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Thermophilic *Campylobacter* spp. Occurrence on Chickens at Farm, Slaughter House and Retail

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Abstract: The aim of this study was to investigate the occurrence of campylobacters in chicken at farms (close-house system and open-house system), slaughtering (conventional slaughterhouse and processing plant) and retail (wet market and supermarket). *Campylobacter* spp. was not found in cloacal swabs in chickens aged of 4 weeks in farms with close-house system. *Campylobacter* spp. was found in cloacal swabs (95.0%) in four weeks old chicken in farms with open-house systems. End-slaughtering samples from conventional slaughterhouse and processing plant were contaminated with *Campylobacter* spp. at 84.0% and 94.0%, respectively. *Campylobacter* contamination on wet market and supermarket samples with 78.0% and 92.0%, respectively. Close-house system at farm level was able to prevent or delay *Campylobacter* spp. colonization in chickens but contamination by *Campylobacter* spp. at retail level was still high. Therefore, monitoring of *Campylobacter* spp. in chicken products at retail level is crucial to reduce risk of human ingestion of *Campylobacter* spp. through chicken products.

Key words: *Campylobacter* spp., farm, slaughter house, retail

INTRODUCTION

Campylobacter spp. had been recognized as the most common food-borne pathogens to cause gastroenteritis (Park and Sanders, 1992; Nachamkin *et al*., 1998; Saleha *et al*., 1998; Corry and Atabay, 2001). Cases of campylobacteriosis mostly associated with handling of raw poultry and consumption of contaminated, raw or undercooked foods (Corry and Atabay, 2001).

Campylobacter spp. is a small and extremely sensitive organism that requires microaerophilic condition to grow. Campylobacters are known to be slow growers and are poor competitors when growing together with other organisms. Despite being unusually sensitive and poor competitors, *Campylobacter* spp. had been recognized to cause human campylobacteriosis and also occurred at high prevalence in chickens at farms (Denis *et al*., 2001; Saleha, 2002), slaughterhouses (Denis *et al*., 2001) and at retail level (Denis *et al*., 2001; Sallam, 2007). Denis *et al*. (2001) reported 79.2% of campylobacters were detected in poultry houses. Saleha (2004) had shown that *campylobacter* colonization in broiler farm was possibly associated with untreated water, the presence of other animals and unhygienic management practices. She also indicated flying birds could also be the source of campylobacters as the birds were found to harbour campylobacters. Denis *et al*. (2001) had shown that campylobacter occurs at all stages of French chicken production.

In this study, we aim to investigate the prevalence of the thermophilic *Campylobacter* spp. in different set up from farm to retail level: 1) Farm (open-system and close-system), 2) slaughterhouse (conventional and automated plant) and 3) retail (wet market and supermarket).

MATERIALS AND METHODS

Bacterial Strain: *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 43478) were used in this study to serve as positive control.

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Sample collection: A total of 577 samples obtained from different stages of chicken production were analyzed. All samples were collected from different locations within Selangor, Malaysia (i) One hundred and fifty two cloacal swabs samples were obtained from three close-house system farms (a total of five houses). Similarly, a total of one hundred cloacal swabs samples were obtained from two open-house system farms (a total four houses). All cloacal swabs were transported in Cary and Blair Transport Medium (Becton, Dickinson and Company, Sparks, MD 21152 USA) in a cool box with ice packs. All cloacal swabs were taken from chickens with age of four weeks old. (ii) All slaughterhouse samples were end-slaughtering samples from slaughter houses or plants. One hundred and fifty chicken samples with skin (breasts, keels, wings, thighs and drumsticks) were obtained from three conventional slaughterhouses while 50 packaged chicken samples with skin (breasts, keels, wings, thighs and drumsticks) were obtained from one supermarket which was supplied directly from two processing plants. (iii) Fifty chicken samples with skin (breasts, keels, wings, thighs and drumsticks) were obtained from two wet markets while seventy five loose chicken samples with skin (breasts, keels, wings, thighs, and drumsticks) displayed on crushed ice were obtained from three supermarkets.

Samples enrichment and *Campylobacter* spp. isolation: All samples were enriched in Bolton Selective Broth (Merck). From the sample enrichments, isolations of *Campylobacter* spp. were done using mCCDA agar as described previously in Tang *et al.* (2009).

DNA extraction and PCR assay: DNA extraction from enrichment samples were carried as described by Tang *et al.* (2009). All enriched samples were examined for the presence of *Campylobacter* spp., *C. jejuni* and *C. coli* by PCR assay using three selective primers used in previous study (Tang *et al.*, 2009). The three selective primers for *Campylobacter* spp., *C. jejuni* and *C. coli* were *16S* rRNA gene (Linton *et al.*, 1996), the *hip* gene (Linton *et al.*, 1997) and the *ceuE* gene (Gonzalez *et al.*, 1997), respectively. DNA from reference cultures, *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 43478), were included as a positive control in every PCR assay. PCR amplification was performed in the same conditions described in Tang *et al.* (2009).

RESULTS

Table 1 shows the detection of campylobacter in various chicken samples using conventional plating as well as PCR method. Conventional plating method detected 25.5% campylobacter in chicken samples while PCR detected 65.2% campylobacter presence in chicken samples. PCR detection showed a greater sensitivity in campylobacter detection than conventional plating methods.

Campylobacter spp. was not detected any of the 152 cloacal swabs samples collected from three close-house system rearing farm. In contrast, there was a high prevalence of campylobacter presence in the cloacal swabs samples collected from two open-house system rearing farms with a prevalence of 93.9% and 96.1%, respectively (Table 1).

Out of the 150 end-slaughtering chicken samples from conventional slaughterhouse, 84.0% were positive with campylobacters (Table 1). Similarly, 94.0% of 50 chicken samples from processing plant were found positive with campylobacters (Table 1).

Table 1: Prevalence of campylobacter on chicken samples using conventional plating and Polymerase Chain Reaction (PCR)

		Plating		PCR	
Samples/Location	n	n	%	n	%
Farm					
Farm 1 (1 house)	50	0	0.0	0	0.0
Farm 2 (2 houses)	51	0	0.0	0	0.0
Farm 3 (2 houses)	51	0	0.0	0	0.0
Farm A (2 houses)	49	28	57.1	46	93.9
Farm B (2 houses)	51	36	70.6	49	96.1
Slaughter house					
S1	50	0	0.0	39	78.0
S2	50	0	0.0	46	92.0
S3	50	0	0.0	41	82.0
PP1	25	19	76.0	23	92.0
PP2	25	15	60.0	24	96.0
Retail					
W1	25	1	4.0	18	72.0
W2	25	3	12.0	21	84.0
SP1	25	17	68.0	24	96.0
SP2	25	15	60.0	22	88.0
SP3	25	13	52.0	23	92.0
Total	577	147	25.5	376	65.2

Note: Farm: Close-house system: Farm 1, Farm 2, Farm 3; Open-house system: Farm A, Farm B
Slaughterhouse: Conventional slaughterhouse: S1, S2, S3; Processing plant: PP1, PP2
Retail: Wet market: W1, W2; Supermarket: SP1, SP2, SP3

Table 2: Prevalence of *Campylobacter* spp., *C. jejuni*, *C. coli* and both *C. jejuni* and *C. coli* on chicken samples at farm, slaughtering and retail

		Campylobacter spp.		*C. jejuni* only		*C. coli* only		*C. jejuni* and *C. coli*	
Samples/Location	n	n	%	n	%	n	%	n	%
Farm									
Farm (Close-house system)	152	0	0.0	0	0.0	0	0.0	0	0.0
Farm (Open-house system)	100	95	95.0	94	94.0	1	1.0	0	0.0
Total	252	95	37.7	94	37.3	1	0.4	0	0.0
Slaughtering									
Conventional slaughterhouse	150	126	84.0	112	74.7	1	0.7	13	8.7
Processing plant	50	47	94.0	19	38.0	0	0.0	28	56.0
Total	200	173	86.5	131	65.5	1	0.5	41	20.5
Retail									
Wet market	50	39	78.0	35	70.0	0	0.0	4	8.0
Supermarket	75	69	92.0	52	69.3	2	2.7	15	20.0
Total	125	108	86.4	87	69.6	2	1.6	19	15.2

A total of 50 chicken samples purchased from wet markets and 75 chicken samples purchased from supermarket; campylobacters occurrence were 78.0% and 92.0% of the 50 wet market and 75 supermarket chicken samples, respectively (Table 1).

Table 2 summarized the prevalence of *Campylobacter* spp., *C. jejuni*, *C. coli* and both *C. jejuni* and *C. coli* in chicken samples from farm, slaughtering and retail level. *C. jejuni* was predominant in the chicken samples from farm, slaughtering and retail level with occurrence at 37.3, 65.5 and 69.6%, respectively. *C. coli* was less contaminating chicken samples from farm, slaughtering and retail with percentage of 0.4, 0.5 and 1.6%, respectively. Chicken samples from farm, slaughtering and retail level contaminated with both *C. jejuni* and *C. coli* at 0.0, 20.5 and 15.2%, respectively.

DISCUSSION

Isolation of *Campylobacter* spp. from retail poultry was shown to vary from 0-71.2% (Willis and Murray, 1997; Cloak *et al*., 2001; Dominguez *et al*., 2002; Whyte *et al*., 2004; Saito *et al*., 2005; Tang *et al*., 2009). Whyte *et al*. (2003) reported that isolation of campylobacters is media-dependent. The Polymerase Chain Reaction (PCR) technique had been proven to be useful for campylobacter detection and confirmation of *C. jejuni* and *C. coli* in naturally contaminated poultry samples (Mateo *et al*., 2005). PCR technique was proven to be more sensitive compare to conventional plating method (Chai *et al*., 2007).

Several studies showed no evidence of vertical transmission of campylobacters to the broiler chickens (Jacobs-Reitsma *et al*., 1995; Newell and Fearnley, 2003; Workman *et al*., 2008). Workman *et al*. 2008 suggested horizontal transmission is the most significant mode of broiler flock colonization. In this study, open-house system had a high prevalence of campylobacter colonization in chickens. This finding is expected as open-house system is more susceptible to birds and animals entering the chicken houses which might carry campylobacter. The present study showed 93.9-96.1% of chickens in open-house farm were campylobacters positive. This finding is similar to the findings of Evans and Sayers (2000) that showed once the campylobacters colonized the chicken, the whole flocks will be infected within a week. As the cloacal swab was taken from the chicken at the age of 4 weeks, there is a high possibility of campylobacters colonizing all the chickens by the time of slaughtering (5-6 weeks). Close-house system at farm level was shown to effectively prevent colonization of campylobacter in chickens. Close-house system kept the chickens within well ventilated close-houses which effectively prevented wild birds and other animals from entering the house. Such approach reduces the chances of campylobacter being carried by those animals from entering the chicken houses and colonizing the chickens.

The present study showed high prevalence of campylobacters occurred in chicken samples during slaughtering process regardless the samples were from conventional slaughterhouse or packaged of chicken samples from automated chicken processing plant. As there are many steps involved during slaughtering process in conventional slaughterhouse or automated processing plant, campylobacter cross-contamination is very likely to occur. Although the present study showed 3 close-house chicken farms (a total of 5 houses) was campylobacter-negative in the cloacal swabs; all chicken samples after slaughtering showed high prevalence of campylobacter contamination. This result is not surprising as cross-contamination of campylobacter during processing is very common and unavoidable. There are various ways where campylobacter contaminate chicken carcasses during processing which includes scalding, defeathering, evisceration, washing, cooling and packaging. The primary source of contamination to chicken carcasses is from their intestinal content

(Oosterom *et al*., 1983; Berndtson *et al*., 1992; Ono and Yamamoto, 1999). Rivoal *et al*., 1999 suggested there is cross-contamination to successive flocks as they found same genotypes of *C. jejuni* with isolates from intestinal contents were obtained from carcasses from successive flock. The findings were supported by Miwa *et al*. (2003) as they found campylobacter-negative flocks were positive with campylobacter isolates which have RAPD PCR type the same as those isolates from cecal contents of previously processed *C. jejuni*-positive flock. Various studies showed there a different ways of cross-contamination during processing includes transport crates (Newell *et al*., 2001; Slader *et al*., 2002), scalding (Stern *et al*., 2001), defeathering (Oosterom *et al*., 1983; Genigeorgis *et al*., 1986; Ono and Yamamoto, 1999; Berrang *et al*., 2000; Berrang *et al*., 2001) and carcass chillers (Sanchez *et al*., 2002; Whyte *et al*., 2002).

In this study, *Campylobacter* spp. occurrence on retail chicken meats ranged from 72-96%. Savasci and Özdemir, (2006) reported 83.4% of the chicken samples analyzed were contaminated with *Campylobacter* spp. Sallam (2007) also found high prevalence (64%) of campylobacters in retail chicken products despite general cleanliness and general level of sanitation practiced in Japan. The study reported contaminations of campylobacters on chicken products are unavoidable as poultry is the main reservoir for campylobacters.

C. jejuni was predominant in the chicken samples analyzed in this study compared to *C. coli* (Table 2). The results were similar to those of other studies which also found *C. jejuni* was more commonly found than *C. coli* in chickens (Willis and Murray, 1997; Denis *et al*., 2001; Whyte *et al*., 2004; Savasci and Özdemir, 2006; Sallam, 2007).

This study concluded that although close-house system may prevent or delay the colonization of *Campylobacter* spp. in chickens at farm level, the contamination by campylobacters in chicken samples at slaughtering and retail level are still very high. Such high occurrence of *Campylobacter* spp. in chicken products will pose high risk of human infections with the organisms. Thus, monitoring campylobacters spp. contamination at all levels of chicken production would be desirable.

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