can be compiled automatically and fingerprint data can be compared and exchanged. For this reason, using AFLP analysis of *Campylobacter* spp. would make it possible to create a national database. It is essential to provide an accurate reflection of bacterial populations from different sources in Thailand. Such a database would represent a useful tool for the development of control strategies for *Campylobacter* spp. and to trace the infection source.

CONCLUSION

The genetic fingerprint, as determined by AFLP from seven commercial poultry farms and two slaughterhouses of 109 isolates of *C. jejuni* and 205 isolates of *C. coli* revealed 48 AFLP strains of *C. jejuni* and 95 AFLP strains of *C. coli*. In most flocks, a broad diversity of *C. jejuni* and *C. coli* strains was found and the distribution of AFLP types changed during the slaughter line processes. Some genotypes could have provided the contamination source of both species in chicken intestines and by direct contamination of chicken meat during the slaughtering and cutting processes in the slaughterhouse. AFLP fingerprinting is an effective method to discriminate between *C. jejuni* and *C. coli* strains and to distinguish between strains that are genetically unrelated or related.

ACKNOWLEDGEMENTS

This research was financially supported by the Center of Excellence on Agricultural Biotechnology, Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education and the Intervet Research Fund, Faculty of Veterinary Medicine, Kasetsart University.

LITERATURE CITED

Alter, T., F. Gaull, A. Froeb and K. Fehlhaber. 2005. Distribution of *Campylobacter jejuni* strains at different stages of a turkey slaughter line. **Food Microbiol.** 22: 345-351.

Coker, A.O. 2000. Incidence, trends and sources of Campylobacteriosis in developing countries - An overview, pp. 44-48. *In* **Proceedings of a WHO Consultation of Experts**. 21-25 November 2000, Copenhagen, Denmark.

Denis, M., C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat and P. Colin. 1999.

Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. **Lett. Appl. Microbiol.** 29: 406-410.

Duim B., P.A.R. Vandamme, A. Rigter, S. Laevens, J.R. Dijkstra and J.A. Wagenaar. 2001. Differentiation of *Campylobacter* species by AFLP fingerprinting. **Microbiol.** 147: 2729-2737.

Duim, B., T.M. Wassenaar, A. Rigter and J. Wagenaar. 1999. High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. **Appl. Environ. Microbiol.** 65: 2369-2375.

European Food Safety Authority. 2006. *Campylobacter*, pp. 96-116. *In* **Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the Europeon Union in 2004.** European Food Safety Authority, Parma, Italy.

Food Safety Authority of Ireland. 2002. **Control of *Campylobacter* Species in the Food Chain.** Food Safety Authority of Ireland. Lower Abbey Street, Dublin.

Hiett, K.L., N.A. Cox and N.J. Stern. 2002. Direct polymerase chain reaction detection of *Campylobacter* spp. in poultry hatchery samples. **Avian Dis.** 46: 219-223.

International Organization for Standardization. 2006. **Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for Detection and Enumeration of *Campylobacter* spp., ISO 10272-1:2006(E).** Geneva, Switzerland.

Jacobs-Reitsma, W. 2000. *Campylobacter* in food supply, pp. 467-481. *In* I. Nachamkin and M. J. Blaser (eds.). ***Campylobacter.*** 2nd ed. ASM Press, Washington, D.C.

Keller, J., B. Wieland, M. Wittwer, R. Stephan, and V. Perreten. 2007. Distribution and genetic variability among *Campylobacter* spp. isolates from different animal species and humans in Switzerland. **Zoonoses and Public Health** 54: 2-7.

Klein, G., F. Reich, L. Beckmann and V. Atanassova. 2006. Quantification of thermophilic *Campylobacter* spp. in broilers during meat processing. **Antonie van Leeuwenhoek** 92: 267-273.

Lindstedt, B.A., E. Heir, T. Vardund, K.K. Melby and G. Kapperud. 2000. Comparative fingerprinting analysis of *Campylobacter jejuni* subsp. *jejuni* strains by amplified-fragment length polymorphism genotyping. **J. Clin. Microbiol.** 38: 3379-3387.

Newell, D.G., J.A. Frost, B. Duim, J.A. Wagenaar, R.H. Madden, J. van der Plas and S.L.W. On. 2000. New developments in the subtyping of *Campylobacter* species, pp. 27-44. *In* I. Nachamkin and M.J. Blaser (eds.). ***Campylobacter.*** 2nd ed. ASM Press, Washington, D.C.

Newell, D.G. and J.A. Wagenaar. 2000. Poultry infections and their control at the farm level, pp. 497-509. *In* I. Nachamkin and M.J. Blaser (eds.). ***Campylobacter.*** 2nd ed. ASM Press, Washington, D.C.

On, S.L.W. and C.S. Harrington. 2000. Identification of taxonomic and epidemiological relationships among *Campylobacter* species by numerical analysis of AFLP profiles. **FEMS Microbiol. Lett.** 193: 161-169.

Sambrook, J. and D.W. Russell. 2001. **Molecular Cloning: A Laboratory Manual.** 3rd ed. Spring Harbor Laboratory Press. New York.

Stern, N.J. and J.E. Line. 2000. *Campylobacter* spp., pp. 1040-1056. *In* B.M. Lund, T.C. Baird-Parker and G.W. Gould (eds.). **The Microbiological Safety and Quality of Food Volume II.** Aspen Publishers Inc., Gaithersburg, Maryland.

Takahashi, R., F. Shahada, T. Chumaand and K.Okamoto. 2006. Analysis of *Campylobacter* spp. contamination in broilers from the

farm to the final meat cuts by using restriction fragment length polymorphism of the polymerase chain reaction products. **Int. J. Food Microbiol.** 110: 240-245.

Tauxe, R.V. 2000. Major risk factors for human Campylobacteriosis–An overview, pp. 65-66. *In* **Proceedings of a WHO Consultation of Experts**. 21-25 November 2000, Copenhagen, Denmark.

Wassenaar, T.M. and D.G. Newell. 2000. Genotyping of *Campylobacter* spp. **Appl. Environ. Microbiol.** 66: 1-9.