**NOTE** *Public Health*

# Comparison of *Campylobacter* Contamination Levels on Chicken Carcasses between Modern and Traditional Types of Slaughtering Facilities in Malaysia

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ABSTRACT. A total of 360 samples including fresh fecal droppings, neck skins, and swab samples was collected from 24 broiler flocks and processed by 12 modern processing plants in 6 states in Malaysia. Ninety samples from 10 traditional wet markets located in the same states as modern processing plants were also collected. Microbiological isolation for *Campylobacter* was performed following ISO 10272–1:2006 (E). The overall rate of contamination for *Campylobacter* in modern processing plants and in traditional wet markets was 61.1% (220/360) and 85.6% (77/90), respectively. *Campylobacter jejuni* was detected as the majority with approximately 70% for both facilities. In the modern processing plants, the contamination rate for *Campylobacter* gradually declined from 80.6% before the inside-outside washing to 62.5% after inside-outside washing and to 38.9% after the post chilling step. The contamination rate for *Campylobacter* from processed chicken neck skin in traditional wet markets (93.3%) was significantly ($P<0.01$) higher than in modern processing plants (38.9%).

KEY WORDS: *Campylobacter*, chicken carcasses, Malaysia, slaughtering facilities.

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*Campylobacter* is a zoonotic bacterium frequently associated with acute gastroenteritis in humans both in developing and industrialized countries [15]. Human campylobacteriosis is predominantly caused by *Campylobacter jejuni* and its close relative *Campylobacter coli* [7].

*Campylobacter* spp. is frequently found in the intestinal tract of poultry where colonization leads to the contamination of carcasses during processing, especially at the defeathering and evisceration stages. Furthermore, it has been suggested that cross-contamination of carcasses between different flocks occurs during processing [8 10]. The sources of carcass contamination and the mechanisms by which the organisms spread between birds during processing are not fully understood; however, it is extremely difficult to prevent cross-contamination between birds and flocks at slaughter [1].

Malaysia is self-sufficient in meeting the country's demand for broiler meat with approximately 2,500 broiler farms producing over 400 million birds. About 30% of broilers are channeled through modern processing plants while the remaining 70% are sold as live or dressed birds in wet markets. Currently, 17 established, fully automated and modern broiler slaughter plants are in operation throughout the country. The modern plants operated under strict protocol; however, after evisceration, washing, and rapid chilling; *Salmonella* and *Campylobacter* are commonly found.

Studies conducted in Malaysia concerning broiler chicken farms detected *Campylobacter* in 38.2% of 14-day-old chicks and in 45.3% of 21-day-old chickens [13]. Another study reported a prevalence of *Campylobacter* spp. as 97.1% in broiler chickens and as 51.5% in village chickens [14]. The aim of this study was to compare the contamination of *Campylobacter* in chicken carcasses during processing in modern poultry processing plants and in traditional wet markets. *Campylobacter* dispersal during all procedures may play a major role in cross contamination; thus, *Campylobacter* contamination levels in the equipment and the environment of modern processing plants and traditional wet markets were also determined.

Twelve of the 17 modern slaughterhouses and 10 traditional wet markets located in 6 out of 13 states in Malaysia, including Selangor, Malacca, Perak and Negeri Sembilan, Johor and Penang, were investigated. The origins of broiler chickens are intensive farms in Malaysia.

All abattoirs are modern with highly mechanized and sophisticated equipments. Slaughter per day varies between 15,000 birds to 140,000 birds. Electrical stunning is done, consecutively, halal slaughter is carried out. The birds are scalded by immersion in hot water at a temperature between 56°C and 62°C for 2 to 2.5 min. After scalding, the feathers are mechanically removed by a series of online plucking machines. Evisceration is done mechanically. The carcass then passes through an automatic inside-outside bird washer. Immersion chilling is done by a spin chiller, the water being chlorinated to attain a concentration of 20 to 40 ppm. In contrast, slaughter per day for the traditional wet

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market is between 500 to 1,000 birds. The chicken is slaughtered by halal method with a sharp knife. Chickens are scalded in a scalding tank of 58 °C to 60 °C for 2 to 3 min. De-feathering is carried out with a semi-automatic de-feathering machine with rubber fingers. The evisceration process is performed by hand with a knife to open the carcass. No chlorination is used in the water for cleaning the carcasses and no chilling process is practiced. Processed carcasses and organs are displayed on a small concrete table for purchase.

Sample size of the study was estimated based on the total flock number that was slaughtered in all the 12 plants during November 2008 to April 2009. A total of 360 samples was collected. Samples were taken from 24 flocks (15 samples each) from 12 processing plants by taking broiler samples and equipment swab samples. Broiler samples were taken at 4 per flock and 3 duplicates were taken at selected sampling sites.

Samples were collected from 7 sites; 4 corresponding to the broiler and 3 to swabs. Upon flock arrival, randomly collected, 25 g fresh fecal broiler samples were obtained from the transport crates. At the remaining three sampling sites, before inside-outside washing, after inside-outside washing and post chilling, 25 g of neck skin was taken. Swab samples were taken from the scalding tank, chilling tank and conveyer belt before chilling. Swab samples were collected from the internal part of equipment early morning before commencing slaughter procedure.

Samples were also collected from 10 traditional wet markets. A total of 9 samples was collected from each wet market, 3 pooled fresh droppings from the crates, 3 neck skin samples from processed carcasses and 3 swab samples from display tables.

Samples were put in a sterile stomacher bag, kept in an icebox, and sent to the laboratory within 1–2 hr. Since *Campylobacter* is very sensitive to freezing but survives best at low temperature, samples were stored at 3°C ± 2°C and were analyzed within 24 hr.

*Campylobacter* isolation was done according to the procedure for isolation and identification of *Campylobacter* spp. as described by ISO 10272–1:2006 (E) [3]. In brief, 25 g of neck skin/feces were added to 225 m*l* of enriched *Campylobacter* selective medium (Bolton broth; Oxoid, CM983) with 5% lysed horse blood, Polymyxin B (10,000 IU/*l*), rifampicin (20 mg/*l*), trimethoprim (20 mg/*l*), cycloheximide (0.2 mg/m*l*). For swab samples, one swab sample was added to 90 m*l* of Bolton broth, and incubated under micro aerobic conditions (7% $O_2$, 10% $CO_2$ and 83% $N_2$) at 37°C for 4 hr and then at 42°C for 2 days. From the cultures obtained, one loop of suspension was inoculated with a sterile loop onto the surface of a selective medium, modified charcoal cefoperazone deoxycholate agar (Oxoid, CM739) as the first, and Karmali Agar (Oxoid, CM935) with sodium pyruvate (0.1 mg/*l*), cefoperazone (0.032 mg/*l*), vancomycin (0.02 mg/*l*), amphotericin (0.01 mg/*l*) as the second medium. Cultures were incubated under micro aerobic conditions for 44 ± 4 hr at 42°C to detect the presence of colonies from thermophilic *Campylobacter*.

Typical colonies are greyish on mCCD agar, often with a metallic appearance, and are flat and moist with a tendency to spread. Suspected colonies were also streaked onto Brucella Medium Base agar (Oxoid, CM169) with 5% inactivated sheep blood and incubated under the above conditions for 24 hr. Typical colonies from agar were harvested and the hanging drop motility test was performed to detect the cork screw motility movement typical for *Campylobacter*. Gram staining and biochemical tests (catalase and oxidase,) were also performed to confirm the genus level. The Campy Latex agglutination test (Oxoid, Dry Spot *Campylobacter* Test) was also conducted to confirm 4 species levels of *Campylobacter* (*C. jejuni, C. coli*, *C. lari* and *C. upsaliensis*). Finally, the hippurate hydrolysis test was done to confirm the *C. jejuni* species. The *Campylobacter* isolates were stored by mixing in Bolton broth (Oxoid, CM983) with supplement and 20% (1 Vol/1Vol) Glycerine in cryovials at –80°C.

The overall rate of contamination for *Campylobacter* in modern processing plants was 61.1% (220/360). Prevalence of *Campylobacter* from the swab samples taken from scalding tanks, chilling tank and conveyer belt prior to slaughter was 40.3%. The prevalence of *Campylobacter* in live broiler chickens at the arrival area (droppings) was 83.3% (60/72). The contamination rate for *Campylobacter* gradually declined from 80.6% (58/72) before inside-outside washing to 62.5% (45/72) after inside-outside washing and to 38.9% (28/72) at the post chilling position.

The overall rate of contamination for *Campylobacter* in traditional wet markets was 85.6% (77/90). The live broiler chickens for traditional wet markets are supplied by similar farms which supply to modern processing plants. There was no decrease in the contamination level of *Campylobacter* in the processed chicken carcasses; instead, the contamination level increased. The contamination rate for *Campylobacter* from processed chicken neck skin in traditional wet markets was also significantly higher than in modern processing plants ($P<0.01$) (Table 1).

The high prevalence of *Campylobacter* spp. in traditional wet markets is attributable to conditions which allow the agent to be transferred more easily. This is in agreement to an earlier study carried out in Malaysia [12] where the prevalence was 87%.

In our study, sampling was not carried out at farm level; however, the high prevalence of *Campylobacter* at arrival (droppings) in the abattoir (83.3%) suggests a high prevalence in the birds at the farm level. Prior to scalding, it is not possible to clean the birds. Fecal contamination may occur during shackling, stunning and bleeding, scalding and defeathering. During scalding, the birds are washed by the turbulence of water and transfer a certain amount and quality of bacteriological burden into the scalding tank. Some *Campylobacter* appear to survive the scalding because of their attachment to chicken skin [2]. Survival is due to a change in the skin surface as a result of scalding temperatures, facilitating the firm attachment of bacteria [5]. Yet, Izat and his

Table 1. *Campylobacter* from different sampling sites in modern poultry processing plants (MPP) and in traditional wet markets (TWM)

| Type of processing Facilities | Sampling site | Sample type | *Campylobacter* contamination level (%) | 95% CI |
|---|---|---|---|---|
| MPP | Equipment | Swabs (Scalding tank, chilling tank and conveyer belt before chilling) | 40.3 (29/72) | 28.9–51.6 |
| MPP | Crates at arrival | Fresh fecal droppings | 83.3(60/72) | 74.7–91.9 |
| MPP | Before inside outside washing | Processed chicken neck skin | 80.6(58/72) | 71.4–89.6 |
| MPP | After inside outside washing | Processed chicken neck skin | 62.5(45/72) | 51.3–73.6 |
| MPP | Post Chilling | Processed chicken neck skin | 38.9(28/72)[a)] | 27.6–50.1 |
| | | Overall | 61.1(220/360) | 56.0–66.1 |
| TWM | Environment | Table swabs | 80.0(24/30) | 65.6–94.3 |
| TWM | Crates | Fresh fecal droppings | 83.3(25/30) | 69.9–96.6 |
| TWM | Processed broiler | Processed chicken neck skin | 93.3(28/30)[b)] | 84.4–100 |
| | | Overall | 85.6(77/90) | 78.2–92.8 |

Data with difference superscript are statistically different ($P$<0.01) by Chi-Square test.

colleagues found a significant reduction of *Campylobacter* counts (1.84 logs) and concluded that the scalding operation is the most effective process for decreasing overall microbial levels on the surface of poultry carcasses [4]. However de-feathering, evisceration and harvesting of giblets brought an increase in carcass contamination. This can be due to viscera rupture during the evisceration process [11] and is further supported by the finding of a high proportion of *Campylobacter* contamination before inside-outside washing, just after the evisceration step.

The findings of *Campylobacter* in equipment prior to slaughter in modern processing plants suggest that *Campylobacter* was still present after cleaning and disinfection. Persistence is possibly because of an insufficient period between cleaning and disinfection and the next slaughter. One study indicates that *C. jejuni* is able to survive overnight on food processing equipment surfaces and after cleaning and disinfection procedures and that these strains may contaminate carcasses during the slaughter process [9].

In wet markets, the environment samples were also highly positive. Samples were taken from the display table where whole carcasses cut chicken parts and internal organs were kept for sell. Tables are made of concrete or mosaic and surfaces are rough. The table condition is wet and pooled with blood from the carcass. Such practices in the traditional wet market aggravate the *Campylobacter* contamination in the final processed carcasses [6].

This study reveals that *Campylobacter* contamination is widespread in live chickens before slaughter, in processing plant environments, and on the processed chicken carcasses. The high prevalence of *Campylobacter* in chickens from wet markets revealed poor hygienic environment. Appropriate control measures for reduction of *Campylobacter* in flocks on carcasses, and as a result of cross-contamination between the flocks during processing and during preparation are highly recommended.

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