Campylobacter contamination during broiler processing

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Introduction

The *Campylobacter* spp. are reported as the most common gastrointestinal bacterial pathogen in humans. According to the European Food Safety Authority (EFSA) report the number of cases in EU in 2005 was 197 363 (EFSA 2006). Comparing to 2004 a 7.8 % increase was observed. Poultry meat products are considered to be a major source of campylobacteriosis (EFSA 2005). The final product contaminations are determined by the *Campylobacter* load in the primary production at farm level. It is claimed that the reduction of the *Campylobacter* concentration on the broiler carcass may lead to decrease in number of poultry associated human campylobacteriosis (Johannessen et al. 2007). Not always interventions in broilers' stables are sufficient enough, which urges to explore alternative solutions at slaughterhouses. The interventions in the chicken meat production chain including for example improved hygiene at farm level, logistic slaughter and improved kitchen hygiene, are presented in CARMA study (Havelaar et al. 2006).

The bacterial load on carcasses changes during poultry processing. The variable *Campylobacter* level on carcasses at different steps of poultry processing was investigated in several studies (Johannessen 2007; Klein 2007). Detailed data of bacterial loads on broilers, especially carcasses within processing line are very important for risk management of *Campylobacter* in poultry meat chain. Good knowledge of *Campylobacter* contamination in poultry processing is required for a proper risk assessment (Nauta et al. 2005).

The main goal of the research was to observe the development of the *Campylobacter* contamination not only in the slaughter line and during further processing but also at farms. The results of *Campylobacter* log cfu counts should primarily be used to confirm the relationship between concentration levels in faeces at farm level and final retail product. Data from this experiment can also be used for validation of the risk assessment model of CARMA.

Materials and Methods

Campylobacter positive flocks were selected by pre-screening at the farms. Fresh faeces samples were taken there, no longer than one week before the final slaughter. After pre-screening at the farms the samples were taken in five different Dutch slaughterhouses at the six sampling locations along the slaughter line: (1) Faecal material from containers, (2) carcasses with feathers after killing and bleeding but before scalding, (3) whole carcasses and (4) neck skin samples before chilling, after final inside/outside washing machine, (5) skin samples from final product (usually thighs) and (6) final product itself without skin (usually deboned thighs without skin). The numbers of samples taken from different processing steps are shown in table 1.

Table 1. *Campylobacter* prevalence of different samples in *Campylobacter* positive flocks.

Sampling material	Total samples number	*Campylobacter* positive	*Campylobacter* positive in %
Faeces farm	118	107	90.68
Faeces containers	225	205	91.11
Carcasses before scalding	225	221	98.22
Carcasses before chilling	224	219	97.77

Neck skin before chilling	224	215	95.98
Final product skin	225	211	93.78
Skinless final product	105	90	85.71
Total	1346	1268	94.21

One gram of faeces was used to prepare required dilutions. The *whole-carcass rinse method* was used to sample carcasses before scalding and chilling. The carcasses were removed from processing line and placed in sterile plastic bag together with 500 ml of peptone water. After closing the bag, it was shaken vigorously for 60 s. A sample of the rinse water was poured into plastic cans. That sample was examined for *Campylobacter* in the laboratory. In case of neck skin and final product, with or without skin, 25 g pieces were used. Samples were placed in bags with 1:9 ww of diluents (BPW) and shaken in stomacher for 60 s. The homogenized fluid was used for further analysis.

The analytical technique was based on plating the samples at CCDA plates (*Campylobacter* Blood-free Selective Medium (Modified CCDA-Preston Agar, OXOID CM 0739; with 2 vials per liter SR 0155 (OXOID)).

Preston enrichment technique (Nutrient Broth Nr 2: (OXOID CM 0067)) with 2 vials Modified Preston *Campylobacter* Selective Supplement SR 0204E (OXOID) and 2 vials *Campylobacter* Growth Supplement SR 0232E (OXOID) per liter) was used to detect *Campylobacter* presence below direct count detection level (Figure 1C). Appropriate dilutions of sampling material were plated directly at CCDA plates and after the incubation time (48 h in 42ºC, microaerobic conditions) the *Campylobacter* colonies were counted and the cfu counts per ml of sample were calculated (Figure 1AB). These data were transformed into log cfu of *Campylobacter*. In case of faeces, the *Campylobacter* concentration was performed as log cfu per gram of faeces, whereas for carcasses before scalding and chilling per product. In case of neck skin and final product the *Campylobacter* load was presented as log cfu per gram of skin or meat.

A **B** **C**

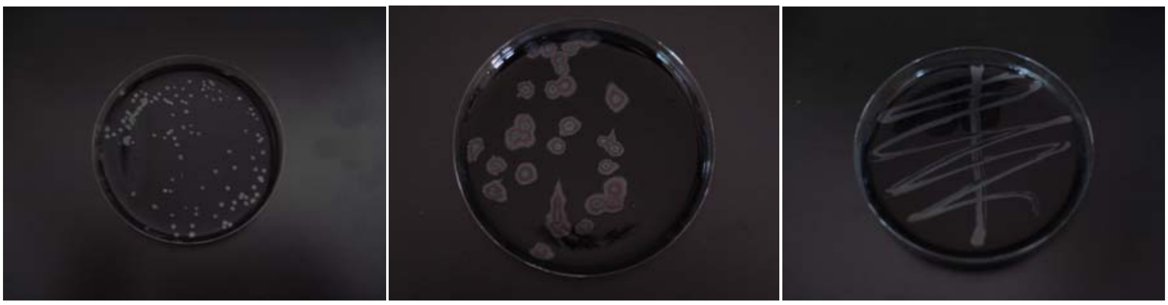

Figure 1. CCDA plates after incubation: *Campylobacter* positive colonies (AB), positive result from Preston enrichment technique (C).

Statistical analysis was performed using SPSS software. The mean, standard deviation, standard error, 95% confidence interval for mean, minimum, maximum and correlation between *Campylobacter* spp. levels recorded at examined sampling locations were determined.

Results and Discussion

The results of 15 examined *Campylobacter* positive flocks showed 94.2 % of *Campylobacter* positive samples on average (table 1). Not in every occasion all sampling locations were tested. In case of faeces from farms and final product without skin the numbers of samples was not completed, due to not complete protocols taken from farms or slaughterhouses.

Table 2 presents the mean value of *Campylobacter* concentrations at analysed sampling locations in 15 examined flocks. In case of 14^{th} flock direct plating gave no *Campylobacter* positive results but after the enrichment technique in Preston some samples were positive. These data could not be log transformed, and subsequently excluded from the statistical analysis.

Although not in every occasion faeces samples at the farm were taken, only *Campylobacter* positive flocks were sampled in the slaughterhouses, which indicates that the *Campylobacter* prevalence in flocks during summer season was high. An increase in *Campylobacter* load in faeces from containers was observed in several flocks, comparing with the load at farms. It is explained by several factors, however transportation could have the strongest impact on the increase.

The research presented high contamination level of carcasses entering the processing. The *Campylobacter* log cfu counts for carcasses before scalding for most analysed flocks range from up 4 to up 7 log cfu. In further processing steps the decrease in *Campylobacter* load was observed. Final product from infected flocks, even without skin, is still considered as an infection threat for consumer. The data showed differences in contamination level in case of final product with and without skin. Although the decrease was not so high in *Campylobacter* level on skinless meat the experiment proved that the chicken meat with skin carries more bacteria than meat with mechanically removed skin. That fact was also confirmed by other studies in that area, by Klein (2007) and Luber et al. (2007).

Table 2. The mean *Campylobacter* concentration in 15 examined flock at different sampling locations. The *Campylobacter* load performed as log cfu counts in case of faeces per gram of faeces; carcasses per ml of wash fluid; neck skin and final product per gram.

Flock No	Farm faeces	Containers faeces	Carcass before scalding	Carcass before chilling	Neck skin	Final product skin	Final product without skin
	Log cfu/g	Log cfu/g	Log cfu/ml	Log cfu/ml	Log cfu/g	Log cfu/g	Log cfu/g
1	5,49	7,70	4,95	2,94	3,13	2,56	2,13
2	*	1,55	4,35	2,13	1,66	2,70	2,21
3	2,07	7,02	4,89	1,92	2,78	3,01	*
4	5,60	5,66	3,99	2,57	2,85	3,76	*
5	4,68	6,24	4,11	3,22	2,52	2,70	*
6	5,96	4,28	5,14	2,26	2,09	1,93	*
7	*	2,00**	4,28	3,50	2,54	2,69	*
8	*	5,73	3,25	2,32	3,32	3,18	2,27
9	*	5,00	5,04	2,70	2,28	2,17	2,00**
10	5,45	4,91	5,24	2,60	2,97	2,00**	*
11	4,82	5,32	4,88	1,34	2,70	3,32	2,29
12	*	6,54	3,99	1,02	1,96	2,88	*
13	4,93	6,02	4,00	2,86	2,77	2,06	*
14	*	***data not transformable into log cfu counts					
15	4,15	4,87	4,82	2,88	3,27	2,48	2,03

* samples were not taken

** *Campylobacter* load in all samples <100 cfu.

*** to many negative samples to transform the load from *Campylobacter* positive one into log cfu.

The analysis of variation in average concentration level in faeces and on product was low. The data analysis could not demonstrate a consistent relationship between concentration levels in faeces and on product. Previous research (Stern and Robach 2003) also did not confirm significant correlation between *Campylobacter* spp. levels for faecal samples and pre chill carcasses. Lack of significant relationship between concentration levels of *Campylobacter* in faeces and on product in this study might be explained by incomplete numbers of samples in case of farm faeces and final product without skin. More research has to be done to find out the occurrence of such relationship. According to Stern and Robach (2003) lack of direct statistical relationship between production and processing can be influenced by factors such as weather or transport time.

In conclusion the *Campylobacter* concentration in infected flocks substantially decreased within the slaughter processes. However the problem of microbiological contamination cannot be eliminated completely within processing line without external interventions. Infected birds are the main source of infected final product. In spite of the fact that during slaughtering carcasses can be contaminated by intestinal content and some processes may reduce the *Campylobacter* significantly, the final products are frequently contaminated with level of 1-4 log cfu/g, cm^2 or per ml of carcasses rinse (EFSA 2005). The preventive actions should be implemented not only within the poultry processing chain but also at the level of domestic kitchen. Insufficient hygiene in the kitchen may cause *Campylobacter* spread from surface of product to another kitchen staff and expose the consumer to the bacteria (Cogan et al. 2002).

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