

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

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Abstract

The prevalence of *Campylobacter* 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 Log₁₀ CFU/mL in faecal, cloacal and breast swabs respectively. Rinsates showed an almost constant concentration of *Campylobacter* (6 Log₁₀ CFU/mL) with a predominance of the presumptive *C. jejuni*. *C. lari* was found in 22% of eviscerated samples. Faecal coliforms and *E. coli*, used as indicators, were detected in all samples (5.46 and 5.15 Log₁₀ CFU/mL, respectively). Final chilling and chlorine (50 ppm) treatments decreased them to acceptable levels, unlike for *Campylobacter*. Further investigation of the dynamics of *Campylobacter* 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 *Campylobacter* (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 (Kudirkiene *et al.*, 2010), while defeathering and evisceration are critical phases (Hue *et al.*, 2010). The objective of this work was to assess the contamination of domestic broilers by *Campylobacter* 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 (5×5 cm²) 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% lysed horse blood (SR048; Oxoid Ltd.). The incubation was carried in a microaerobic environment (85% N, 10% CO₂, 5% O₂) for 48 h at 42°C (CO₂ water-jacketed Incubator). Following enrichment, 20 µL of diluted swab/rinsate 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 9308-1:2000; ISO, 2000).

Results and Discussion

Farm level

Aerobic plate count reached a maximum of 5.71 Log₁₀ CFU/mL in the CS, but scored a lower value (4.71 Log₁₀ CFU/mL) in the FS. *Campylobacter* concentrations were higher

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with 6.26 Log₁₀ CFU/mL in FS, 6.34 Log₁₀ CFU/mL in CS and 5.38 Log₁₀ 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 Log₁₀ CFU/mL) in 50% of FS and CS against 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 Log₁₀ CFU/mL and reached 9.5 Log₁₀ CFU/mL after washing (Figure 2). Its lower number on farm and receiving area may be due to the limited area sampled by swabbing (5×5 cm²), while the peak after washing may be attributed to cross-contamination between basins batches. Chilling reduced the number to 7.5 Log₁₀ CFU/mL, but it was still above the international standards (5-7 Log₁₀ 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 Log₁₀ CFU/mL) supports the scenario of physical damage. Washing reduced them to 3.46 Log₁₀ CFU/mL and chilling to 2.00 Log₁₀ 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 Log₁₀ 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 Log₁₀ CFU/mL, without chlorine, and by 2.5 Log₁₀ 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 slaughtering chain (5.95±0.04 Log₁₀ CFU/mL). The slight increase after evisceration (5.88 to 5.97 Log₁₀ 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 Log₁₀ 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 Log₁₀ 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 Log₁₀ CFU/mL) and the greatest after washing

(6.00 Log₁₀ 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 1.43×10⁶ 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.

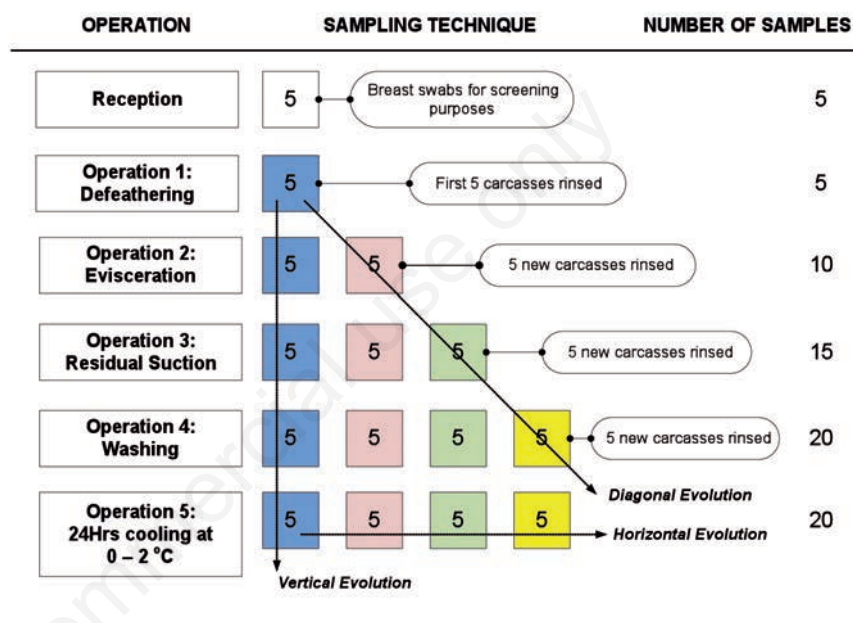


Figure 1. Sampling design for carcass rinsates showing the effect on the microbial load of frequent rinsing (vertical evolution), the number of rinsing (horizontal evolution) and no frequent rinsing (diagonal evolution).

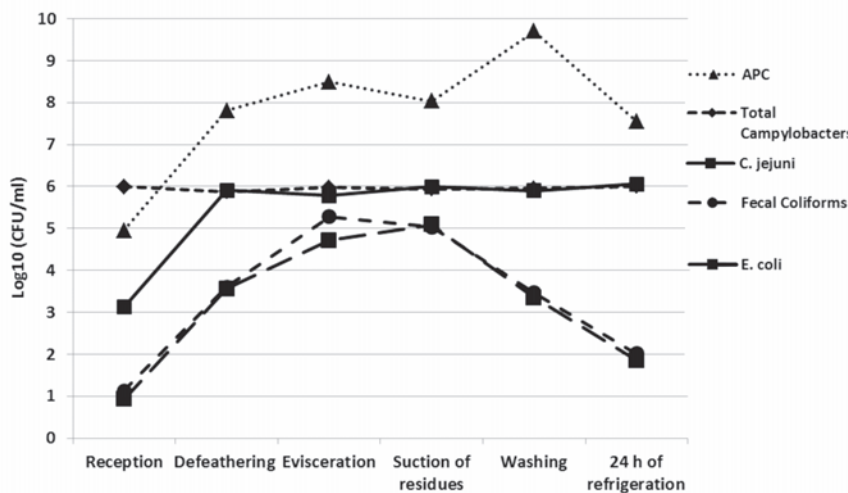


Figure 2. Dynamic of total *Campylobacters*, *C. jejuni* in comparison to aerobia plate counts, faecal coliforms and *E. coli* throughout the slaughtering operations.

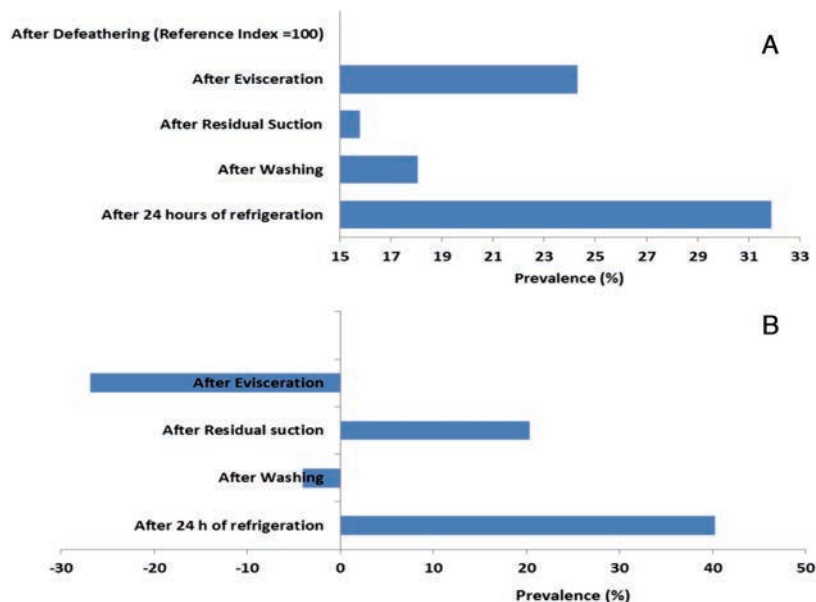


Figure 3. Dynamic index of Campylobacters (A) and *Campylobacter jejuni* (B) calculated relative to defeathering. Counts at defeathering: *Campylobacters*= 7.53×10^5 CFU/mL; *C. jejuni*= 8.2×10^5 CFU/mL.

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 Log₁₀ CFU/mL) throughout the chain. *Campylobacter jejuni* dramatically increased after evisceration, before decreasing in the end product by ≈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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