

PRESENCE OF *CAMPYLOBACTER* AND *SALMONELLA* SPP. IN POULTRY AND ENVIRONMENTAL SAMPLES FROM FARM TO RETAIL IN HUNGARY

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The aim of this study was to investigate the occurrence of *Salmonella enterica* and its most important serovars *Salmonella* Infantis, *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Campylobacter* spp. in the broiler meat production chain. Altogether 110 pooled samples were analysed; environment, cloaca, body surface at the farm, then carcass, offal, and packed meat from the slaughterhouse. The combination of redox potential measurement and real-time PCR was used for the detection of the microbes.

At the farm, the first *Salmonella* positive result came from the water system, then it appeared in most of the samples. In contrast to the absence of *Salmonella* on the birds' body surface before transportation, by the end of the processing it had reached 100%, with the only identifiable serovar being *S. Infantis* (65%). All packed meat samples showed positivity, from which 70% was *S. Infantis*.

Campylobacter appeared at the farm on the 3rd week and remained significant during the breeding. After the slaughtering process, the contamination was 100% in the carcass, offal, and packaged meat samples.

Our results demonstrated the success of the *Salmonella* control program, by the low prevalence of *S. Typhimurium* and Enteritidis.

Keywords: *Salmonella*, *Campylobacter*, poultry, food safety, public health

Campylobacter has been the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union since 2005 (EFSA, 2017). Broiler meat is considered to be the most important source of human campylobacteriosis. *Campylobacter* genotypes found in the farm flock can efficiently survive and persist throughout the food chain (GRUNTAR et al., 2015). The number of *Campylobacter* found in the carcass varies during the processing; usually it is the highest at the defeathering, followed by a decrease along the rest of the steps (GUERIN et al., 2010). But the modern, short processing chain, the dark, moist, and cool storage, and the use of protective plastics contribute to *Campylobacter* survival, especially in large-scale marketing (HARRISON et al., 2001). High contamination level in meat preparations and meat cuts has been described by authors (HUMPHREY et al., 2007; SUZUKI & YAMAMOTO, 2009). The infective dose of *Campylobacter* is low, so carcasses contaminated at low level can also be considered as threat to public health (ROBINSON, 1981; MALHER et al., 2011).

Since the implementation of the National Control Programmes, the declining trend in the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium positive poultry flocks has continued for all groups of animals during their production period in the EU. Salmonellosis, caused by *Salmonella enterica* is one of the most frequently reported foodborne illnesses worldwide. Chicken meat has often been reported as a source of *Salmonella* contamination (ALALI et al., 2014). *Salmonella* Infantis and *S. Enteritidis* were the two most common serovars isolated from broiler meat in the European Union in 2016 (EFSA, 2017).

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While *Campylobacter* and *Salmonella* infections are often asymptomatic in broilers, the colonization of these pathogens in broilers presents a significant risk to human health through ingestion of contaminated meat. Improving our understanding of the on-farm transmission dynamics of *Campylobacter* and *Salmonella* will allow the implementation of better control measures to reduce pathogen prevalence (THAKUR et al., 2013).

The aim of this study was to examine the prevalence of *Campylobacter* and *Salmonella* during the whole food chain. The birds, the environments of the farm, the slaughterhouse, and the processing plant were sampled to determine the spread and contamination possibilities of the two pathogens at the different stages.

1. Materials and methods

1.1. Sample collection

A total of 110 samples were examined between February and April 2017 in Hungary. The samples were collected from the two unseparated halves of a single building. Four feed, 4 bedding, 4 water, 5 cloaca, and 5 body surface samples were collected from both areas and then pooled into two final samples ('A' and 'B' building). The first sampling of the environment happened before the animals' arrival (day 0), then the broilers were sampled immediately after their settling. Afterwards, the same number of samples were taken every week on the same day until the chickens' transportation to the slaughterhouse; altogether 36 from the environment and 24 from the animals. Further samples were obtained on the day of slaughter (n=20) and from the packaged meat (n=30). The samples are summarised in Table 1.

Twenty five grams of meat samples were collected. The examination of the surfaces was carried out by swab and sponge sampling from a 100 cm² area. Each sample was put into different sterile stomacher bags and transferred into the laboratory in a cooler box at 4 °C. The analysis of the samples was performed on the same day.

Table 1. Sampling locations and sampling methods used in the study

	Sampling location	Type of sample	Sampling method	Samples (n)
Farm	Bedding	Faeces	25 g	12
	Cloaca	Faeces	Swab	12
	Feed	Feed	25 g	12
	Drinking system	Surface	Sponge	12
	Body surface	Surface	Sponge	12
Slaughterhouse	Body surface before transport	Surface	Sponge	5
	Body surface after evisceration	Surface	Sponge	5
	Abdominal cavity	Surface	Sponge	5
	Offals	Meat	25 g	5
Retail	MSM*	Meat	10 g	5
	Chicken breast filet	Meat	25 g	5
	Chicken wings	Meat	25 g	5
	Chicken back	Meat	25 g	5
	Drumstick	Meat	25 g	5
Total				110

*MSM: mechanically separated meat

1.2. Detection of *Salmonella*

The enrichment phase was carried out by redox potential measurement method. The redox potential measurement was originally developed for rapid determination of viable count of several microorganisms in water, milk, food, and hygienic samples (REICHART et al., 2007; ERDŐSI et al., 2012; 2014). As a result of the enrichment, the *Salmonella* positive samples could be detected by this rapid technique. Instead of a subsequent biochemical confirmation, further identification was carried out by applying real-time PCR technique (ERDŐSI et al., 2014).

Rappaport-Vassiliadis *Salmonella* selective broth (RVS broth) Merck 107666 was used as culture medium at the incubation temperature of 42 °C. Samples of 25 g were homogenized in 225 ml of RVS broth. Swabs were placed into the measuring cell containing 10 ml broth. The sponges were put into 100 ml of selective medium.

During the incubation in water bath, the redox potential of each cell was continuously monitored. The parts of the redox potential measuring system were: Schott Blue Line 31RX redox electrodes, MicroTester device supplied with a Windows-based software MicroTester Redox v.2.5.16 for data collection and evaluation (REICHART et al., 2007).

DNA was isolated from 1 ml of the enriched samples taken from the redox potential measuring cell at the end of the enrichment process. “Mericon DNA Bacteria Kit” (Qiagen) was used according to the manufacturer’s instructions.

Real-time PCR amplification was performed on SLAN® Real-Time PCR System (Hongshi) using Mericon *Salmonella* Kit (Qiagen) for the detection of *Salmonella* spp. DiaSalmR kit (Diagon) was used in order to detect *Salmonella* Typhimurium, *S. Enteritidis*, and *S. Infantis*. PCR assays contained 10.8 µl Multiplex PCR Master Mix and 9.2 µl DNA isolate.

1.3. Detection of *Campylobacter* spp.

Bolton Broth (Merck 100068) with Bolton Selective Supplement (Merck 100079) was used for selective enrichment.

Samples of 25 g and 225 ml broth were put into 250 ml measuring cells equipped with redox electrodes. Swabs and sponges were placed into the measuring cells containing 10 ml and 100 ml broth, respectively.

To verify the presence of *Campylobacter* spp., real-time PCR technique was used. Genomic DNA was isolated from 1 ml of enriched samples. “MericonDNA Bacteria Kit” (Qiagen) was used according to the manufacturer’s instructions. Real-time PCR amplification was performed with SLAN® Real-Time PCR System (Hongshi) using the Mericon *Campylobacter* spp. kit (Qiagen). The PCR tubes contained 10.8 µl Multiplex PCR Master Mix and 9.2 µl DNA isolate.

1.4. Mathematical-statistical evaluation

The regression analysis for the calibration curves was performed by Microsoft Excel 2016.

2. Results and discussion

2.1. *Salmonella*

The weekly changes of the presence of positive samples at the farm are shown in Table 2. The bacterium first appeared at the 1st week in the water sample similarly as described by other

authors earlier in Spain (MARIN et al., 2011). Then, from the 2nd week positive results were detected from all other materials, except for the feed (only a single positive sample at the 5th week). *S. Infantis* and *S. Typhimurium* were identified by the real-time PCR. According to Regulation No 200/2012 (EU, 2012), the goal regarding broiler flocks is to reduce the prevalence of *S. Enteritidis* and/or *S. Typhimurium* to 1%. The percentage of non-target serovars increased in 2015 due to *S. Infantis* (EFSA, 2017). Our findings were in correlation with the relevant EFSA report.

Table 2. Presence of *Salmonella enterica* at the broiler farm

Date	Bedding		Feed		Drink		Body surface		Cloaca	
	A	B	A	B	A	B	A	B	A	B
Settling	-	-	-	-	-	-	-	-	-	-
Week 1	-	-	-	-	+	+	-	-	-	-
Week 2	+	+	-	-	+SI	+SI	+SI	+SI	+	+ST
Week 3	+	+	-	-	+ST	+ST	+ST	+SI	+	+
Week 4	+	+	-	-	+	+	+	+	+ST	+SI
Week 5	+	+	+	-	+	+	-	-	+ST	+SI

'A', 'B': pooled samples from the two unseparated areas of the building

SI: *Salmonella* Infantis

ST: *Salmonella* Typhimurium

The 0% positivity from before the transportation increased up to 100% in the offal and on the body surface and 80% in the body cavity after the evisceration. Only serovar *S. Infantis* (65.3%) was identified. The results of the slaughterhouse's sampling are found in Table 3.

As can be seen, 100% of the samples taken from chicken meat packages were found positive, from which 70% was *S. Infantis*. *S. Typhimurium* and *S. Enteritidis* have not been detected (Table 4). Other authors have published *Salmonella* prevalence from samples taken from the skin of the skin-on chicken parts and the meat of the skin-off parts with 42.2% and 17.6%, respectively (GURAN et al., 2017). More studies have determined the overall *Salmonella* presence on chicken carcasses and chicken parts around 30% (MAZENGA et al., 2014; WU et al., 2014).

Table 3. Presence of *Salmonella* and *Campylobacter* spp. during the slaughtering process (%)

Sample	<i>Salmonella</i> spp.	<i>Salmonella</i> Infantis	<i>Salmonella</i> Typhimurium	<i>Salmonella</i> Enteritidis	<i>Campylobacter</i>
Body surface before transport	0	0	0	0	60
Body surface after evisceration	100	70	0	0	100
Abdominal cavity after evisceration	80	35	0	0	100
Offal	100	91	0	0	100

Table 4. Presence of *Salmonella* and *Campylobacter* in the packaged meat samples (%)

Sample	<i>Salmonella</i> spp.	<i>Salmonella</i> Infantis	<i>Salmonella</i> Typhimurium	<i>Salmonella</i> Enteritidis	<i>Campylobacter</i>
Wings	100	44	0	0	100
Thighs	100	81	0	0	100
Drumsticks	100	81	0	0	100
Breasts	100	72	0	0	100
Back	100	62	0	0	100
MSM	100	80	0	0	100

2.2. *Campylobacter* spp.

Several studies have revealed that *Campylobacter* contamination is higher in the chicken meat than that of *Salmonella* (HUANG et al., 2016).

Transmission along the food chain (from broilers, through the abattoir, to the retail shop) is generally accepted as a major source of human campylobacteriosis (THAKUR et al., 2010). Over the last decade, the prevalence of *Campylobacter* in the poultry production chain has been widely investigated in many countries. This was in agreement with previous studies that have determined that once *Campylobacter* enters a flock, it spreads quickly and colonizes the entire flock (THAKUR et al., 2013). Although strategies for reducing the incidence of this pathogen in poultry and poultry products are being studied and applied, the prevalence of *Campylobacter* is still high (PRACHANTASENA et al., 2016).

In our study, the first *Campylobacter* positive samples were detected in the 3-week-old flock, when *Campylobacter* was isolated from the drinking system, bedding, and the surface of the animals. From the 4th week of the examination, 100% of the cloaca samples became positive. The feed was proved to be negative throughout the whole experiment. The weekly changes of the presence of the *Campylobacter* positive samples at the farm are shown in Table 5.

Table 5. Presence of *Campylobacter* spp. at the broiler farm

Date	Bedding		Feed		Drink		Body surface		Cloaca	
	A	B	A	B	A	B	A	B	A	B
Settling	-	-	-	-	-	-	-	-	-	-
Week 1	-	-	-	-	-	-	-	-	-	-
Week 2	-	-	-	-	-	-	-	-	-	-
Week 3	+	+	-	-	+	+	+	+	-	-
Week 4	+	+	-	-	+	+	-	-	+	+
Week 5	-	-	-	-	-	-	+	+	+	+

'A', 'B': pooled samples from the two unseparated areas of the building

The 60% positivity of the body surface before the transport has increased to 100% during the slaughtering process, indicating an important cross-contamination rate between poultry meat and the equipment (Table 3). Other authors have found around 50% positivity, showing the high prevalence of *Campylobacter* in the poultry production chain (SANCHEZ et

al., 2017). PRACHANTASENA and co-workers (2016) found a prevalence of 43.5% as an average of five slaughterhouses in Thailand. KUDIRKIENÈ and co-workers (2011) stated that *Campylobacter* can survive in the slaughterhouse for 8 hours. Their examinations showed that the processing area was less contaminated with *C. jejuni* than the slaughterhouse.

All 60 packed poultry meat samples exhibited positivity (Table 4). This is significantly higher than the results of other authors. The prevalence of *Campylobacter* in French retail is high, 76% of chicken meat products were found to be contaminated (GUYARD-NICODÈME et al., 2015). In Italy, STELLA and co-workers (2017) described 34.1% prevalence. In a previous research in Hungary, 76.4 % positivity was described (DAMJANOVA et al., 2011).

3. Conclusions

Based on our results, the *Salmonella* control program in the EU is successful, because the prevalence of *S. Enteritidis* and Typhimurium serovars is low. However, the prevalence of other serovars, especially *S. Infantis*, which can cause similar symptoms in humans as *S. Enteritidis* and Typhimurium, has increased significantly.

An efficient control program should be applied to reduce the occurrence of other *Salmonella* serovars and *Campylobacter* spp. to decrease human foodborne diseases originated from the poultry production chain, with particular regard to the farm's water system and the hygiene of the slaughterhouse.

The 100% positivity of *Salmonella* and *Campylobacter* ssp. shows how important the consumers' awareness is during the processing and home cooking procedures of raw chicken products.

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