ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 7 (12): 1242-1246, 2008
ISSN 1682-8356

Risk Factors for the Presence of *Campylobacter* Sp. in Lithuanian Broiler Flocks

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Abstract: The objective of this study was to define the incidence of *Campylobacte*r in Lithuanian broiler flocks. The incidence of both *Campylobacter* and *Salmonella* and the seasonal fluctuations on the occurrence of pathogens were focused in this study. Faeces, dust and water samples were obtained at the farms 1-2 days before the broiler slaughtering. The cecum was removed after slaughtering. Microbiological study of the faeces and cecum content showed, that 18.4% (95% CI 7.0-29.0) of flocks were colonized with *Campylobacter* sp. However, dust and water samples were found to be free of *Campylobacter*. The study of influence of other pathogens (*Salmonella*) on the prevalence of *Campylobacter* sp. showed, that 12.2% (95% CI 3.0-21.0) of broiler flocks were colonized with both pathogens (*Campylobacter* and *Salmonella*). *Campylobacter* jejuni was predominant among the Campylobacter-positive flocks. The majority of broiler flocks harbored *Campylobacter* in spring (30.7%).

Key words: *Campylobacter, Salmonella*, broiler flocks, seasonal fluctuations

INTRODUCTION

Campylobacteriosis is one of the most commonly reported bacterial foodborne infections worldwide (Allos, 2001). *Campylobacter* sp. are recognized as a major cause of human gastroenteritis in developed countries (Blaser, 1997). Consumption of undercooked poultry has often been identified as a risk for human campylobacteriosis (Kapperud *et al*., 1992; Pearson *et al*., 2000). Trying to avoid the *Campylobacter* contamination of poultry products should reduce the risk of human infection. Over the last decade, the occurrence and spread of *Campylobacter* in broiler flocks has been intensively studied in several countries. Flock prevalence with *Campylobacter* ranging from 18-90% had been reported in Europe (Evans and Sayers, 2000; Refregier-Petton *et al*., 2001; Newell and Fearnley, 2003). There are two possible routes of its transmission in poultry: horizontal and vertical. Horizontal transmission is believed to be mainly through contaminated water, litter, insects, wild birds, rodents, faecal contact or transferred by farm personnel via their boots (Evans and Sayers, 2000). Once *Campylobacter* enters a flock, all the chickens in the flock become colonized and stay colonized till the slaughter time (Lindblom *et al*., 1986). Various factors influencing the prevalence of *Campylobacter* in broiler flocks have been described in several studies. The main factors associated with an increased colonization of *Campylobacter* are the lack of hygiene barriers (Kapperud *et al*., 1993; Evans and Sayers, 2000); the presence of other domestic animals in poultry farms (Kapperud *et al*., 1993; Bouwknegt *et al*., 2004); several poultry-houses on the farm (Refregier-Petton *et al*., 2001; Bouwknegt *et al*., 2004); and the season of the year (Kapperud *et al*., 1993; Wallance *et al*., 1997; Obiri-Danso and Jones, 2000; Refregier-Petton *et al*., 2001; Sari *et al*., 2004). Conversely, broiler flocks that tested negative at the farm for *Campylobacter* were also negative after slaughter (Aho and Him, 1988). However, transporting broilers to the processing plant was shown to increase the prevalence of *Campylobacter* positive birds because of faecal contamination of skin and feathers by neighboured birds during shipping (Stern *et al*., 1995).

Many studies have showed the prevalence of broiler flocks with *Campylobacter* or *Salmonella*. However only few studies have investigated the possible association between the occurrences of both: *Campylobacter* and *Salmonella*. Though no association was found in Danish and Belgian studies (Wedderkopp *et al*., 2001; Rasschaert *et al*., 2007), a possible correlation was reported in Dutch poultry flocks (Jacobs-Reitsma *et al*., 1994; Jacobs-Reitsma *et al*., 1995).

Consumers and farmers have been increasingly interested in organic food products. However, organic meat production involves potentially higher microbiological safety risk due to raising animals outdoors, the use of slow-growing breed, the prohibition to use antimicrobial preparations and exploiting of very small slaughtering facilities (Engvall, 2002). However, little is know about the microbiological status of conventional animal products in different countries.

The objective of this study was to define the incidence of *Campylobacte*r in Lithuanian conventional broiler flocks. We focused this investigation on a possible association

between the incidence of both *Campylobacter* and *Salmonella* depending on the season of the year.

MATERIALS AND METHODS

From August 2005 until April 2007 forty-nine conventional flocks from 4 different Lithuanian broiler farms were sampled. All conventional farms reared Cobb broilers. Faeces, dust and water samples were obtained at the farm from broiler flocks 1-2 days before the slaughtering. Five pooled samples were taken from each flock. The cecum from broiler flocks was removed after slaughtering and kept on ice for the determination of *Campylobacter*. The samples were divided into two parts: one was placed into semisolid enrichment media for *Campylobacter* culture and the other for *Salmonella* culture. The samples for the study of *Campylobacter* were analyzed according to ISO 10272 using mCCDA and Karmali agar for plating and Preston Broth (Oxoid) for enrichment. The qualitative analysis was performed by enrichment of 1g of cecum content or faeces in 9 g of Preston broth (Oxoid) for 48 h at 42°C in a micro-anaerobic atmosphere (85% N_2, 10% CO_2, 5%O_2). The enrichment cultures were streaked onto mCCDA and Karmali plates and later were micro-anaerobically incubated for 48 h at 42°C. Colonies were confirmed by testing for motility and cell morphology, testing for catalase and oxidase activity. Confirmed *Campylobacter* isolates were biochemically differentiated with the API Campy test (BioMérieux). Water samples were filtered on a sterile 0, 45 µm microporous filter prior to adding the remaining broth. After enrichment, the samples were streaked onto selective agar media (Karmali agar) and plates were micro-anaerobically incubated for 48 h at 42°C. Colonies were confirmed by contrast microscopy and identified biochemically with API Campy test (BioMérieux).

Salmonella was isolated according to standard methods (International Organization for Standardization 6579, 1998). Twenty five gram samples of faeces, caecal and dust were homogenized with 225 mL of pre-enrichment medium buffered peptone water (BBL, Le Pont de Claix and France) and incubated for 18 h at 37°C; 25 mL of water sample were filled into a bulb followed by adding 225 mL of BBL and incubated for 18 h at 37°C. The pre-enriched culture (0.1 and 1 mL, respectively) was transferred to Rappaport-Vassiliadis (Oxoid, UK) broth and Selenite broth (Merck, Germany) and incubated for 24 h at 42°C. Following incubations, a loopful from each broth was streaked into XLD full Agar (Oxoid), Brilliant Green Agar (BBL, France), Hectoen Enteric Agar (Merck, Germany), or Rambach Agar (Merck) plates and incubated at 37°C for 24 h. The suspected *Salmonella* colonies were transferred into Klinger Agar (Oxoid CM33) and Urea Agar Base (Oxoid CM53) tubes. Following another overnight incubation at 37°C the *Salmonella* cultures were further identified biochemically, using API 20E system (bio Mérieux, France) and agglutination test using specific O and H antisera (Sifin, Germany, Murex, France and Seiken, Japan).

The 95% Confidence Intervals (CI) for the observed prevalence of *Campylobacter*-positive and *Salmonella*-positive samples were estimated by linear interpolation formula:

$$CI = p\text{-}z[p(1\text{-}p)/n]^{0.5},\ CI = p + z[p(1\text{-}p)/n]^{0.5}$$

where:

- p = Number of positive samples/number of tested samples.
- z = (95%) 1.96.
- n = Number of tested samples.

RESULTS

The results of our investigations showed, that *Campylobacter* sp. colonized differently broiler flocks in separate farms and tested samples. From 4 farms studied *Campylobacter* sp. was detected only in two of them: C and D (Table 1). In farm D according to the results of investigation of ceca samples, 5 flocks from 15 were positive for *Campylobacter* sp. (33.3%). The study of the faeces samples showed, that 3 flocks from 7 were infected with *Campylobacter* (42.5%). In farm C according to the results of cecum samples only one flock was colonized with *Campylobacter*. The results of the cecum and faeces samples showed, that 22.2% of cecum samples and 25.0% of faeces samples were colonized with *Campylobacter*. All samples from dust and water samples proved to be *Campylobacter* negative. Totally, 9 flocks from 49 (18.4%) flocks were colonized with *Campylobacter* sp. The species distribution among Campylobacter-positive flocks showed that *Campylobacter* jejuni was predominant. From 9 positive flocks for *Campylobacter* sp. 8 were colonized with *Campylobacter jejuni* (88.9%) and only one flock with *Campylobacter coli* (11.1%).

The study of the influence of other pathogens on the prevalence of *Campylobacter* sp. showed, that 9 broiler flocks from 49 (18.4%) were colonized with *Campylobacter*, whereas *Salmonella* was isolated from 12 flocks (24.4%) (Table 2). Six flocks were positive for both pathogens *Campylobacter* and *Salmonella* (12.2%).

Considering the seasonal variability of *Salmonella* and *Campylobacter* colonized flocks, the study period was divided into four periods according to the four seasons of the year (January-March, April-June, July-September and October-December).

The majority of *Campylobacter* positive broiler flocks were found in spring (30.7%) (Table 3). The percentage of *Campylobacter* infected broiler flocks was less in winter and summer (23.0 and 20.0%, accordingly). The

Table 1: The results of tested flocks for *Campylobacter* sp. in different farms

		Cecum		Faeces		Dust		Water	
Farm	No. of houses	No. of tested flocks	No. of positive flocks (%)	No. of tested flocks	No. of positive flocks (%)	No. of tested flocks	No. of positive flocks (%)	No. of tested flocks	No. of positive flocks (%)
A	4	3	0	1	0	NT	NT	NT	NT
B	6	5	0	4	0	1	0	1	0
C	50	4	1/25.0%	NT	NT	NT	NT	NT	NT
D	27	15	5/33.3%	7	3/42.5%	4	0	4	0
Total		49	6/12.2%	12	3/25.0%	5	0	5	0

NT-not tested

Table 2: The incidence of *Campylobacte*, *Salmonella* sp. and *Campylobacter* + *Salmonella* in tested broiler flocks

No. of tested flocks for *Campylobacter* (n = 49)		
	Positive (%)	9 (18.4)
	CI (95%)	7.3-28.7
No. of tested flocks for *Salmonella* (n = 49)		
	Positive (%)	12 (24.4)
	CI (95%)	12.1-36.0
No. of the tested flocks for *Campylobacter* + *Salmonella* (n = 49)		
	Positive (%)	6 (12.2)
	CI (95%)	3.0-21.0

similar seasonal influence for the prevalence of *Salmonella* was also detected. The majority of *Salmonella* positive broiler flocks was established in spring (54.0%). The incidence of *Salmonella* in winter, summer and autumn was less (30.0, 10.0 and 14.3%, accordingly).

DISCUSSION

Limited data exist on the prevalence of Campylobacter infection in conventional poultry flocks in different countries. According to the literature, in Europe this prevalence varies from 18 to >90%; in the northern countries it was found to be less than in southern European countries (Newell and Fearnley, 2003). In the Netherlands about 30% of broiler flocks were contaminated with *Campylobacter* (Bouwknegt *et al*., 2004), while in Belgium 73% (Rasschaert *et al*., 2007). However, the prevalence of *Campylobacter* showed higher rates in mid and southern Europe, where up to 91% of positive flocks were found (e.g. in Italy) (EFSA, 2006). According to our results 18.4% of Lithuanian broiler flocks were found to be colonized with *Campylobacter* sp. A possible explanation for our finding of relatively low presence of *Campylobacter* in broiler flocks is the conventional production system used which is the most established housing type in Lithuania. The incidence of *Campylobacter*-positive flocks is generally higher (up to 100%) in organic and free-range flock farms (Berndtson *et al*., 1996; Heuer *et al*., 2001); Rodenburg *et al*., 2004) as compared to intensively reared ones.

The main reservoir of *Campylobacter jejuni* in poultry is the cecum (Rudi *et al*., 2004). Our results showed that cecum and faeces were colonized with *Campylobacter,* however dust and water samples proved to be *Campylobacter* negative. Seleha (2004) failed to isolate *Campylobacter* from swab samples of the walls, floors and dust from Malaysian chicken houses. There is an assumption that *Campylobacter* cannot survive for long period within the dehydrating conditions of dust. Bull (2006) suggest, that drinking water can be contaminated by faecal droppings during the rearing period and can serve as a transmission route. However the data from our investigations showed, that water was free of *Campylobacter* and could not be the risk factor for Campylobacter infection.

The species distribution among the *Campylobacter*-positive flocks showed that *Campylobacter jejuni* was predominant in conventional broiler flocks (88.9%) in Lithuania. These results are in agreement with those of other authors (Bouwknegt *et al*., 2004; Oyarzabal *et al*., 2005).

Although several risk factors for infection of broilers with *Campylobacter* sp. have been identified, knowledge about the various routes by which flocks become infected and their relative influence is still incomplete. One of the risk factors for the prevalence of *Campylobacters* in broiler flocks is the presence of >2 broiler houses on the farm (Refregier-Petton *et al*., 2001; Guerin *et al*., 2007). Our study showed that in farm (D), where the number of houses was 27, the prevalence of broiler flocks colonized by *Campylobacter* was 33.3% while in two other farms (A and B), wherein were only 4 and 6 houses, broiler flocks were found to be negative for *Campylobacter*. Several houses on the same farm may lead to an increased risk of *Campylobacter* through an intensive movement of farm workers between the houses. Dutch authors (Bouwknegt *et al*., 2004) suggest that animals on farms within 1 km have the highest impact on *Campylobacter* presence in Dutch broiler flocks.

There is only limited data on mixed infections on broiler flocks with *Campylobacter* and *Salmonella* (Jacobs-Reitsma *et al*., 1994; Wedderkopp *et al*., 2001; Rodenburg *et al*., 2004; Cui *et al*., 2005). In the study of Cui *et al*. (2005) the majority of conventionally bred chickens were contaminated with *Campylobacter* (74%) and only 44% with *Salmonella*. Other investigators also noted that 13% of organic broiler flocks were positive for *Salmonella* and 35% for *Campylobacter* (Rodenburg *et*

Table 3: Distribution of *Campylobacter* sp. and *Salmonella* spp. according to the season of the year

	Season of the year			
	Winter	Spring	Summer	Autumn
No. of tested flocks for *Campylobacter*	13	13	10	2
No. of positive flocks for *Campylobacter* (%)	3 (23.0%)	4 (30.7%)	2 (20.0%)	0
No. of tested flocks for *Salmonella*	27	13	10	7
No. of positive flocks for *Salmonella* (%)	7 (30.0%)	7 (54.0%)	1 (10.0%)	1 (14.3%)

al., 2004). However, in our study the number of *Salmonella*-positive flocks was bigger (24.4%) than that of *Campylobacter* infected ones (18.4%). Six flocks were positive for both pathogens *Campylobacter* and *Salmonella* (12.2%). It is difficult to explain which of these pathogens is "primary" or "secondary". Further studies are needed to determine mixed infections in broiler flocks.

Seasonal variations in the occurrence of *Campylobacter* in broiler chickens have been described in several reports (Heuer *et al*., 2001; Wedderkopp *et al*., 2001; Huneau-Salaün *et al*., 2007). Although the seasonal aspect of broiler colonization by *Campylobacter* is often reported, the reason thereof is still unknown. In the study of Heuer *et al*. (2001), the highest prevalence of colonization was found from May to October. The risk of a flock being colonized with *Campylobacter* was higher in spring/summer period as compared to winter (Kovats *et al*., 2004; Huneau-Salaün *et al*., 2007). Other investigators (Wedderkopp *et al*., 2001) noticed that the majority of *Campylobacter* positive cloacae swabs were found in July, August and September while the lowest number of positive samples was found from January to April. The Danish scientists (Hald *et al*., 2004) suggest that flies may be an important source of Campylobacter infection of broiler flocks in summer. Our results showed that the risk of *Campylobacter* excretion by broiler chickens was increased in spring period. The geographical variation in the timing of the seasonal peak suggests that climate may be a contributing factor to *Campylobacter* transmission. On the other hand, the climate in Lithuania is very variable and changes from year to year. Taking into account the data of seasonal scattering in the occurrence of *Campylobacter*, we postulate, that warm period in comparing to cold one is more favourable to spreading *Campylobacter* infection in broiler flocks. The strong seasonal effect certainly made it more difficult to highlight other risk factors of bacteria colonization in our study.

Conclusion: In conclusion, no significant correlation between occurrence of Campylobacter and Salmonella infections in Lithuanian broilers was found, however the incidence of campylobacteriosis salmonellosis infection was associated with the season of the year.

ACKNOWLEDGEMENT

The study was part of the research project POULTRYFLORGUT funded by the European Commission, Contract no.: FOOD-CT-2005-007076.

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