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ORIGINAL ARTICLE

Impact of the slaughter line contamination on the presence of *Salmonella* on broiler carcasses

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Abstract

Aims: The aim of the study was to assess the impact of *Salmonella* present on the slaughter line before processing on broiler carcass contamination during processing.

Methods and Results: Three Belgian broiler slaughterhouses were each visited twice. Samples were taken from the slaughter line after the cleaning and the disinfection process and before slaughter of the first flock. During the slaughter of the first flock, feathers and neck skins were collected at various points of the slaughter process. Swab samples were also taken from the crates in which the birds were transported. In two slaughterhouses, the slaughter line was contaminated with *Salmonella* before the onset of slaughter, especially the shackles, conveyer belt and the plucking machine in the dirty zone. During slaughter, the carcasses of the first *Salmonella*-free flock became contaminated with the same strains as isolated previously from the slaughter line.

Conclusion: Contamination of the slaughter line with *Salmonella* leads to carcass contamination.

Significance and Impact of the Study: Implementation of logistic slaughter is only successful when the cleaning and disinfection process completely eliminates the *Salmonella* contamination of the slaughter line. Only if this is achieved, will the slaughter of *Salmonella*-free flocks result in the absence of *Salmonella* on the carcasses after slaughter.

Introduction

Salmonella enterica subsp. *enterica* is one of the major foodborne causes of gastroenteritis in most industrialized countries. In Belgium, 9543 *Salmonella* isolates from human infections were sent in 2004 for further characterization to the National Reference Centre for *Salmonella* and *Shigella* (NRSS 2004). The serotypes mostly isolated in 2004 were *Salmonella* serotype Enteritidis (64%) and *Salmonella* serotype Typhimurium (26%) (NRSS 2004). According to van Pelt *et al.* (1999), eggs and poultry meat are responsible for 39% and 21% of human salmonellosis cases, respectively, whereas human salmonellosis is caused by pork in 25% of the cases and by beef in about 10% of the cases. Contamination of poultry products can occur through the whole production chain, but until now, most studies have been focusing on the primary production. Several risk factors for *Salmonella* contamination have already been identified in the farm such as vertical transmission from breeder flocks to their offspring, contamination of equipment in the hatchery, a poor level of hygiene in the farm, the presence of rodents and insects on the farm, inadequate cleaning between rotation of flocks and contamination of the feed and drinking water (Davies and Wray 1995,1996; Davies *et al.* 1997; Rose *et al.* 2000, 2001; Davies and Breslin 2003; Doyle and Erickson 2006). Several control measures have been implemented to reduce *Salmonella* contamination of poultry flocks at farm level such as vaccination of the breeder flocks, application of competitive exclusion, the use of prebiotics, acidification of feed and water and strict hygiene measures on the farm (Doyle and Erickson 2006). At slaughter age, it is important to maintain the birds *Salmonella*-free during transport and slaughter. However, transport in inadequately cleaned and disinfected containers (Rigby *et al.* 1980), cross-contamination by the

slaughter environment or by *Salmonella*-contaminated flocks to the carcasses of *Salmonella*-free flocks are identified as possible risk factors at this stage of production (Corry *et al.* 2002; Olsen *et al.* 2003). To reduce cross-contamination, logistic slaughter has been applied since 1999 in Belgium. Flocks with a *Salmonella*-free status are slaughtered first followed by *Salmonella*-positive flocks. The *Salmonella* status is determined by collecting faecal material in the broiler house using two pairs of overshoes within 3 weeks before slaughter. In a previous Belgian study, 18 broiler flocks were followed from hatching to slaughter. Though eight flocks had a *Salmonella*-free status, the carcasses of seven of these flocks were contaminated by *Salmonella* after slaughter while four of these flocks were slaughtered first on the sampling days (Heyndrickx *et al.* 2002). This may indicate that the *Salmonella* contamination originated from the slaughter environment.

The aims of the present study were first to assess the presence of *Salmonella* on the slaughter line before processing the first flock and second, to determine the impact of *Salmonella* present on the slaughter line on the carcass contamination of the first flock slaughtered.

Materials and Methods

The poultry-processing plants

The study was conducted in three Belgian broiler slaughterhouses (A, B and C) from June to November 2005. The slaughterhouses were visited on a Tuesday or a Wednesday after at least one day of operation in the week. Each slaughterhouse was visited twice with a minimum interval of 3 weeks between visits. A similar slaughter procedure was applied in the three slaughterhouses. In the living area, the birds were unloaded, hanged manually, electrically stunned and killed. In a second separated area, the birds were scalded in a counter current flow scalding tank at a temperature of ±51°C before they were mechanically plucked. The head of the bird was removed before the carcasses were hung over on the evisceration line. Finally, the mechanical evisceration took place in a third room. During processing, only potable water was used in the three slaughterhouses. Plants A, B and C had processing capacities of 9000, 6000 and 6000 birds per hour, respectively. Slaughterhouse A was the only slaughterhouse with two killing lines, but only one killing line was included in the sampling plan.

Table 1 Overview of the number of samples taken in the three slaughterhouses before and during processing

Samples	Number of samples
Hanging area before processing	
Three shackles before the hanging area	1
Two wheels and 25 cm conveyer belt before the hanging area	1
Three shackles after the hanging area	1
Two wheels and 25 cm conveyer belt after the hanging area	1
Three shackles after stunning	1
Two wheels and 25 cm conveyer belt after stunning	1
Scalding tank before processing	
Three shackles	3
Two wheels and 25 cm conveyer belt	3
Doors (400 cm^2)	3
Roof (400 cm^2)	3
Just above the water surface (10 cm^2)	3
25 ml scalding water	4
Plucking machine before processing	
Three shackles	3
Two wheels and 25 cm conveyer belt	3
Plucking fingers – one element	3
Plastic bands between fingers (400 cm^2)	3
Construction (400 cm^2)	3
Evisceration before processing	
Three shackles	3
Two wheels and 25 cm conveyer belt	1
Neck breaker – one element	1
Vent cutter – one element	1
Abdominal cavity opening machine – one element	1
Scoops – one element	1
Cropper – one element	1
Neck cutter – one element	1
Neck remover – one element	1
Neck skin cutter – one element	1
Lung remover – one element	1
Inside/outside bird washer – one element	1
Samples during processing the first flock	
Feathers before scalding (25 g)	3
Feathers after scalding (25 g)	3
Feathers from the plucking machine (25 g)	3
25 ml scalding water	6
Neck skins after plucking (25 g)	30
Neck skins after evisceration (25 g)	30
Four crates of a transport container (4 × 400 cm^2)	6
Pooled sample of 10 duodena	6
Pooled sample of 10 caeca	6

Sample collection

An overview of the sampled slaughter equipment and the samples taken from the first poultry flock slaughtered at each visit are shown in Table 1. The samples from the slaughter line were taken 1 h before the slaughter activities started and several hours after the cleaning and disinfection process had ended. All samples, except the water scalding samples, consisted of one swab moistened with sterile peptone water (0·1%). From each scalding tank,

different water samples (25 ml) were collected before and during slaughter. During slaughter of the first flock, feathers from the breast and the wings were collected while the birds were hanging on the shackles before scalding, after scalding and from the plucking machine. Thirty neck skin samples were collected immediately after plucking and 30 neck skin samples after evisceration. Up to three neck skins were pooled to obtain a sample of at least 25 g. All samples collected during processing of the first flock were taken at evenly distributed intervals over the time needed to process the complete flock. The six flocks had all received a *Salmonella*-negative status as stated on the transport documents. To check this status at the moment of slaughter, 60 gastrointestinal tracts from each flock were collected just after evisceration (95% confidence interval to detect a prevalence of 5% in a flock). Furthermore, six containers used to transport the flock were sampled just before the crates were washed and disinfected. Of each transport container, four samples were taken (*c.* 400 cm^2) with four swabs and pooled to one sample. All samples were transported to the laboratory under cooled conditions and processed immediately.

Bacteriological examination

Twenty-five millilitre of each water sample was mixed with 25 ml of double-strength buffered peptone water (BPW; Oxoid CM509, Basingstoke, UK). Forty millilitre of the pre-enrichment media BPW was added to all swab samples, except for the swabs of the transport containers to which 100 ml of BPW was added before homogenizing in a stomacher blender at normal speed. Twenty-five grams of the feather samples was mixed with 225 ml of BPW. Each neck skin sample (25 g) was stomachered in 225 ml of BPW. From each of the 60 gastrointestinal tracts, 1 g of the duodenum and 1 g of the caecum were aseptically collected. These samples were pooled to create six subsamples of 10 g caeca content and six subsamples of 10 g duodenum content. These subsamples were homogenized with 90 ml of BPW in a stomacher blender.

After incubation of the pre-enrichment media at 37°C for 18 h, 100 μl was plated onto Diagnostic Semi-Solid *Salmonella* Agar (Diassalm; LabM 537, Lancashire, UK) and 100 μl was added to 10 ml of Rappaport-Vassiliadis boullion (RV; Oxoid CM669). After incubation for 24 h at 42°C, a loopful from the edge of the purple migration zone from the Diassalm plates was plated onto Xylose Lysine Desoxycholaat (XLD; Oxoid CM469). If the plates showed a large migration zone (complete discolouration), two loopfuls of the zone were plated on two XLD plates. Ten microlitre of each RV tube was plated on XLD. All XLD plates were incubated at 37°C for 24 h. From the XLD plates streaked out from the RV tubes, two morphologically typical colonies were picked. That way, a maximum of four colonies per sample were further examined. Presumptive *Salmonella* colonies were confirmed at genus level by polymerase chain reaction (PCR) using the primers described by Aabo *et al.* (1993). The reaction mixture and amplification protocol were as described by Botteldoorn *et al.* (2003).

Characterization of the *Salmonella* isolates

All *Salmonella* isolates were characterized by enterobacterial repetitive intergenic consensus (ERIC) PCR as previously described by Rasschaert *et al.* (2005). ERIC-PCR can be used to limit the number of strains that have to be serotyped as different strains belonging to the same serotype cluster together at a delineation level of 95%. At least two isolates per cluster were subsequently serotyped by the Belgian *Salmonella* reference laboratory. Randomly selected isolates of each serotype were characterized at strain level by pulsed-field gel electrophoresis (PFGE). The isolates were grown for 18 h on tryptone soya agar (TSA; Oxoid CM0131) at 37°C. The cells were suspended in cold Pett IV buffer (1 mol l^{-1} of NaCl, 10 mmol l^{-1} of Tris-HCl pH 8, 10 mmol l^{-1} of Na_2 EDTA) and adjusted to an optical density (OD_{600}) value of 0·8. The method of Olsen *et al.* (1994) was followed for preparing the plugs. Plug slices were digested for 18 h with 30 U of *Xba*I and *Not*I (Invitrogen, Paisley, UK) in single digestion reactions. DNA fragments were separated by Chefmapper in a 1% Seakem Agarose gel (Biowhittaker Molecular Applications, Rockland, Maine, USA). The running conditions were 6 V cm^{-1} at 14°C in 0·5 × Tris-Borate-EDTA buffer for 22 h with a ramping time from 4 to 40 s for the *Xba*I enzyme or 24 h with a ramping time from 2 to 12 s for the *Not*I enzyme. PFGE profiles were clustered with GelCompar 3·0 (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient (1% position tolerance) and the unweighted-pair group method using the arithmetic averages algorithm (UPGMA). A PFGE genotype was assigned on the basis of a difference in the absence or presence of at least one band in at least one of the two profiles (*Xba*I and *Not*I). Genotypes within serotypes were indicated by the capital of the name of the serotype followed by a number (e.g. *Salmonella* Montevideo genotype 1 is idicated as M1). A small shift of one band in a maximum of one of the two profiles was indicated by an apostrophe.

Salmonella isolates from flocks slaughtered in the week before the sampling day

As there is a legal obligation to determine the *Salmonella* status of all Belgian broiler flocks before slaughter, the

slaughterhouses were able to give an overview of all *Salmonella*-positive flocks slaughtered in the week before the sampling days. Some isolates from these *Salmonella*-positive flocks could be recuperated from the laboratories to which the overshoes were sent to determine the *Salmonella* status. These isolates were characterized by ERIC-PCR and PFGE as described before.

Results

In total, 881 samples were collected in the three slaughterhouses. Two hundred and twenty-one samples were *Salmonella*-positive (25%) and 553 *Salmonella* isolates were further characterized.

Slaughterhouse A

The first sampling day

On the first sampling day in slaughterhouse A, 17 out of 50 samples (34%) taken from the slaughter line were contaminated by *Salmonella*. Nine genotypes belonging to six serotypes were found on the slaughter equipment (Table 2). *Salmonella* Typhimurium O5+ genotype T1 and *Salmonella* Paratyphi B genotype P1 were most commonly isolated from the slaughter environment. In the plucking and scalding area, the plucking machine was the most contaminated. The plastic bands (between the rows of the plucking fingers) in the plucking machine were contaminated with seven different genotypes.

The *Salmonella*-free status of the first slaughtered flock was confirmed by the absence of *Salmonella* in the intestines. Nevertheless, the transport crates, the feathers before and after scalding, the feathers collected during plucking and the neck skins samples after plucking and after evisceration were contaminated by *Salmonella*. These samples were contaminated with the same strains as previously isolated from the slaughter line before processing, except *Salmonella* Minnesota genotype Mi1 which was isolated from 10 neck skin samples after evisceration. Only *Salmonella* Indiana strain I1 which was isolated from different places from the slaughter line was not found during slaughter of the first flock (Table 2).

In the week before the first sampling day, two flocks with a *Salmonella*-positive status were slaughtered. A flock slaughtered 4 days before the sampling day was colonized by *S.* Typhimurium O5+ strain T1.

The second sampling day

On the second sampling day in slaughterhouse A, 23 out of 56 samples (41%) of the slaughter line were contaminated by *Salmonella*. The shackles and wheels were the most contaminated. Only in the evisceration room were the shackles and wheels *Salmonella*-free. Seven genotypes belonging to five serotypes were found in the slaughter environment (Table 2). Although this flock had a *Salmonella*-free status, two strains were isolated from the duodenal content of this flock: *S.* Paratyphi B strain P5 and *S.* Typhimurium O5+ strain T1. The former was not found during slaughter of the flock, whereas the latter was found on the slaughter line before slaughter and during slaughter of the first flock and was also frequently isolated on the first sampling day. *Salmonella* Blockley strain B1, *S.* Minnesota strain Mi1 and *S.* Montevideo strain M1 were also found on both sampling days (Table 2). Again, the crates, the feather samples and the neck skin samples were contaminated with the same strains as previously isolated from the slaughter line (Table 2).

Four flocks with a *Salmonella*-positive status were slaughtered in the week before the second sampling day. Two flocks slaughtered five and six days before the second sampling day were colonized by *S.* Typhimurium O5+ strain T1. These two flocks and the flock from which the same strain was isolated in the week before the first sampling day originated all from the same farm.

Slaughterhouse B

The first sampling day

On the first sampling day, only 4 of the 54 samples (7%) taken from the slaughter line were *Salmonella*-positive (Table 3). The *Salmonella*-negative status of the flock was confirmed by the absence of *Salmonella* in the intestines. During processing the flock, *Salmonella*-positive samples were collected from the feathers before scalding, the scalding water and five neck skin samples (Table 3). All isolates belonged to *Salmonella* Livingstone genotype L1. Two days before the first sampling day, one *Salmonella*-positive flock was slaughtered. No isolates of this flock were available.

The second sampling day

On the second sampling day, 23 of the samples (43%) taken from the slaughter equipment were *Salmonella*-positive (Table 3). Especially the scalding tank, inclusive of the scalding water was contaminated by *Salmonella*. Only two strains were isolated from the slaughterhouse equipment: *S.* Indiana strain I2 and *Salmonella* Virchow strain V1. No *Salmonella* was isolated from the intestines of the flock slaughtered first, though *S.* Indiana strain I2 was isolated from one crate. The feathers after scalding and during plucking, and 29 of the neck skin samples after plucking and 15 neck skin samples after evisceration were contaminated by *Salmonella*. The same two strains were isolated from the skin samples as from the slaughter line before processing. *Salmonella* Agona strain A3 was found on the feathers collected from the plucking machine and on the

Table 2 *Salmonella* contaminated samples on the slaughter line and during processing of the first flock in slaughterhouse A

	First sampling day			Second sampling day		
	No.	Serotype	Genotype	No.	Serotype	Genotype
Cleaned slaughter equipments						
Hanging area						
Shackles before the hanging area	–			1/1	Paratyphi B	P2
					Kentucky	K1
Wheels and conveyer belt before the hanging area	–			1/1	Paratyphi B	P2
					Blockley	B1
Shackles after the hanging area	–			1/1	Paratyphi B	P2
Wheels and conveyer belt after the hanging area	–			1/1	Paratyphi B	P2
Shackles after stunning	–			1/1	Paratyphi B	P2
Wheels and conveyer belt after stunning	–			1/1	Paratyphi B	P2
					Blockley	B1
Scalding tank						
Shackles	2/3	Typhimurium O5+	T1	1/3	Typhimurium O5+	T1
		Blockley	B1		Blockley	B1
		Paratyphi B	P1			
Wheels and conveyer belt	1/3	Typhimurium O5+	T1	3/3	Typhimurium O5+	T1
					Paratyphi B	P3
					Blockley	B1
Doors	1/3	Typhimurium O5+	T1	0/3		
Roof	1/3	Typhimurium O5+	T1	1/3	Paratyphi B	P2
Just above the water surface	1/3	Indiana	I1	1/3	Paratyphi B	P2
Plucking machine						
Shackles	1/3	Paratyphi B	P1	2/3	Paratyphi B	P2
Wheels and conveyer belt	3/3	Typhimurium O5+	T1	3/3	Typhimurium O5+	T1
		Paratyphi B	P1		Paratyphi B	P2
		Agona	A1		Blockley	B1
		Indiana	I1			
Fingers	1/3	Paratyphi B	P1	1/3	Paratyphi B	P2
Bands between fingers	3/3	Typhimurium O5+	T1	3/3	Paratyphi B	P2
		Paratyphi B	P1		Montevideo	M1′
		Agona	A2			M4
		Indiana	I1			
		Montevideo	M1			
			M2			
			M3			
Construction	1/3	Paratyphi B	P1	0/3		
Evisceration						
Neck breaker	1/1	Paratyphi B	P1	0/1	Paratyphi B	P2
Vent cutter	0/1			1/1	Blockley	B1
Scoops	1/1	Typhimurium O5+	T1	1/1	Paratyphi B	P2
					Blockley	B1
First flock						
Crates	1/6	Typhimurium O5+	T1′	2/6	Paratyphi B	P2
Feathers before scalding	3/3	Typhimurium O5+	T1	3/3	Paratyphi B	P2
		Rissen			Blockley	B1
Feathers after scalding	2/3	Typhimurium O5+	T1	3/3	Paratyphi B	P2
		Paratyphi B				
Feathers from plucking machine	3/3	Typhimurium O5+	T1	3/3	Paratyphi B	P2
		Agona	A2		Blockley	B1
		Montevideo	M1		Typhimurium O5+	T1
					Montevideo	M1
						M4
Scalding water during processing	0/6			1/6	Indiana	I1

Table 2 Continued

	First sampling day			Second sampling day		
	No.	Serotype	Genotype	No.	Serotype	Genotype
Neck skins after plucking	13/30	Typhimurium O5+ (5)*	T1	20/30	Typhimurium O5+ (3)	T1
		Paratyphi B (5)	P1		Paratyphi B (10)	P2
		Blockley (5)	B1		Blockley (5)	P3
					Montevideo (2)	B1
					Tennessee (1)	M1
Neck skins after evisceration	18/30	Typhimurium O5+ (4)	T1	17/30	Typhimurium O5+ (1)	T1
			T2		Paratyphi B (14)	P2
		Paratyphi B (6)	P1			P2′
		Blockley (1)	B1			P4
		Minnesota (10)	Mi1		Blockley (3)	B1
					Minesota (1)	Mi1
Duodenum	0/6			2/6	Paratyphi B	P5
					Typhimurium O5+	T1

*Within brackets is the number of isolates. On some occasions (see Materials and methods), more than one colony was picked from a *Salmonella*-suspected plate; therefore, the total number of isolates exceeds the number of *Salmonella*-positive samples.

neck skins after plucking, but was not isolated from the slaughter line before processing.

Two *Salmonella*-positive flocks were slaughtered eight and six days before the second sampling day. These two flocks originated from the same farm and harboured *S.* Virchow strain V2 in the intestines. On the day before the second sampling day, a flock colonized by *S.* Agona strain A3 was slaughtered.

Slaughterhouse C

No *Salmonella* was isolated on the first sampling day in slaughterhouse C. On the second sampling day, only two neck skin samples after plucking and one neck skin sample after evisceration were contaminated with *S.* Livingstone strain L2. In the week before the first sampling day, no flocks with a known positive *Salmonella* status were slaughtered. However, a few foreign flocks were slaughtered for which the status was not determined. In the week before the second sampling day, one *Salmonella*-positive flock and some flocks with an unknown status were slaughtered.

Discussion

Slaughtering broiler flocks colonized with *Salmonella* can lead to a contamination of both carcasses and slaughter line (Lillard 1990; Corry *et al.* 2002; Olsen *et al.* 2003). The cleaning and disinfection process performed after the slaughter activities is expected to remove the existing *Salmonella* contamination from the slaughter environment. In the present study however, in two slaughterhouses the slaughter equipment was found to be still contaminated when slaughter activities started. In both slaughterhouses, the slaughter equipment in the plucking and scalding area was more contaminated than in the evisceration room. This may indicate that in the evisceration room the bacterial load is lower than in the plucking and scalding area or that the cleaning and disinfection process is more effective in the evisceration room.

Two strains, *S.* Typhimurium strain T1 and *S.* Blockley strain B1, were isolated from the slaughter line in slaughterhouse A on both sampling days. Some *Salmonella*-positive flocks, all colonized with the same *S.* Typhimurium strain T1 and reared on the same farm, were slaughtered in the week before both sampling days. Slaughtering these flocks may be the source for the contamination of the slaughter equipment on both occasions. It is possible that strain B1 also re-entered the slaughterhouse, as it is a strain that circulates in Belgian flocks (unpublished data) or survived on the processing line. A flock colonized by *S.* Agona strain A3 was slaughtered in slaughterhouse B the day before the second sampling day. This strain was not recovered from the slaughter line before slaughter, although it was isolated from the feathers collected from the plucking machine and carcasses after plucking. This observation indicates that this strain may have survived the cleaning and disinfection process but was not picked up by the sampling and isolation method applied.

Slaughtering *Salmonella*-positive flocks can lead to a contamination of the slaughter line as demonstrated in slaughterhouse A. However, the *Salmonella* strains (V2 and A3) from the positive flocks slaughtered before the second sampling day did not correspond with those found on the slaughter equipment before slaughter activities started. According to Olsen *et al.* (2003), some

Table 3 *Salmonella*-contaminated samples on the slaughter line and during processing of the first flock in slaughterhouse B

	First sampling day			Second sampling day		
	No.	Serotype	Genotype	No.	Serotype	Genotype
Cleaned slaughter equipments						
Scalding tank						
Shackles	0/3			1/3	Indiana	I2
Wheels and conveyer belt	1/3	Livingstone	L1	3/3	Indiana	I2
					Virchow	V1
Doors	0/3			3/3	Indiana	I2
					Virchow	V1
Roof	0/3			1/3	Indiana	I2
					Virchow	V1
Just above the water surface	1/3	Livingstone	L1	2/3	Indiana	I2
					Virchow	V1
Scalding water	0/4			2/4	Indiana	I2
					Virchow	V1
Plucking machine						
Wheels and conveyer belt	2/3	Livingstone	L1	3/3	Indiana	I2
					Virchow	V1
Bands between fingers	0/3			2/3	Indiana	I2
					Virchow	V1
Construction	0/3			2/3	Indiana	I2
Evisceration						
Wheels and conveyer belt	0/3			1/3	Indiana	I2
Neck breaker	0/1			1/1	Indiana	I2
Scoops	0/1			1/1	Indiana	I2
Cropper	0/1			1/1	Indiana	I2
First flock						
Crates	0/6			1/6	Indiana	I2
Feathers before scalding	1/3	Livingstone	L1	0/3		
Feathers after scalding	0/3			1/3	Indiana	I2
Feathers from plucking machine	0/3			3/3	Indiana	I2
					Agona	A3
Scalding water	1/6	Livingstone	L1	3/6	Indiana	I2
Neck skins after plucking	4/30	Livingstone	L1	29/30	Indiana (24)*	I2
					Virchow (11)	V1
					Agona (4)	A3
Neck skins after evisceration	1/30	Livingstone	L1	15/30	Indiana	I2

*Within brackets is the number of isolates. On some occasions (see Materials and methods), more than one colony was picked from a *Salmonella*-suspected plate; therefore, the total number of isolates exceeds the number of *Salmonella*-positive samples.

Salmonella strains can survive up to 5 days in the slaughter environment despite the daily cleaning and disinfection procedures. The results may indicate that some *Salmonella* strains can better survive in the slaughter environment than others.

In the present study, 11% of the crates used to transport the flocks were contaminated by *Salmonella*, notwithstanding that the flocks were *Salmonella*-negative. Even in the case that the flock was infected (slaughterhouse A, second sampling day) other *Salmonella* genotypes were isolated from the crates. Different studies have shown that the cleaning and disinfection process is often inadequate in eliminating *Salmonella* from crates. In the study of Rigby *et al.* (1982), 99% of the washed and disinfected crates examined were still contaminated with *Salmonella*. More recently, *Salmonella* was isolated from 13% to 87% of disinfected crates at eight Danish poultry slaughterhouses (Olsen *et al.* 2003). According to Rigby *et al.* (1980) and Corry *et al.* (2002), more crates were contaminated by *Salmonella* after washing and disinfection than after unloading the birds. Even more, during this process, the crates may become contaminated with other *Salmonella* serotypes (Corry *et al.* 2002). Rigby *et al.* (1980) have shown that the transport of broilers in *Salmonella*-contaminated crates led to the contamination of the exterior of the birds. Therefore, the contamination of the feathers before scalding may have originated from the contaminated crates. This indicates that inadequately cleaned and disinfected crates can maintain a *Salmonella* contamination cycle during transport and slaughter.

During slaughter, *Salmonella* on the slaughter equipment can be spread out on the carcasses by the process water. This was demonstrated in the plucking machine, where most of the *Salmonella* strains found on the feathers collected during slaughtering were present on these machines before slaughter. Scalding and plucking in a contaminated environment resulted in contaminated carcasses leaving the dirty zone. The number of contaminated carcasses at this point in the slaughter process seemed to be related to the number of contaminated sampling points of the slaughter line as demonstrated on both sampling days in slaughterhouse B. The contamination of the evisceration line caused no further increase in the number of positive carcasses, even a reduction was observed on different occasions.

In slaughterhouse A, *S.* Minnesota isolated from carcasses after evisceration was not found either from the environment or at the slaughter stage before. This serotype may have originated from the second scalding and plucking line as this line was not sampled during the investigation. On both sampling days, strains belonging to this serotype were genetically undistinguishable indicating that this strain probably survived for a long time in this part of the slaughterhouse.

In conclusion, the separation in time between the slaughter of *Salmonella*-infected and noninfected broiler flocks is a good control measure to prevent cross-contamination during processing. However, two conditions have to be fulfilled. First of all, the status must be determined correctly, which is difficult as the status is determined a few weeks before slaughter. In the time span between status determination and slaughter, the birds can become (apparently) clear of infection (Heyndrickx *et al.* 2002) or the flock can acquire a new infection, e.g. during transport (Rigby and Pettit 1980). There can also be an increased rate of shedding owing to the stressful transportation to the slaughterhouse (Rigby and Pettit 1980). Second, the slaughterhouse environment must be *Salmonella*-free at the start of the day. The present study has demonstrated that contamination of the transport containers and the slaughter environment may lead to the contamination of the end product. Only the application of a daily cleaning and disinfection process which eliminates any *Salmonella* contamination can assure that the slaughter of *Salmonella*-free flocks when applying logistic slaughter will result in the absence of *Salmonella* on the carcasses after slaughter.

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