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Original article

Poultry flocks as a source of *Campylobacter* contamination of broiler carcasses

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Abstract

Campylobacter infection is the leading foodborne bacterial gastroenteritis worldwide and the bacteria are frequently isolated from the intestines of chickens. The broiler meat contamination with *C. jejuni* or *C. coli* may occur during slaughter processing. The aim of the study was to investigate the prevalence of *Campylobacter* in poultry flocks and the corresponding broiler carcasses in 15 districts (voivodeships) all over Poland. A total of 128 samples from broiler flocks and the corresponding carcasses were collected between February 2011 and April 2013. The *Campylobacter* isolation and species identification were performed according to ISO 10272-1 standard and with PCR. It was found that 112 flock (96.5%) were contaminated with campylobacters, either *C. jejuni* (77 samples; 68.7%) or *C. coli* (35 flocks; 31.3%). Analysis of the corresponding chicken carcasses tested after chilling revealed that 77 out of 128 (60.2%) samples were positive for *Campylobacter*, either *C. jejuni* (58; 75.3%) or *C. coli* (19; 24.7%). Most of the carcasses were contaminated with the same *Campylobacter* species as identified in the corresponding flock before slaughter. As tested by PCR, out of the 77 crops with *C. jejuni* 58 were positive for the same bacterial species. On the other hand, out of the remaining 35 flocks infected with *C. coli*, only 19 corresponding carcass samples were contaminated with *C. coli*. In three cases in the slaughtered flocks *C. jejuni* was identified but in the same carcasses *C. coli* was found. The opposite findings (flock positive for *C. coli* but the corresponding carcasses contaminated with *C. jejuni*) were seen in six voivodeships. It was also observed that several carcass samples were negative for *C. jejuni* and *C. coli* although the original flocks were *Campylobacter*-positive before slaughter (total 36 of the 77 samples; 46.7%). On the other hand, some carcasses were contaminated with *Campylobacter* although the flocks were negative for these bacteria (9 samples; 11.7%) which may also be due to internal contamination during slaughter of broilers.

Key words: *Campylobacter*, Poland, broiler flocks, carcasses, cross-contamination

Introduction

Campylobacter infection is the leading foodborne bacterial gastroenteritis worldwide, and during last couple of years it has been the most commonly reported zoonosis in the European Union (EFSA 2014). In Poland, the number of laboratory confirmed campylobacteriosis cases is low but has increased during last few years (EFSA 2014). *Campylobacter jejuni* and, in a less extend *C. coli*, are commonly associated

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with human infections and several epidemiological studies showed that food, particularly contaminated poultry meat and chicken meat food products, are mainly connected with a risk of human campylobacteriosis (Adkin et al. 2006, Humphrey et al. 2007, Nauta et al. 2007, Riddle et al. 2012). Poultry are asymptomatic carriers of *Campylobacter* and the infected flocks cannot be identified by clinical symptoms in birds (Berry et al. 1988, Newell et al. 2001, Newell and Fearnley 2003, Wieczorek and Osek 2005, Adkin et al. 2006, Bull et al. 2006, Allen et al. 2007, 2008, Wirz et al. 2010, Hue et al. 2011, Ridley et al. 2011, Habib et al. 2012). The contamination of broiler meat with *C. jejuni* or *C. coli* from the chicken intestine may occur during slaughter processing through several routes, such as the air, water, previously slaughtered *Campylobacter*-positive flocks, equipment used in abattoirs, insects or slaughterhouse personnel (Rivoal et al. 1999, Newell et al. 2001, Nauta et al. 2007, Normand et al. 2008, Reich et al. 2008, Wirz et al. 2010, Hue et al. 2011, Habib et al. 2012). Many studies have evaluated *Campylobacter* diversity in poultry and the significance of cross-contamination at the slaughterhouse level (Rivoal et al. 1999, Newell et al. 2001, Nauta et al. 2007, Hue et al. 2011, Habib et al. 2012).

The aim of the study was to investigate the prevalence of *Campylobacter* in poultry flocks and the corresponding broiler carcasses as well as possible impact of cross- and self-contamination during slaughter.

Materials and Methods

Sample collection

During the study, 15 poultry broiler farms located in 15 out of 16 voivodeships in Poland have been selected for sampling at the slaughterhouse level. Sampling took place between February 2011 and April 2013. The mean number of birds in flocks from which the samples were taken was ca. 13,000, with the age of broilers at slaughter (mean ± SD) 43 ± 4 days. The intact caeca from 10 randomly selected birds from one slaughtered flock were taken at the time of evisceration, pooled and defined as a sample for further analysis. Additionally, the swab samples were collected directly after immersion chilling (0 to 4°C) from the neck skin and the skin surface under the wings. The caecal and swab samples were immediately transported to a laboratory in Amies transport medium (Medlab, Poland) and examined for the presence of *Campylobacter* spp. Altogether, 128 caecal and 128 swab samples from 128 broiler flocks were collected. The highest number of flocks were tested in the following voivodeships: lubuskie (12 samplings from farm no. 238) as well as lubelskie (farm no. 233), podkarpackie (farm no. 79), podlaskie (farm no. 145), and śląskie (farm no. 117) – 11 samplings from each holding. In the remaining voivodeships the samples were collected from 10 (pomorskie) to 4 (mazowieckie and świętokrzyskie) broiler flocks (Table 1).

Bacterial isolation and identification

In the laboratory, caecal samples were streaked directly onto two selective solid media: Karmali agar (Oxoid, UK) and *Campylobacter* blood-free agar (Oxoid) with CCDA selective supplement (Oxoid). The swabs from carcasses were placed in 5 ml of Bolton enrichment broth (Oxoid, UK) supplemented with 5% lysed horse blood and modified Bolton broth supplement. The cultures from both types of samples were incubated at 41.5°C for 48 h under microaerobic conditions using the CampyGen kit (Oxoid). *Campylobacter* bacteria were isolated and identified according to the ISO 10272-1:2006 standard. Briefly, after the enrichment step, the cultures from swab samples were plated onto Karmali agar (Oxoid) and *Campylobacter* blood-free agar (Oxoid) with CCDA selective supplement (Oxoid) and incubated at 41.5°C for 48 h under microaerobic conditions. The plates with caecal and carcass bacterial cultures were then examined for morphologically typical *Campylobacter* colonies (grayish, often with a metallic sheen, flat, and moist with a tendency to spread) and from each sample, one presumptive *Campylobacter* isolate was confirmed by PCR assay as previously described (Wieczorek et al. 2013). Furthermore, the isolated strains were identified as *C. jejuni* or *C. coli* by PCR (Wieczorek and Osek 2005).

Reference strains

Two *Campylobacter* reference strains were included in the study: *C. jejuni* ATCC 33560 and *C. coli* ATCC 43478.

Results

A total of 128 sampling visits during a two-year period were performed in 15 broiler farms located all over Poland. Poultry flocks in most of the voivodeships (n = 12) were infected with both *Campylobacter* species, either *C. jejuni* or *C. coli*; however, in three districts (mazowieckie, opolskie, and wiel-

Table 1. Prevalence of *Campylobacter* in broiler flocks and corresponding carcasses.

Farm ID	Voivodeship	No. of samplings	Prevalence of *Campylobacter*	Positive (+) or negative (–) results		No. of respective results
				Flock	Carcass	
1	2	3	4	5	6	7
25	Kujawsko-Pomorskie	10	*C. jejuni*	+	+	3
			C. jejuni	+	–	2
			C. jejuni	–	+	1
			C. coli	+	–	2
			Negative	–	–	2
233	Lubelskie	11	*C. jejuni*	+	–	2
			C. coli	+	+	6
			C. coli/*C. jejuni*[a]	*C. coli* +	*C. jejuni* +	3
238	Lubuskie	12	*C. jejuni*	+	+	1
			C. jejuni	+	–	4
			C. coli	+	+	3
			C. coli	+	–	1
			C. coli/*C. jejuni*[a]	*C. coli* +	*C. jejuni* +	3
201	Łódzkie	6	*C. jejuni*	+	+	2
			C. jejuni	–	+	1
			C. coli	+	+	1
			C. coli	–	+	1
			Negative	–	–	1
119	Małopolskie	7	*C. jejuni*	+	+	2
			C. jejuni	+	–	3
			C. coli	+	+	1
			C. jejuni/*C. coli*[b]	*C. jejuni* +	*C. coli* +	1
205	Mazowieckie	4	*C. jejuni*	+	+	2
			C. jejuni	+	–	1
			Negative	–	–	1
133	Opolskie	6	*C. jejuni*	+	+	2
			C. jejuni	+	–	2
			Negative	–	–	2
79	Podkarpackie	11	*C. jejuni*	+	+	4
			C. jejuni	+	–	1
			C. jejuni	–	+	2
			C. coli	+	–	1
			C. coli/*C. jejuni*[a]	*C. coli* +	*C. jejuni* +	1
			Negative	–	–	2
145	Podlaskie	11	*C. jejuni*	+	+	3
			C. jejuni	+	–	2
			C. coli	+	+	2
			Negative	–	–	2
33	Pomorskie	10	*C. jejuni*	+	+	3
			C. jejuni	+	–	3
			C. jejuni	–	+	1
			C. coli	+	+	1
			C. jejuni/*C. coli*[b]	*C. jejuni* +	*C. coli* +	1
			Negative	–	–	1
117	Śląskie	11	*C. jejuni*	+	+	6
			C. jejuni	+	–	3
			C. coli	+	–	1
			Negative	–	–	1
51	Świętokrzyskie	4	*C. jejuni*	+	+	2
			C. jejuni/*C. coli*[b]	*C. jejuni* +	*C. coli* +	1
			C. coli/*C. jejuni*[a]	*C. coli* +	*C. jejuni* +	1

cont. table 1

1	2	3	4	5	6	7
164	Warmińsko-Mazurskie	9	*C. jejuni*	+	+	2
			C. jejuni	+	–	1
			C. jejuni	–	+	2
			C. coli	+	–	1
			C. coli/*C. jejuni*[a]	*C. coli* +	*C. jejuni* +	1
			Negative	–	–	2
127	Wielkopolskie	8	*C. jejuni*	+	+	6
			C. jejuni	+	–	1
			C. jejuni	–	+	1
207	Zachodniopomorskie	9	*C. jejuni*	+	+	1
			C. jejuni	+	–	2
			C. coli	+	+	1
			C. coli	+	–	3
			Negative	–	–	2

Explanation: [a] – *C. coli* was identified in flock and *C. jejuni* in carcass; [b] – *C. jejuni* was identified in flock and *C. coli* in carcass

kopolskie) only *C. jejuni* was identified. In the majority of the holdings (10 out of 15 tested) one or two broiler crops were negative for *Campylobacter* during the study period (Table 1).

It was found that out of the total 128 samples collected from the poultry flocks, 112 (96.5%) were positive for *Campylobacter*. Most of them were identified as *C. jejuni* (77; 68.7%) whereas the remaining 35 (31.3%) flock-positive samples were classified as *C. coli*. Analysis of the chicken carcasses tested after chilling revealed that 77 out of 128 (60.2%) samples were positive for *Campylobacter*, either *C. jejuni* (58; 75.3%) or *C. coli* (19; 24.7%). Most of the carcasses were contaminated with the same *Campylobacter* species as identified in the corresponding flock before slaughter. As tested by PCR, out of the 77 crops with *C. jejuni* 58 were positive for the same bacterial species. On the other hand, out of the remaining 35 flocks infected with *C. coli*, only 19 corresponding carcass samples were contaminated with *C. coli*. In three cases (małopolskie, pomorskie, and świętokrzyskie voivodeships), in the slaughtered flocks *C. jejuni* was identified but in the same carcasses *C. coli* was found. The opposite findings (flock positive for *C. coli* but the corresponding carcasses contaminated with *C. jejuni*) were seen in warmińsko-mazurskie, podlaskie, lubelskie, lubuskie, świętokrzyskie, and podkarpackie voivodeships (Table 1).

Discussion

In the present study *C. jejuni* and *C. coli* were identified in broiler flocks all over Poland and the prevalence was compared to contamination of the corresponding carcasses at the slaughter level. In 15 voivodeships one farm was selected and during a longitudinal investigation all grown flocks (from four to 12 cycles) were investigated for the presence of *Campylobacter*. Most of the flocks (96.5%) were positive, mainly for *C. jejuni* (68.7%) and, to lower extent, for *C. coli* (31.3%). The obtained results may suggest that the chicken carcasses were cross-contaminated during the slaughter process or the original flocks were infected with another or not only these *Campylobacter* species than that identified in the study. It was also observed that several carcass samples were negative for *C. jejuni* and *C. coli* although the original flocks were *Campylobacter*-positive before slaughter (total 36 of the 77 samples; 46.7%). On the other hand, some carcasses were contaminated with *Campylobacter* although the flocks were negative for these bacteria (9 samples; 11.7%). Similar finding has also been reported by Allen at al. (2007) who found a cross-contamination of carcasses from two of five *Campylobacter*-negative flocks, even they were processed in the slaughterhouse after negative birds. Others authors also found carcasses contaminated with campylobacters at the end stage of processing, even when the bacteria were not isolated from the chickens upon arrival to the abattoir (Newell et al. 2001, Miwa et al. 2003, Reich et al. 2008). However. contamination of broiler carcasses during processing can occur at various points such as scalding, plucking, defeathering, evisceration or chilling operations (Allen et al. 2007, Reich et al. 2008).

The numbers obtained in the present survey are higher than those described in the European Union baseline study performed in 2008 in which the prevalence of *Campylobacter* in broiler flocks in Poland was 79.0% as compared to 71.2% at the EU level, with the range between 2% (Estonia) and 100% (Luxembourg) (EFSA 2010). The bacterial species identification revealed that 60.8% campylobacters from the

broiler flocks were classified as *C. jejuni* which was a little less that found in the present study. Other investigations also clearly demonstrated that poultry flocks are often infected with *Campylobacter* and therefore, broiler meat may be contaminated with these bacteria during commonly automated slaughter processing through several routes, such as air, water, previously slaughtered flocks or abattoir equipment (Rivoal et al. 1999, Newell et al. 2001, Nauta et al. 2007, Wirz et al. 2010, Hue et al. 2011, Habib et al. 2012).

As detected in the present study, *Campylobacter* was identified in 60.2% of carcass samples tested which originated from the broiler flocks, both positive and negative for this pathogen. The prevalence of the bacteria was much lower as obtained from the mentioned EU baseline studies in 2008 where 75.8% of similar chicken carcass samples were contaminated (EFSA 2010). During that study it was also found that in the EU percentage of positive tests ranged from a minimum of 4.9% for Estonia to a maximum of 100% for Luxembourg. In Poland, 81.0% such samples were contaminated with *Campylobacter* spp. *C. jejuni* was predominant bacterial species (67.9% of the isolates) and it was detected on broiler carcasses in all EU Member States. On the other hand, *C. coli* (39.4% of positive samples) was identified in most of EU countries with the exception of Estonia, Finland, and Sweden (EFSA 2010). As it was identified in the present study, a lower prevalence of this *Campylobacter* species was detected but it may be due to a lower number of samples tested as compared to the EU baseline study.

In conclusion, the present longitudinal study on 15 chicken farms with several crop cycles has clearly demonstrated the widespread contamination of broilers with campylobacters. The *Campylobacter*-infected flocks may be a source of these bacteria for the corresponding carcasses, although the presence of the same bacterial species in the paired samples (flock – carcass) might also be due to cross-contamination during a slaughter process. These kind of transmission was also confirmed by other authors (Allen et al. 2007, Ellerbroek et al. 2010). Furthermore, the identification of other *Campylobacter* species on carcasses than those in the original flocks may also suggest a different contamination sources and routes. These findings should be further tested using methods for molecular characterization of the *Campylobacter* isolates of the same species (Normand et al. 2008). During processing, the spread of *Campylobacter* and the cross-contamination of broiler carcasses by the bacteria present in the intestinal content may create a hygiene problem (Ellerbroek et al. 2010). The results of the present and other studies suggest that control mechanisms at slaughterhouses may be more promising than countermeasures being applied at the farm level only; however, the complete elimination of *Campylobacter* during processing is probably not possible (Allen et al. 2007, Reich et al. 2008).

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