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Prevalence of *Campylobacter jejuni* in chicken meat and chicken-based products

(Prevalens *Campylobacter jejuni* di dalam daging ayam dan produk berasaskan ayam)

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Abstract

A study was conducted to determine the prevalence of *Campylobacter jejuni* in raw chicken (fresh and chilled), marinated raw chicken (fresh and chilled) and chicken based products (frozen) from wet markets and supermarkets in Hulu Langat area. Samples were collected randomly for 8 months from February–September 2007. Isolation of *C. jejuni* was done using conventional methods in which 25 g of each sample was homogenized with 225 ml of Bolton Broth (supplemented with Bolton Broth Selective Supplement and Laked Horse Blood) and incubated in microaerophilic condition at 37 °C for 4 h followed by 42 °C for 48 h. Samples were then streaked onto *Campylobacter* blood-free selective agar base supplemented with CCDA selective supplement and was incubated at 42 °C for 48 h. Final confirmation was carried out using CAMP-ID, the *Campylobacter* identification system which comprises three biochemical tests for the presumptive identification of thermophilic *Campylobacter* spp. Results showed that *C. jejuni* can be found in various parts of fresh and chilled raw chicken meat such as the breast, wings, thigh, neck, vent, liver, gizzards and feet. Analysis done on marinated raw chicken meat and frozen chicken-based products (chicken nuggets, burger, frankfurter, chicken meat ball, chicken mosaic and minced chicken) showed the absence of *C. jejuni*.

Introduction

The most important pathogenic strains belonging to the group of thermo-tolerant *Campylobacter* is *C. jejuni*. This species is most often isolated from human. *Campylobacter jejuni* is most often implicated as the causative agent of Campylobacteriosis (Skirrow 1998). *Campylobacter* occurs as a commensal microorganism in the microflora of the gastrointestinal tract of poultry and is likely to occur on the exterior of the bird as well as from the contamination of the growth environment. The high optimal growth temperature of the organism, which approximates the normal body temperature of poultry, encourages gastrointestinal establishment (Rob et al. 2003). *Campylobacter* infections in human are usually associated with the ingestion of improperly handled or undercooked food, mainly poultry products (Ruberg et al. 1998).

Even though *Campylobacter* occurs as natural microbial flora in the gastrointestinal tract of poultry but after slaughtering it can spread to poultry carcasses, parts and products. Due to the fact that *C. jejuni*

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survives well in modified atmosphere and vacuum packaging and also under refrigerated temperatures (Rob et al. 2003), there are possibilities that it could occur in packaged chicken products that has gone through poor handling and processing. Therefore, this project was undertaken to evaluate the prevalence of *C. jejuni* in raw chicken meat (fresh and chilled), marinated raw chicken (fresh and chilled) and also packaged chicken-based products that have been stored in freezing condition.

Materials and methods

Sample collection

Samples of whole chicken and chicken parts were randomly collected from various wet markets (for all fresh raw chicken meat) and supermarkets (for marinated samples, all chilled and frozen samples) in Hulu Langat area. Summary of analysed samples are mentioned in *Table 1*. Samples were taken to the laboratory on the day of collection in insulated boxes containing thermafreeze ice replacement and analysed within 24 h. Collection of samples was done randomly for 8 months from February to September 2007.

Isolation of Campylobacter jejuni

Twenty five g of each sample was homogenized with 225 ml of Bolton Broth (BB; Oxoid, UK) supplemented with half a vial of Bolton Broth Selective Supplement (BBSS; Oxoid, UK) and 5% Laked Horse Blood (Oxoid, UK). The homogenates were transferred to screw-capped sterile bottles and placed into anaerobic jars containing CampyGen pack (Oxoid, UK) that produced suitable gaseous atmosphere for microaerophilic condition. The incubation period was at 37 °C for 4 h followed by 42 °C for 48 h. After incubation period, samples were streaked onto *Campylobacter* blood-free selective agar base (CCDA; Oxoid, UK) supplemented with one vial of CCDA selective supplement (Oxoid, UK). Plates were incubated for 24–48 h at 42 °C under microaerophilic condition.

Identification of isolated C. jejuni

Colonies that grew on CCDA agar were examined based on morphological appearance and Gram-staining. They were grey in colour, moist, glossy, flat spreading colonies with or without a metallic sheen. The *C. jejuni* ATCC 33291 bought from American Type Culture Collection was used as a reference and control during the identification of the colonies. Through microscopic examination, it was Gram-negative with curved or spiral-shaped rods. Suspected colonies were then sub-cultured onto Columbia Blood Agar (CBA; Oxoid, UK) supplemented with 25 ml of Laked horse blood and Campylobacter Growth Supplement Liquid (CGSL; Oxoid, UK). Plates were incubated for 24–48 h at 42 °C under microaerophilic condition. Biochemical tests were conducted for confirmation of the species using the *Campylobacter* identification system, CAMP-ID (Mast Diagnostics, UK) which comprises three biochemical tests; namely the urease test, indoxyl acetate test and hippurate hydrolysis test for the presumptive identification of thermophilic *Campylobacter* spp. (Colles et al. 2003).

Table 1. Summary of samples analysed

Sample	Number of samples	Number of positive samples
Raw chicken meat and marinated raw chicken (*fresh)	94	48
Raw chicken meat and marinated raw chicken (chilled)	35	9
Chicken-based products (frozen)	22	0
Total	151	57

*Fresh samples refer to samples that were neither chilled nor frozen

Results and discussion

A total of 151 samples were analysed and 57 samples were found to be contaminated with *C. jejuni* (*Table 1*). All fresh samples were collected from the wet market. Fresh samples refer to samples that have been kept in normal ambient temperature without chilling or freezing. All chilled, frozen as well as marinated raw samples were collected from the supermarket. The wet market in the Hulu Langat area do not provide any chiller or freezer for displaying the chicken, thus no temperature control is practised. However, the supermarkets provide facilities for raw chicken storage and displaying in chilled or frozen condition. This is proof that efforts are being taken to control the quality and safety of the raw chicken sold in those premises.

Due to the condition of the premises (wet markets without chiller or freezer) where most fresh samples were taken, a very high prevalence of *C. jejuni* has been isolated. About 51% of fresh samples collected were contaminated with *C. jejuni* (48 out of 94 samples) (*Table 2*). All parts of fresh raw chicken (breast, wings, thigh, neck, vent, liver, gizzards and feet) were found contaminated with *C. jejuni*. It is often that if one part of the chicken has been contaminated, there are chances that the organism will spread to other parts as well. Thus, it is not surprising to find that even the inner parts of the chicken were also contaminated, most likely due to cross contamination during processing. However, among the fresh samples, only marinated chicken were found free from the organisms. A marinade is a liquid mixture in which food is soaked prior to cooking and contains spices, herbs, salts, oil and an acid (Faridah 2002). In marinated raw chicken, failure of isolating *C. jejuni* could be due to a high concentration of salt and other ingredients contained in the marinades. The *Campylobacter* can usually survive up to 2.0% of salt (Rob et al. 2003). So in this case, it was possible that the samples might have been contaminated by *C. jejuni*, but later was killed by the use of marinades.

There was a low contamination of *C. jejuni* in chilled chicken samples which comprised of whole chicken, chicken wings, thighs, vent, feet, liver, gizzard as well as marinated chicken. The chilled samples were taken from various supermarkets where chicken meat was stored and displayed in chilled condition. Supermarkets, usually offer chicken meat under conditions that appear more hygienic than those in wet markets with better control of the storage temperatures. For whole chicken or chicken

Table 2. *Campylobacter jejuni* isolated from fresh raw chicken and fresh marinated raw chicken

Sample (*Fresh raw chicken, and *fresh marinated raw chicken)	*C. jejuni* positive samples/ total no. of samples plated	% of *C. jejuni* positive samples/ total no. of samples plated
Whole chicken	12/32	37.05
Chicken breasts	4/6	66.67
Chicken wings	6/8	75.00
Chicken thighs	6/8	75.00
Chicken neck	3/4	75.00
Chicken vent	6/10	60.00
Chicken liver	4/6	66.67
Chicken gizzards	1/4	25.00
Chicken feet	6/10	60.00
Marinated chicken vent	0/2	0.00
Marinated chicken wings	0/2	0.00
Marinated chicken thighs	0/2	0.00
Total	48/94	51.06

*Fresh samples refer to samples that were neither chilled nor frozen

pieces sold in the supermarkets, they are usually placed on polystyrene trays and covered with a cling film. In order to catch any excessive liquid released from the meat, an absorbent (a kind of chemical placed behind the plastic material) was used and put into the tray together with the chicken or chicken pieces.

Results indicated that only nine samples (25.71%) of chilled raw chicken out of total 35 samples were contaminated with the organism (*Table 3*). *Campylobacter jejuni* can still be isolated and found in the chicken meat, even though the supermarkets have chillers and refrigeration system for displaying and storage of the chicken samples. There are many possibilities as to how this could happen. Refrigeration and freezing are used to control bacterial growth in foods. It is understood that *Campylobacter* has an optimal growth temperature in the range of 37 °C to 42 °C and do not grow below 30 °C (Saumya and Bryan 2004). So obviously, chicken meat stored or displayed in chilled condition should be *Campylobacter* free. However, Hazelegar et al. (1998) reported that *C. jejuni* displayed physiological activity at 4 °C and Skirrow (1998) reported that *Campylobacter* could survive in water for several weeks at 4 °C. Although microbial growth is arrested during low temperatures and some of the bacteria may be killed, but a fraction of it may survive or are severely injured. This could be the answer as to why *Campylobacter* was isolated from chilled samples in this study.

If raw chicken meat brought to the supermarkets are already contaminated or was contaminated anywhere along the process before storing at low temperatures, the chances of *C. jejuni* or some of it to survive is still there. Even though foodstuffs in supermarkets appear more hygienic, the preparation before packing could be unhygienic and the packing facilities itself could be in poor conditions. So there is actually a chance of *C. jejuni* occurring in this kind of foodstuff due to cross-contamination. Bryan and Doyle (1995) and Baumgartner et al. (1995) also reported the prevalence of *C. jejuni* in chilled and frozen uncooked chicken in their study. Rob et al. (2003) concluded from his study that once the food has been contaminated, *C. jejuni* has the ability to survive at low temperatures. He also observed that the survival of *C. jejuni* is better under refrigeration than at room temperature. The survival rate is 15 times better at 2 °C than at 20 °C

Results on analysis of 22 frozen chicken products (chicken nuggets, burger, frankfurter, chicken meat ball, chicken mosaic and minced chicken) indicated that all samples were free of the organism. There are very limited studies done on the prevalence of *Campylobacter* in these kinds of frozen chicken products worldwide. Even though frozen chicken products may be at

Table 3. *Campylobacter jejuni* isolated from chilled raw chicken and chilled marinated raw chicken

Sample (Chilled raw chicken and marinated raw chicken)	*C. jejuni* positive samples/ total no. of samples plated	% of *C. jejuni* positive samples/ total no. of samples plated
Whole chicken	0/6	0.00
Chicken wings	2/4	50.00
Chicken thighs	3/5	60.00
Chicken vent	2/5	40.00
Chicken feet	2/6	33.33
Chicken liver	0/3	0.00
Chicken gizzard	0/2	0.00
Marinated chicken chops	0/2	0.00
Marinated chicken wings	0/2	0.00
Total	9/35	25.71

a lower risk in supporting the growth of *Campylobacter*, there are possibilities of isolating *C. jejuni* from contaminated frozen products (Rob et al. 2003). This is because the numbers of *C. jejuni* decline slowly at normal freezing temperatures and therefore does not instantly activate the organism in foods.

However, *C. jejuni* was not detected in the 22 types of frozen chicken-based products because they had undergone minimal cooking process prior to freezing. This may have reduced the risk of occurrence of *Campylobacter*. This result is in line with most of the findings involving cooked-chilled chicken products including a study done by Joint Food Safety and Standard Group of United Kingdom (1996). They also found that 758 cooked-chilled chicken products analysed were free from *Campylobacter*.

Heating at 55 °C and above, rapidly inactivates the organism (Rob et al. 2003). Thorough cooking kills *Campylobacter* as indicated by Rob et al. (2003). The level of contamination of *Campylobacter* in ready-to-eat chicken products that has undergone thorough cooking is relatively low. However, undercooked chicken may represent a considerable source of risk. Findings by Quinones-Ramirez et al. (2000) showed that 27% out of 100 samples of undercooked roasted chicken tacos were contaminated with *Campylobacter*. Humphrey et al. (2001) indicated that the high level of contamination of raw chicken with *C. jejuni* has been shown to contaminate food preparation surfaces and subsequent re-infection of the cooked chicken or other food. Thus, sterile chicken products due to thorough cooking or low risk group of cooked-chilled chicken products could still be re-contaminated by the raw chicken with high load of *Campylobacter* through unhygienic practices.

The results of the prevalence study involving *Campylobacter* in foods may also depend on the methods used for analysis. Isolating *Campylobacter* from food products required enrichment procedure to determine the level of contamination. However, according to Rob et al. (2003) the use of a conventional enrichment method for isolation of *Campylobacter* was not sufficient for the recovery of freeze-injured organisms. This could lead to false negative results where the frozen poultry were largely free of *C. jejuni* as in this study. Freezing is thought to damage the outer membrane of *Campylobacter* and make the cell resistant for isolation (Humphrey 1988). Therefore, Lay et al. (2007) suggested the use of Polymerase Chain Reaction (PCR)-based methods that have been successfully used in isolation of the organism from vegetables for *Campylobacter* detection. The use of PCR could probably help to improve the isolation technique in frozen based products as well.

Conclusion

Campylobacter jejuni can be found in all parts of fresh raw chickens. However, a very low prevalence of *C. jejuni* was observed in chilled raw chickens. *Campylobacter jejuni* was not detected in fresh and chilled marinated raw chicken as well as in frozen chicken-based products.

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Abstrak

Kajian telah dijalankan untuk menentukan kehadiran *Campylobacter jejuni* di dalam ayam mentah segar dan disejuk dingin, ayam mentah yang diperap segar dan disimpan sejuk dingin serta produk berasaskan ayam yang disejuk beku. Sampel diperoleh daripada pasar basah dan juga pasar raya di sekitar Hulu Langat. Sampel diambil secara rawak selama 8 bulan bermula dari Februari hingga September 2007. Pemencilan *C. jejuni* dijalankan dengan kaedah konvensional dengan setiap 25 g sampel dihomogenkan dengan 225 ml kaldu Bolton (ditambah dengan kaldu Bolton terpilih dan darah kuda) dan dieramkan pada 37 °C selama 4 jam diikuti pada 42 °C selama 48 jam di dalam keadaan mikroaerofilik. Sampel yang telah dieram kemudiannya dipindahkan ke atas agar CCDA yang mengandungi suplemen menggunakan kaedah coretan agar. Agar CCDA ini kemudiannya dieramkan pada suhu 42 °C selama 48 jam. Pengesahan koloni yang tumbuh dilakukan menggunakan sistem pengesahan CAMP-ID yang terdiri daripada tiga ujian biokimia. Keputusan menunjukkan kehadiran *C. jejuni* pada pelbagai bahagian ayam mentah segar dan disejuk dingin (dada, kepak, paha, leher, ekor, hati, hempedu dan kaki). Walau bagaimanapun, *C. jejuni* tidak terdapat di dalam sampel daging ayam mentah yang diperap serta produk berasaskan ayam (nugget, burger, frankfurther, bebola ayam, mozek ayam, daging ayam cincang) yang disejuk beku.

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