Dynamic of *Campylobacter* species contamination along a poultry slaughtering chain

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**Abstract**

The prevalence of Campylobacters was studied in a poultry farm and along the slaughtering chain. Fifteen swabs from a farm and 75 samples (swabs and rinsates) from its slaughterhouse were collected. All the faecal and cloacal farm swabs were contaminated by *Campylobacter jejuni* and *C. coli* against 50% for breast swabs. *C. jejuni* had a concentration of 6.26, 6.34 and 5.38 Log$_{10}$ CFU/mL in faecal, cloacal and breast swabs respectively. Rinsates showed an almost constant concentration of Campylobacters (6 Log$_{10}$ CFU/mL) with a predominance of the presumptive *C. jejuni*. *C. lari* was found in 22% of mixed cultures. Faecal coliforms and *E. coli*, used as indicators, were detected in all samples (5.46 and 5.15 Log$_{10}$ CFU/mL respectively). Final chilling and chlorination (50 ppm) treatments decreased them to acceptable levels, unlike for Campylobacters. Further investigation of the dynamics of Campylobacters and their response to prevention and treatment measures is required.

**Introduction**

In the European Union Summary Report, undercooked chicken meat was estimated to cause 220,209 cases of confirmed campylobacteriosis in 2011 (EFSA, 2013). Ninety percent of these were caused by *Campylobacter jejuni* and 5-10% by *C. coli* (Tam et al., 2003). Despite an earlier description, it was not until 2011 that the United States Department of Agriculture (USDA) set the levels (WattAgNet, 2011). This may explain the absence of standards in Lebanon, where broiler production aims to meet international standards. Research work on *Campylobacter* human infections is very limited in Lebanon. However, Talhouk et al. (1998) reported rare contaminations in hospitalised humans, whereas Dabboussi et al. (2012) found 11% of *Campylobacter* cases in sick children.

In slaughterhouses, preventive measures are focusing on *Salmonella* and faecal coliforms. Yet, 75.8% of broiler carcasses were contaminated by Campylobacters (EFSA, 2011). Contamination may occur through the contact of faecal matter in overcrowded means of transport of animals. *Campylobacter*-negative batches are contaminated by the surface of equipments (Kudrikiene et al., 2010), while defaecation and evisceration are critical phases (Hue et al., 2010). The objective of this work was to assess the contamination of domestic broilers by Campylobacters at the farm level and throughout the slaughtering operations.

**Materials and Methods**

**Sampling**

Samples were collected from an open system farm and its slaughterhouse (12,000 birds/night) in one single sampling batch from a homogeneous flock. From 5 birds, cotton swabs (5x5 cm$^2$) were taken in duplicates from the breast (BS), cloacae (CS) and fresh faeces (FS). The sampling at the slaughterhouse is presented in Figure 1. Rinsates were obtained by shaking the whole carcasses in peptone water (0.1%) for 1 min in a sterile bag.

**Microbiological analysis**

*Campylobacter* was detected according to ISO 10272-1:2006 (ISO, 2006a). Swabs and rinsates (1 mL) were first enriched with Bolton Selective Enrichment Broth (CM0983+SR0183 E; Oxoid Ltd., Basingstoke, UK) and 5% lyed horse blood (SR048; Oxoid Ltd.). The incubation was carried in a microaerobic environment (85% N, 10% CO$_2$, 5% O$_2$) for 48 h at 42°C (CO$_2$ water-jacketed Incubator). Following incubation, 20 mL of diluted swab/rinsates were seeded on mCCDA-Preston (CM0739+SR0155E; Oxoid Ltd.). *Campylobacter* spp. was identified by testing for oxidase, catalase, indoxyl acetate hydrolysis and hippurate (Hendriksen et al., 2003). *C. jejuni* (ATCC 33291; Microbiologics, St. Cloud, MN, USA) served as a positive control. In parallel, rinsates and swabs were tested for aerobic plate count (APC) (ISO 4833:2003; ISO, 2003), faecal coliforms (ISO 4832:2006; ISO, 2006b) and *E. coli* (ISO 9308-1:2006; ISO, 2000).

**Results and Discussion**

**Farm level**

Aerobic plate count reached a maximum of 5.71 Log$_{10}$ CFU/mL in the CS, but scored a lower value (4.71 Log$_{10}$ CFU/mL) in the FS. Campylobacter concentrations were higher with 6.26 Log$_{10}$ CFU/mL in FS, 6.34 Log$_{10}$ CFU/mL in CS and 5.38 Log$_{10}$ CFU/mL in BS. Cross-contamination among broilers may have caused the detection of *Campylobacter* species in 100% of FS and CS against 50% in BS. The greatest prevalence of *Campylobacter jejuni* was observed in FS (37.5%) followed by CS (25%), then by BS (16.67%). *C. coli* were present in 25% of CS, 16.67% of BS and 12.5% of FS. Predominance of *C. jejuni* meets previous results obtained by Newell and Fearnley (2003), but disagrees with those from Greek farms contaminated only with *C. coli* (Marinou et al., 2012). Unidentified *Campylobacter* species were found in high counts (6.58 Log$_{10}$ CFU/mL) in 50% of CS and 16.67% of BS. The levels recorded for *C. jejuni* exceed USDA standards of 10.4% of contaminated raw chickens (WattAgNet, 2011). This contamination may be due to the coprophagic tendency of the birds (Newell and Fearnley, 2003) and the hot season (June-August).

**Slaughterhouse level**

Aerobic plate count started at 5.00 Log$_{10}$ CFU/mL and reached 9.5 Log$_{10}$ CFU/mL after washing (Figure 2). Its lower number on farm and receiving area may be due to the limited area sampled by swabbing (5x5 cm$^2$), while the peak after washing may be attributed to cross-contamination between basins batches. Chilling reduced the number to 7.5 Log$_{10}$ CFU/mL, but it was still above the international standards (5-7 Log$_{10}$ CFU/g) reported by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986).

The increase of faecal coliforms between
defeathering and evisceration (3.7 to 5.2 Log$_{10}$ CFU/mL) supports the scenario of physical damage. Washing reduced them to 3.46 Log$_{10}$ CFU/mL and chilling to 2.00 Log$_{10}$ CFU/mL. However, ICMSF (1986) considered unnecessary to have microbiological criteria for these organisms because they are part of the natural flora of poultry. In the end product, E. coli was below 2 Log$_{10}$ CFU/mL in 80% of carcasses rinsates, reaching the USDA standard. Following James et al. (2006), the water/immersion chilling reduces the number of indicator organisms by 1.1 Log$_{10}$ CFU/mL, without chlorine, and by 2.5 Log$_{10}$ CFU/mL with chlorine. Cross-contamination in immersion chilling was also considered a major problem in the EU, hence the suggestion to use dry chilling.

Unlike for faecal coliforms, Campylobacters remained constant throughout the slaughter- ing chain (5.95±0.04 Log$_{10}$ CFU/mL). The slight increase after evisceration (5.88 to 5.97 Log$_{10}$ CFU/mL) may be due to contamination caused by rupture of viscera and/or due to bacterial self-protection mechanisms (Teh et al., 2010), where the formation of biofilm potentially enhances the survivability in poultry environment. This level of contamination is higher than 2.4 Log$_{10}$ CFU/mL reported in carcasses (Chemaly et al., 2012).

Campylobacters were isolated from 95% of rinsates with the highest prevalence for C. jejuni (25%), followed by C. lari (22%), and C. coli (13%). In spite of its fastidious nature, favourable conditions for the pathogenic C. jejuni were encountered along the processing line. As shown by other studies, poultry slaughterhouses were variably contaminated starting from 50% in the UK and 51.9% in Belgium (Habib et al., 2012) to 87.5% in French abattoirs (Hue et al., 2010). In this slaughterhouse, the constant levels of Campylobacters (~6 Log$_{10}$ CFU/mL) indicate that hygienic treatments were ineffective, keeping percentages above the acceptable 10.4% USDA standard (WattAgNet, 2011). A systematic review showed an increase of Campylobacter by 10 to 72% after defeathering and by 15% after evisceration, while after washing results were inconsistent with a margin of 23% decrease to an increase of 13.3% (Guerin et al., 2010).

Unlike C. coli and C. lari, C. jejuni showed a maximum after evisceration, to be reduced later by about 35%. Campylobacters present in the intestines of slaughtered chickens would contaminate their carcasses throughout the processes. C. lari, absent at the reception, was detected after defeathering, while unidentified Campylobacters appeared to increase after washing.

Repeated rinsing of carcasses, designated as the vertical evolution (Figure 1) had no clear effect on Campylobacters. The lowest value recorded was after residual suction (5.73 Log$_{10}$ CFU/mL) and the greatest after washing (6.00 Log$_{10}$ CFU/mL). Similar observations were recorded for horizontal and diagonal evolution.

**Dynamic index**

Dynamic index (DI) was calculated in relation to an arbitrary reference index equal to 100 at defeathering. The DI showed a positive increase throughout the process with the greatest value after refrigeration (Figure 3A). The cumulative DI reached 190.03, which is equivalent to $1.43 \times 10^6$ CFU/mL. Despite its sensitivity to environmental factors, Campylobacter may have developed survival mechanisms such as the transition to a coccus shape, or through the development of a biofilm (Mihaljevic et al., 2007) adhering to the skin. The DI of C. jejuni (Figure 3B) decreased after evisceration (-26.83%) and slightly after washing (-4.03%). It increased by 20.37% after residual suction reaching its maximum of 40.24% after refrigeration. Globally, C. jejuni increased by 29.75%. The results showed that rearing and slaughtering conditions, mainly hygiene and chlorine treatment, were not adequate to render the end product safe.

**Table 1.** Sampling design for carcass rinsates showing the effect on the microbial load of frequent rinsing (vertical evolution), the number of rinsing (horizontal evolution) and no frequent rinsing (diagonal evolution).

| OPERATION               | SAMPLING TECHNIQUE | NUMBER OF SAMPLES |
|-------------------------|--------------------|-------------------|
| Reception               |                    | 5                 |
| Operation 1: Defeathering |                   | 5                 |
| Operation 2: Evisceration | 5          | 15               |
| Operation 3: Residual Suction | 5            | 20               |
| Operation 4: Washing    | 5                  | 20               |
| Operation 5: 24Hrs cooling at 0 – 2 °C | 5     | 5                |

![Figure 1](image1.png)

**Figure 1.** Sampling design for carcass rinsates showing the effect on the microbial load of frequent rinsing (vertical evolution), the number of rinsing (horizontal evolution) and no frequent rinsing (diagonal evolution).

![Figure 2](image2.png)

**Figure 2.** Dynamic of total Campylobacters, C. jejuni in comparison to aerobia plate counts, faecal coliforms and E. coli throughout the slaughtering operations.
Counts at defeathering: Campylobacters = $7.53 \times 10^5$ CFU/mL; C. jejuni $8.2 \times 10^6$ CFU/mL.

**Conclusions**

Sanitary practices and treatment conditions in the slaughterhouse tested in this study were effective against APC, faecal coliforms and E. coli only. Rearing system and slaughtering conditions were not able to reduce Campylobacters to an acceptable level. These last showed a high prevalence (95%), with an almost constant count ($6 \log_{10}$ CFU/mL) throughout the chain. Campylobacter jejuni dramatically increased after evisceration, before decreasing in the end product by $\approx 35\%$. Since the final load of Campylobacter in the end product was higher than recommended standards and may pose potential risk to consumers, more effective measures and hygienic practices should be investigated.

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