POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

http://journal.pan.olsztyn.pl
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Pol. J. Food Nutr. Sci.
2010, Vol. 60, No. 3, pp. 265-271

THE CAECA – NICHE SUPPORTING SURVIVAL OF *CAMPYLOBACTER* SPP. IN COMMERCIALLY REARED BROILER CHICKENS

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Key words: campylobacters, broilers, caecum, slaughter

The aim of the study was to find out how efficient the feed withdrawal prior to slaughter is in clearing the commercial broilers caeca from campylobacters. It was confirmed for 68.3% of the broiler lots tested, deprived of feed 24 h prior to slaughter to carry *C. jejuni* in their caeca.

The results obtained showed statistically significant differences ($p \leq 0.001$) between the prevalence of *Campylobacter* spp. in the birds' caeca and the rearing site, with the main suppliers delivering campylobacter-positive broiler lots significantly more often than the smaller ones. The number of campylobacters inhabiting caeca of fasted broilers prior to slaughter exceeded 10^7 cfu/g being by one order of magnitude higher in deliveries from large scale breeders. The distances broilers were to cover from rearing site to processing plant (5 to ≤154 km) have no effect on the prevalence and carriage rate of campylobacters in commercial broiler chickens caeca.

The dominance and high numbers of *C. jejuni* in the caeca of commercial broilers states, in our opinion, indirect evidence for the caecum to be the environment supporting survival and favoring growth of *C. jejuni* under deprivation of competing microflora. Also the ability of *C. jejuni* to displace other colonizers confirm the caeca to constitute the main reservoir of *C. jejuni* in the broiler chickens.

INTRODUCTION

Poultry is one of the most frequently indicated sources of *Campylobacter* spp. Under intensive rearing, campylobacters can be transferred on birds, mainly, horizontally either through the litter, unchlorinated tap water [Shreeve *et al.,* 2000], equipment and utensils used by farm workers [Evans & Sayers 2000], by farm/domestic animals [Giessen *et al.,* 1992], rodents, wild birds [Evans & Sayers 2000], flies [Rosef & Kapperud 1983; Wright, 1982], *etc*. Healthy birds may shed $10^2 – 10^7$ cfu of *C. jejuni* in 1 g of droppings [Wallace *et al.,* 1998; Sahin *et al.,* 2003].

When present in the environment, colonization of birds with campylobacters is simply a matter of time. Wallace *et al*. [1998] suggested for colonization of birds to start 5-7 days after hatching, while Idris *et al*. [2006] detected DNA of *C. coli* in ileum, cecum and yolk contents of the newly hatched chicks already. According to Calderón-Gómez *et al*. [2009], among the strains of *C. jejuni*/*C. coli* inhabiting chicken guts there are dominant colonizers able to displace others, irrespective of the day of infection.

Diverse molecular structure of epithelial mucins lining various parts of the gastrointestinal tract (GT) of broiler chickens may affect adhesive abilities of campylobacters [Lengsfeld *et al.,* 2007]. Thus numbers of *Campylobacter* spp. in various sections of the birds GT can differ essentially [Rudi *et al.,* 2004; Wallace *et al.,* 1998] with bacterial floras of the intestines to vary with different diets and to change as the birds mature [Lu *et al.,* 2003].

Despite numerous data on the carriage rate of *Campylobacter* spp. in chickens, based on analysis of cloacal swabs, droppings, intestinal contents, carcass rinses, skin and meat samples, none, so far, has been addressed to caeca of commercial broiler chickens subjected to a routine feed withdrawal prior to slaughter and processing. Stop feeding birds up to 24 h prior to transportation to the abattoir is to empty their guts of contents and residing bacteria, reducing possible contamination of carcasses during processing [Northcutt *et al.,* 2006; Wesley *et al.,* 2005].

The aim of the study was to find out how efficient the birds starvation prior to slaughter is in clearing out the commercial broiler chickens caeca from campylobacters and if type of supplier and the distance to cover when transporting the birds affects the carriage rate of campylobacters in the chicken broilers' caeca.

MATERIAL AND METHODS

Sampling

The subject of analysis were caeca of commercial 6-week-old broiler chickens at slaughter. The gastrointestinal tracts (GIT) were collected directly from one of the local poultry processing plants in West Pomeranian District, Poland, at the manual evisceration operation stage of broilers. Each lot of birds from particular supplier was represented by 2 pooled samples each consisting of 3 GIT's. GIT's, collected at random, when put on a disposable, polystyrene tray were placed

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into a disposable plastic bag, then in the thermoinsulated box, at ±4°C and transported to the laboratory.

Prior to analysis, up to 4 h after collecting the samples, each pair of intact caecum was first surface cleaned with a sterile cotton then sterilized by swabbing with 70% methanol industrial alcohol and flamed, then cut off aseptically onto sterile Petri dish, cut lengthwise to open with a sterile scalpel and transferred into a sterile stomacher bag. Three pairs of caeca from birds representing one supplier, treated as pooled sample were weighed and subjected to analysis.

Between December and March 2002, a total of 240 pooled samples representing 120 different lots of broiler chickens were subjected to analysis. Working in one-shift system the poultry processing plant under surveillance processed ~80.000 birds daily, delivered, on a daily basis, by 1 to 4 suppliers. The broiler chickens tested originated from 38 different rearing sites and were delivered by 18 main contractors (3-9 deliveries each) and 20 smaller suppliers (1-2 deliveries).

Confirmation of the presence of campylobacters and their enumeration

The presence of *Campylobacter* spp. in pooled caeca samples as well as the numbers of campylobacters inhabiting caeca were estimated using procedures including 24-48 h preenrichment of initial dilution in Preston broth prior to isolation on modified Cefoperazone Charcoal Desoxycholate Agar (mCCDA) as well as by direct plate counting on mCCDA medium according to ISO 10272:1995.

Initial (1:2) and serial 10-fold dilutions prepared in Buffered Peptone Water [ISO 10272:1995] were spread on mCCDA medium in duplicate and incubated at 42°C under microaerophilic atmosphere for 48 h. Characteristically growing colonies were counted. Typically, growing colonies, selected at random, were tested for purity and subjected to identification according to ISO 10272:1995.

As for the pre-enrichment step, the initial material (2 mL of 1:2 of initial dilution) was transferred into 10 mL of the Preston broth supplemented with sterile lysed defibrinated horse blood and antibiotic solution (SR 204E, Oxoid) and incubated for 24-48 h at 37°C prior to isolation on modified Cefoperazone Charcoal Desoxycholate Agar (mCCDA).

Colonies typically, growing on mCCDA medium were isolated at random and subjected to further identification steps only if the direct analysis gave negative result and/or types of colonies differed visibly. The detection level of the methods applied was 1 cfu/g.

Positive result for one pooled sample was to treat the lot as positive for campylobacters.

Confirmation tests

Biochemical identification of the isolated strains was based on a simplified set of tests according to ISO 10272:1995 with a set of strains identified by the apiCAMPY tests (bioMerieux). The identification of all the strains classified as *C. jejuni* was confirmed by the nested PCR method with two pairs of primers: C-1 + C- 4 and C-1 + C-2 [Daczkowska-Kozon *et al.*, 2003; Winters *et al.,* 1998]. For genetic identification, the isolated strains were stored in AUX Medium (apiCAMPY – bioMerieux) supplemented with 10% glycerol (growth turbidity -4-6 in Mc Farland's scale) at -20°C.

Statistical analysis

Statistical analyses based on the one-way analysis of variance (ANOVA) and the chi square (χ^2) test [Petrie & Watson, 1999] were used to compare the differences in the incidence rate and numbers of campylobacters in the caeca of broilers at slaughter between the birds' suppliers and the transportation distance from the rearing farm to the poultry processing plant. Differences were considered significant at $p \leq 0.05$.

RESULTS

Prevalence and numbers of campylobacters in caeca of broiler chickens at slaughter

Analysis of the caeca samples collected from 6-week-old broiler chickens deprived of feed 24 h prior to slaughter confirmed 82 of 120 (68.3%) broilers lots tested were positive for *Campylobacter* spp.

Results showed statistically significant differences ($p \leq 0.001$) between the prevalence of *Campylobacter* spp. in the birds caeca and the rearing site the broiler chickens originated from. The number of campylobacter-positive broilers lots from the main contractors was significantly higher compared to that from smaller suppliers (Table 1). Of the 91 lots delivered by 18 large suppliers, 70 (75.9%) contained birds carrying high number of campylobacters in their caeca. Only one (No. 8) of the 18 main contractors, had all the four birds' lots, delivered in February 2003, free of campylobacters. Majority of broilers' lots from smaller breeders did not carry *Campylobacter* spp. in their caeca, at the level detectable by the method applied (Table 1).

Numbers of *Campylobacter* spp. inhabiting caeca of fasted broiler chickens at slaughter were mostly 10^7 cfu/g and higher (Table 2). The campylobacter-positive birds from smaller suppliers carried mainly 10^7 cfu/g of caeca, while broilers from the main contractors, usually, 10^8 cfu/g (36 of 70 positive lots) followed by 10^9 cfu/g (12/70).

A spread in the carriage rate of *Campylobacter* spp. in caeca of commercial broilers delivered to processing plant by the main contractors is presented in Figure 1. Fluctuation in the number of campylobacters inhabiting the broilers caeca differed, usually, by 1 to 3 orders of magnitude both, between suppliers and particular lots of one supplier. For instance for nine, all campylobacter-positive, lots delivered, successively, in December, January and March 2003, by one supplier (No. 1), the number of *Campylobacter* spp, expressed as lg_{10} per 1 g of caeca, ranged between 7.0–8.8, 7.1–8.5 and

TABLE 1. Prevalence of *Campylobacter* spp. in the caeca of broiler chickens at slaughter.

Type of contractor (No.)	No. of lots tested	No. of lots	
		positive	negative
Main (18)	91	70 (76.9%)	21 (23.1%)
Occasional (20)	29	12 (41.4%)	17 (58.6%)
Total	120	82 (68.3%)	38 (31.7%)

TABLE 2. The carriage rate of *Campylobacter* spp. in the caeca of broiler chickens (n= 180) from different lots at slaughter.

Numbers of campylobacters in caeca (cfu/g)	Campylobacter-positive broilers' lots			Number (%) of broilers' caeca inhabited by *Campylobacter* spp.	
	Number (%) of positive lots				
	main suppliers	small suppliers	total	when identified biochemically	when confirmed by nested PCR
$< 10^2$	2	0	2 (2.4%)		
$10^2 - < 10^5$	0	0	0	*C. jejuni*: 118 (65.6%)	*C. jejuni*: 146 (81.1%)
$10^5 - < 10^7$	2	1	3 (3.6%)	*C. coli*: 26 (14.4%)	
$10^7 - < 10^9$	54	9	63 (76.8%)	*C.* spp.: 29 (16.1%)	*C.* spp.: 1 (0.6%)
$\geq 10^9$	12	2	14 (17.1%)	*C. lari*: 7 (3.9%)	
Σ	70	12	82		

8.6–9.2, respectively. Some of the poultry breeders delivered alternatively campylobacter-negative and campylobacter-positive lots. *E.g.* after three campylobacter-negative lots delivered in February (No. 10 supplier), the next two, delivered in March, were highest in number of campylobacters, reaching 9.4-9.6 lg_{10}. per 1 g of caeca (Figure 1).

Although, the caeca samples from commercial broilers at slaughter were, mostly, high in number of *Campylobacter* spp., there were also few pooled caeca samples with numbers as low as $<10^2$ and 10^5-10^6 cfu/g of caeca (Table 2). Nonetheless the differences noted in the number of campylobacters inhabiting caeca of broiler chickens between different suppliers and/or month of delivery were insignificant statistically.

Campylobacters dominating the broiler chickens caeca

From among 180 strains isolated most of the strains were classified, based on the simplified biochemical identification according to ISO 10272:1995 (E), as *C. jejuni* (118) followed by unidentified *Campylobacter* species (29/180), *C. coli* (26/180) and *C. lari* (Table 2). Strains classified as unidentified were able to hydrolyse hippurate (HIP +), were resistant to nalidixic acid (NA-R) and were either cephalotin resistant (CF-R) or cephalotin sensitive (CF-S) – data not shown. The biochemical profile of the majority (28/29) of unidentified *Campylobacter* species was (HIP +, NA-R, CF-R). Primary biochemical identification confirmed by the nested PCR assay identified that group of strains as *C. jejuni* (Table 2).

Distance to cover and the carriage rate of campylobacters

The transportation time lasting less than 3 h and the distance from the rearing sites to the processing plant up to 160 km (Figure 2) did not seem to affect visibly the campylobacters' number in the broilers caeca. Yet, two lots of broilers from one supplier (No. 4), delivered in January 2003, containing birds with less than $<10^2$ campylobacters in their caeca, resulted, presumably, from incidental infection taking place somewhere between collecting birds at the farm and slaughtering them at the abbatoir. The differences in the number of campylobacters in the caeca of broiler chickens based on the distances birds were to cover (or the transportation time) were, also, insignificant statistically.

DISCUSSION

Prevalence and numbers of campylobacters in caeca of broilers at slaughter – what's behind the phenomenon

Processing 80.000 broiler chickens at a daily basis, 5 days a week, requires from poultry processing plant to cooperate with many commercial broiler breeders. Yet to keep poultry meat attractive for consumers, both the breeders and processors have to meet the standards and comply with the EU regulations 91/628 [1991] and 93/119 [1993].

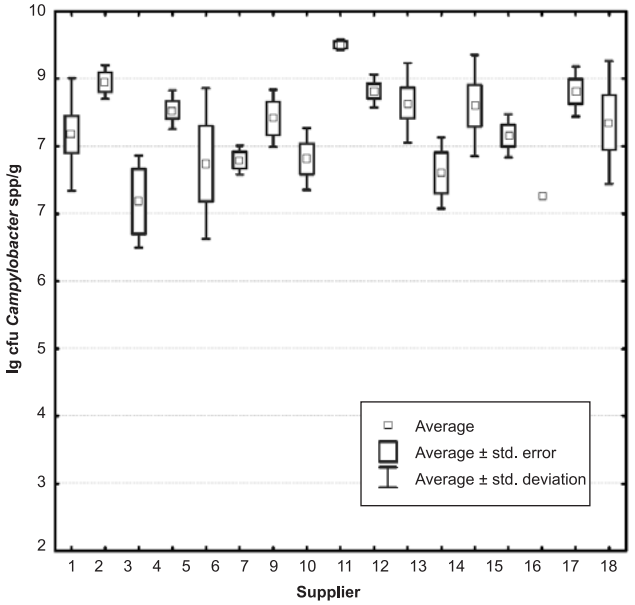

FIGURE 1. Spread in numbers of *Campylobacter* spp. in caeca-positive broilers from the main suppliers of one poultry processing plant.

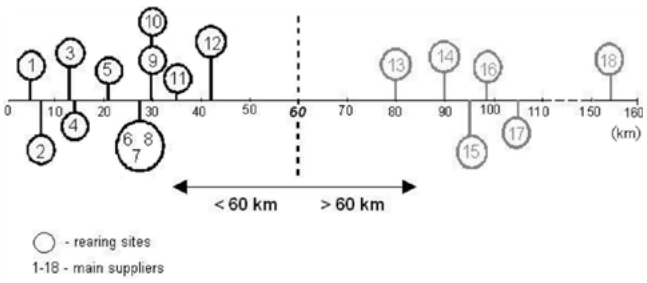

FIGURE 2. The distances between rearing broiler farms of the main suppliers and the poultry processing plant.

As to avoid secondary contamination of carcasses during poultry processing it has been a routine practice for the contractors of the poultry processing plant under surveillance to deliver broilers deprived of feed 24 h prior to slaughter [PN-92/R-78550]. Stop feeding birds prior to their transportation to the abattoir was to empty their guts, caeca included, of contents and residing bacteria, thus reducing possible contamination of carcasses [Mulder 1999; Northcutt *et al.,* 2006; Wesley *et al.*, 2005]. Thus, the conduced surveys were based on the assumption that feed withdrawal should remove or at least partially empty the gastrointestinal tracts of its digesta and residing bacteria, including campylobacters.

Results of our surveys conducted between December 2002 and March 2003, confirmed the caeca of commercial broiler chickens at slaughter to be colonized with *Campylobacter* spp. in 68.3%, with the colonization rate for the lots of broiler chickens from the larger contractors being significantly higher then from the smaller ones(Table 1). Similar results, though based on analysis of the caecal digesta, were presented by Reich *et al.* [2008]. From among 40 conventionally reared flock of broilers, 70%, tested for over 18 months period, carried campylobacters in their caeca contents at slaughter.

Our results indicated for the feed withdrawal (FW) prior to slaughter not necessarily mean getting rid of *Campylobacter* spp. from the broilers caeca. Though, based on visual examination, the broilers intestines collected for testing, were deprived of the contents, which was not identical with lacking the campylobacters in their caeca lining and mucous.

Severe preslaughter stressors, the broiler chickens are being exposed to, FW including, except for affecting the birds' welfare and meat quality, make birds more vulnerable to colonization with pathogenic microbes [Delezie *et al*., 2007]. Besides, the FW, by disturbing the guts peristalsis, may add to intestine wall fragility, making the penetration of microbes into deeper tissues or carcass contamination easier [Mulder, 1999].

According to Lengsfeld *et al*. [2007] bacterial adhesion is mediated by epithelial mucins, which may differ substantially in molecular structure between the gastrointestinal sections which can explain differences in adhesive abilities of *C. jejuni* to different sectors of an intestinal tract. The adhesion assay conducted *in vitro* on campylobacters isolated from animals excreta proved the adhesion rate to be basal on gastric tissue, marginal in duodenal and strong and stable at jejunum section. In their opinion *C. jejuni* strain bound strongly to cloacae tissue with ileum and caecum materials indicating no affinity to *C. jejuni*.

Changes in physical and chemical structure of the guts due to FW as well as deprivation of native, protective microflora containing mostly lactic acid bacteria [Lu *et al.,* 2003; Souza *et al.,* 2007], can make the gastrointestinal tract of broilers more vulnerable to colonization with enteropathogens such as campylobacters. Thus it seems quite possible for the fasting process to favour colonization of the broilers' caeca mucous and/or lining with campylobacters.

It was confirmed for the bacterial floras of broilers GIT to be very diversed [Lu *et al.,* 2003], with each part developing its own bacterial community as the bird matures, and the GIT microbiota composition to vary with the diet applied. 16SrRNA based analysis of caeca microbiota [Lu *et al.,* 2003] indicated, *e.g.* for the -proteobacteria to dominate in the broilers caeca. Surveys conducted by Hinton *et al.* [2000] on the 6-week-old commercial broilers indicated fasting for 12 to 24 h to increase the population of aerobes, *Enterobacteriaceae* and *S. typhimurium* in the crop.

The chicken infection trials conducted by Calderón-Gómez *et al*. [2009] signaled, that when infected with different *C. jejuni* / *C. coli* strains, it was a matter of a short time, for one or two strains to establish themselves as dominant colonizers of the chicken guts, displacing others, irrespective of the day of inoculation. Besides, it is quite possible for *C. jejuni* to avoid being expelled from intestines by temporal invasion and evasion of the epithelial cells and rapid multiplication in the mucous helps *C. jejuni* to survive in birds GIT [Deun *et al.,* 2008]. Based on the findings that when invaded the epithelial cells *C. jejuni* strains were not able to proliferate intracellularly and when evaded from the cells were capable of replication in chicken intestinal mucous Deun *et al.* [2008] suggested it to be the strategy of *C. jejuni* for colonizing the chickens' intestines.

In the present study the fasted commercial broilers carried mostly 10^7 to 10^9 cfu of campylobacters per 1 g of caeca (Table 2). The majority of the isolated strains inhabiting the caeca were identified as *C. jejuni* (81.1%) followed by *C. coli* and *C. lari.* The biochemical differentiation of the three species, according to ISO 10272:1995, based, practically, on the ability to hydrolyse hippurate (HIP+) and on resistance/sensitivity to nalidixic acid (NA-R or S) and cephalotin (CF – R or S) left some isolated strains unidentified (Table 2). All 29 but 1 HIP+ and NA-R strains unidentified biochemically were classified as *C. jejuni* by the nested PCR method applied.

A limited reliability of biochemical identification for atypical strains as well as discrepancies between identification based on biochemical and genetic methods was suggested, also, by Wainø *et al* [2003] and Steinhauserova *et al.* [2001]. Among the strains identified, by the PCR methods, as *C. jejuni* [Wainø *et al*., 2003], were the hippurate-negative, catalase-negative and cephalotin sensitive ones. According to both authors the discrepancies in identification were more frequent for *Campylobacter* species less often represented in particular environment. From among 15 strains isolated from cloacal samples of pigs *e.g.* and identified biochemically as *C. lari* none was confirmed *C. lari* by the PCR-RFLP method, with 14 classified as *C. coli* and 1 as *C. jejuni* [Steinhauserova *et al.,* 2001].

Also biochemical differentiation between *C. jejuni* and *C. coli* based on ability (*C. jejuni*)or lack of ability (*C. coli*) to hydrolyse hippurate can be illusive for the *hip*O gene encoding hippuricase does not always mean for strain to express this ability. Among 97 strains identified, based on PCR method, as *C. jejuni,* 13 (13.4%) were hippurate negative yet representing 9 different genetic (PFGE; *Sma*I) profiles [Steinhauserova *et al*., 2001].

Based on the above it can be stated for the atypical campylobacters to be minority in the commercial broilers caeca, yet not to misjudge the species it is advisable to enforce the biochemical classification with the PCR one.

Undoubtfull dominance of *C. jejuni* strains occupying the caeca of commercial broiler chickens in our experiment, indicate this species to show high affinity to broilers caeca, with high numbers of *C. jejuni* in the caeca lacking the competitive microflora suggesting the caeca to be the site supporting multiplication of this species.

The caeca as the site attractive for campylobacters were also indicated by other authors [Raschaert *et al*., 2007; Wallace *et al*., 1997]. Yet, when colonized, the numbers of *Campylobacter* spp. were usually high.

Wallace *et al.* [1997] indicated the small intestine and the caeca to be the main sites for campylobacters amplification, with the numbers of *Campylobacter* spp. in caeca ranging from 10^7 to 10^{12} MPN/g and being significantly higher than in the small intestine ($p<0.001$).

Quantification of *C. jejuni* in chicken fecal and caecal samples by direct RT-PCR method showed most of the cecal samples to give DNA signals corresponding to 7.5–8.5 lg_{10} of cfu/g of caecal digesta with values for the fecal samples being lower by two orders of magnitude [Rudi *et al*., 2004].

Fla-DGGE analysis confirmed for the naturally contaminated broiler caeca to contain different genotypes of *C. jejuni* and *C. coli* with cfu of campylobacter per 1 g of caecal digesta to range from 3.0 to 8.2 lg_{10} [Najdenski *et al*., 2008].

According to our results it seems obvious for the caeca to be the reservoir of *Campylobacter* spp. in broilers, infected while alive with these bacteria.

Based on prevalence and numbers of campylobacters in commercial broiler chickens' caeca at the point of delivery to slaughterhouse, the 3 groups of birds' lots can be distinguished:

1. A quite frequently noted one, including birds with high numbers of *Campylobacter* spp. – effect of colonization taking place at the rearing farm, with the numbers to vary with the size of the rearing site.

2. A most desired one with commercially reared broilers free of campylobacters in their caeca.

3. The flocks with low numbers of *Campylobacter* spp. resulting from incidental infections during period prior to slaughter.

Less than 10^2 campylobacters per 1 g of caeca in broilers in one of the two pooled samples [the other being negative one] present in two lots of one supplier (No. 4) gives evidence, in our opinion, to incidental character of birds infection taking place somewhere between the rearing site and slaughterhouse. As the subject for testing were the caeca, the small numbers of campylobacters suggested the colonization to take place not long ago.

According to results presented by others the numbers of campylobacters in GIT of commercially reared birds are correlated with the sampling season, with higher prevalence noted, usually, in warmer months of the year [Allen *et al*., 2007; Johannessen *et al*., 2007].

For our experiment took place between December and March, the prevalence noted was high and numbers of campylobacters in the caeca exceeded, in majority of lots, 10^7 cfu/g, it can be assumed for seasonal changes to fluctuation in exchanging the birds in the shed rather, than the season. Indoor rearing of commercial broiler chickens makes, in our opinion, the seasonal changes less pronounced in affecting the carriage rate of *Campylobacter* spp., for the temperature, humidity, ventilation and other conditions essential for rearing quality and safety are, usually, strictly controlled.

Distance to cover and the carriage rate of campylobacters

The rearing broiler farms providing the poultry processing plant under surveillance were spread up to 154 km with most of the main suppliers (12/18) operating within 50 km distance from the plant. For the close vicinity between the majority of the broiler breeders and the abbatoir, the distances the birds were to cover did not seem to cause increase in the number of campylobacters, for their numbers in the caeca were similar, no matter the 5 or 150 km distance were covered.

According to Whyte *et al*. [2001], transportation to the slaughterhouse increased the level of *Campylobacter* spp. in caecal digesta from 6.0 and 6.6 to 6.8 and 7.3 cfu/g of caecal material.

Data presented by Wesley *et al.* [2005] indicated generally, a statistically significant increase ($p<0.01$) in prevalence of *Campylobacter* spp. in cloacal swabs after loading and transport of turkey to slaughter compared to results obtained for the same flocks on farm prior to loading.

Contrary to results of other surveys based on the cloacal swabs or guts/caeca contents [Wesley *et al*., 2005; Whyte *et al*., 2001], the chi square analysis of our own data indicated there to be no statistically significant differences between the distance and the prevalence or numbers of campylobacters in the caeca of transported broiler lots.

It seems obvious for the 24 h feed withdrawal prior to slaughter, transportation including, to add to reduction in shedding rate of GIT contents. However, even with reduced defecation, broilers, caprophagic in nature, may become secondarily infected with excreted campylobacters present within the birds reach.

The stressors birds are exposed to, may trigger temporal invasion/evasion reaction accompanied by rapid multiplication of campylobacters at the site they normally colonize – the caeca, for example. Statistically significant increase in excretion rates of *Campylobacter* spp. ($p<0.05$) by broilers following transportation to slaughterhouse compared to those at the farms noted by Whyte *et al*. [2001] could have been explained, *e.g.* by the stress-triggered survival strategy of campylobacters.

With birds carrying *Campylobacter* spp. in their GIT cross-contamination of processed carcasses is practically unavoidable. Surveys carried out during slaughter operations of poultry confirmed intestinal digesta to be the main source of raw poultry contamination or cross-contamination of successively processed flocks with campylobacters [Miwa *et al*., 2003; Rosenquist *et al*., 2006]. Besides the prevalence of campylobacters on carcasses and the poultry cuts was significantly higher ($p<0.05$) for campylobacter–positive flocks than for the negative ones [Reich *et al.,* 2008].

The correlation between numbers of campylobacters in the intestines and on carcasses of chickens at slaughter, noted by Rosenquist *et al*. [2006], indicated for the numbers on carcasses to be over 4 log lower.

With majority of broiler chickens carrying 10^7-10^9 cfu *Campylobacter* spp. per 1 g of caecum., the contamination

of broiler carcasses with *Campylobacter* spp. produced from broilers of the same main suppliers (though not the same flocks) throughout the year, ranged, mostly, between $\geq 10^2$ and $< 10^4$ cfu/g [Daczkowska-Kozon *et al*., 2008]. Yet the differences noted, both, in the incidence rate and numbers of campylobacters between the months in the annual cycle were statistically significant.

CONCLUSIONS

High numbers of *Campylobacter* spp. in the caeca of commercial broilers in our experiment, with the dominance of *C. jejuni* may constitute an indirect evidence for the caecum to be the environment supporting survival and promoting growth of *C. jejuni* under deprivation of competing microflora and presence of intermittent residents less adjusted to the caeca environmental conditions. Moreover, ability to displace other colonizers as well as strategy of *C. jejuni* for colonizing the chickens' guts based on temporal invasion and evasion of the epithelial cells and rapid multiplication in the mucous may suggest, in our opinion, the caeca to be the main reservoir of *Campylobacter* spp. in broiler chickens.

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Received July 2009. Revision received and accepted January 2010.