# *Salmonella* prevalence and persistence in industrialized poultry slaughterhouses

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**ABSTRACT** *Salmonella* contamination sources and transmission routes were studied in 5 Belgian poultry slaughterhouses. Samples from the slaughter and cutting line after cleaning and disinfection were collected, as well as neck skin samples and thighs during slaughter of the first flock. In total, 680 swab and water samples were taken from the slaughter line before slaughter. In all slaughterhouses, *Salmonella* was notwithstanding cleaning and disinfection still isolated from the slaughter line before start of activities. The prevalence of *Salmonella* in the plucking area was 10.4% (38/365) (hanging area: 5.0%, scalding tank: 5.8%, plucking machine: 17.0%); in the evisceration room, 1.5% (2/138); and in the cutting area, 2.0% (3/149). No *Salmonella* (0/28) was found in samples from the chilling line. On neck skin samples taken from the various lines, *Salmonella* prevalence was 16.1% (48/299) after plucking, 16.0% (48/300) after evisceration, 23.3% (70/300) after chilling; on thighs, prevalence was 10.0% (24/240). Nine *Salmonella* serotypes were identified of which *Salmonella* Infantis was the most common serovar (53.8%), especially in slaughterhouse A. Two contamination causes were identified; first, although all flocks had an official *Salmonella* negative status, this was in one case incorrect and led to an enormous contamination of the neck skins of the flock and the slaughterline (i.e., cooling water). Second, molecular typing revealed cross-contamination from flocks slaughtered 1 d before sampling. *Salmonella* was apparently not always eliminated by the cleaning and disinfection process and able to contaminate the carcasses of the first slaughtered flock. In conclusion, the results of this study provided practical insights for poultry production to further improve their *Salmonella* control, for example, *Salmonella* status determination closer to the slaughter date, to adapt cleaning and disinfection protocols especially for critical machinery and better hygienic designed equipment.

**Key words:** *Salmonella*, prevalence, persistence, poultry slaughterhouse

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## INTRODUCTION

*Salmonella* (***S.***) *enterica* subspecies *enterica* is one of the most important food-borne pathogens. It was the most frequently reported causative agent in bacterial food-borne outbreaks and caused the highest number of deaths due to food-borne illnesses in the European Union in 2017 (EFSA and ECDC, 2018). Most human cases of salmonellosis are attributed to the consumption of food contaminated with *Salmonella*; poultry meat has been identified as one of the most important sources (FAO and WHO, 2009; Jackson et al., 2013; EFSA, 2014; Bula-Rudas et al., 2015). In the EU in 2018, the top 5 most commonly reported serovars causing human salmonellosis were *Salmonella* Enteritidis, *Salmonella* Typhimurium, monophasic *Salmonella* Typhimurium, *Salmonella* Infantis, and *Salmonella* Derby (EFSA and ECDC, 2019). *Salmonella* Enteritidis was reported to be mainly associated with eggs and broiler meat, whereas *S.* Infantis was especially associated with broiler meat. As the consumption of poultry meat is increasing every year (OECD, 2019), prevention of *Salmonella* contamination in the poultry meat production chain remains very important.

As per previous studies, poultry and poultry meat can become contaminated with *Salmonella* during the entire poultry production chain, that is from the breeder farm, production farm, transportation, slaughterhouse, and retail (Hue et al., 2011; Marin et al., 2011; Choi et al.,



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2014; Bai et al., 2015; Panzenhagen et al., 2016; Ramírez-Hernández et al., 2017; Zhu et al., 2017; Hardie et al., 2019; Shang et al., 2019). In the studies of Corry et al. (2002), Olsen et al. (2003), Heyndrickx et al. (2007), and Rasschaert et al. (2007), the slaughterhouse has been identified as a potential source for *Salmonella* contamination of poultry meat. During slaughter, carcasses may become contaminated by bacteria found in the intestinal content of the animals, either from within the flock or in previously slaughtered flocks. Such cross-contamination can occur via slaughter equipment, transport crates, and water (Rouger et al., 2017). Steps such as scalding, plucking, and evisceration could increase the chance of contamination and cross-contamination (FAO and WHO, 2009).

To control and eradicate *Salmonella* in slaughterhouses, it is crucial to gather detailed information about the contamination within the slaughterhouse, more specifically the main sources or routes of contamination during slaughter (Heyndrickx et al., 2007; Marin et al., 2011).

To minimize the chance of cross-contamination from a *Salmonella* positive flock to a *Salmonella* negative flock, logistic slaughter is implemented in several EU countries. This is dependent on determination of the *Salmonella* status of the flocks before slaughter. Flocks with a *Salmonella*-free status are slaughtered first, followed by flocks with a positive status. Logistic slaughter may be only effective when the slaughter line and equipment before starting the slaughter activities is actually free of *Salmonella* after effective cleaning and disinfection.

The goal of the study was to investigate the degree of contamination and possible contamination source and transmission routes for *Salmonella* in broiler and spent hen poultry slaughterhouses in Belgium. Despite published results of similar studies performed in Belgium and other industrialized countries (Corry et al., 2002; Olsen et al., 2003; Rasschaert et al., 2007) specifically for broilers, *Salmonella* contamination problems are still present. The need to evaluate the progress/status after more than 10 yr is clear and evaluation has been requested by Belgian slaughterhouses. Studies concerning the *Salmonella* contamination in spent hen slaughterhouses are lacking. This is the first detailed investigation of *Salmonella* in spent hen slaughterhouses.

## MATERIALS AND METHODS

### Poultry Slaughterhouses and Cutting Plants

This study was conducted in 5 Belgian poultry slaughterhouses: 3 broiler slaughterhouses (A-C) and 2 spent hen slaughterhouses (D-E). Sampling was conducted at each slaughterhouse twice in the period from December 2017 to June 2018 with a minimum interval of 1 mo between both visits. All slaughterhouses were sampled on Tuesday or Wednesday to ensure that at least one processing day was completed after the weekend.

Plants A, B, C, D, and E were industrialized slaughterhouses with a capacity of between 8,000 and 14,000 birds per hour. Slaughter procedures were similar in all 5 slaughterhouses, except that a different chilling system was used for broilers and spent hens. The procedure began with birds being hung manually on the slaughter line. Birds were either stunned with $CO_2$ before hanging or electrically stunned after hanging. In a separate area, the bled carcasses were scalded in water baths with counter flow at a temperature of approximately 52°C for the broilers and 60°C for the spent hens before they were defeathered by a series of plucking machines. Finally, mechanical evisceration took place in evisceration room by a series of devices: the vent was opened, and intestines were removed. After final washing, broiler carcasses were air-chilled for 1.5-2 h, whereas spent hen carcasses were water chilled. Afterward, the carcasses were mechanically cut.

### Sampling

The sampling protocol applied in 5 poultry slaughterhouses was similar, with only minor differences in sampling sites depending on the equipment present just before the start of slaughter activities. After cleaning and disinfection and before start of slaughter, the slaughter line was sampled with cotton swabs (a 4 cm diameter ball made from medical cotton and handled with a sterile metal forceps) premoistened with maximum recovery diluent (**MRD**) (Oxoid, Basingstoke, UK). Before sampling, the slaughter line ran empty for approximately 10 min. An overview of the sampling protocol is shown in Table 1. Briefly, samples were taken from shackles, wheels, and transport rail, scalding tanks, plucking machines, all equipment from the evisceration line, chilling tank (slaughterhouses D and E) and all machines and conveyer belts from the cutting line.

Water samples (25 mL) from scalding tank were collected before slaughter, after 30 min and after 60 min of slaughter in all slaughterhouses and from chilling tank in the spent hen slaughterhouses.

During slaughter of the first flock, samples were taken from transport crates used to transport the flock (before cleaning and disinfection); intestines; exterior of the birds before and after scalding; neck skins after plucking, evisceration and chilling; and thighs after cutting. Twenty-four crates from transport containers used to transport the corresponding flock were sampled with sponge swabs (Sponge-Stick, 3M, Diegem, Belgium) moistened with 10 mL of MRD just before the emptied crates were washed and disinfected. Breast feathers of the first flock were swabbed with sponge swabs before and after scalding at 3 times: at the start of slaughter, after 30 min and after 60 min of slaughter. Before scalding samples were taken with swabs premoistened with 10 mL of sterile MRD, while after scalding dry swabs were used. For each sample, 5 birds were swabbed with one sponge swab. Furthermore, at the abovementioned sampling times, 25 g of feathers were collected from plucking machine.

**Table 1.** Overview of the sampling protocol in 5 slaughterhouses before and during the slaughter of the first flock.

| Samples | No. of samples | Samples | No. of samples |
|---|---|---|---|
| Before slaughter of the first flock | | | |
| Hanging area | | Evisceration line cont. | |
| Three shackles before the hanging area | 1 | Evisceration machine | 1 |
| Two wheels and 25 cm transport trail before the hanging area | 1 | Head puller—1 element | 1 |
| Three shackles after the hanging area | 1 | Crop puller—1 element | 1 |
| Two wheels and 25 cm transport rail after the hanging area | 1 | Neck breaker[1]—1 element | 1 |
| Three shackles after stunning | 1 | Neck skin cutter[1]—1 element | 1 |
| Two wheels and 25 cm transport rail after stunning | 1 | Lung remover[1]—1 element | 1 |
| Scalding tank | | Inside and outside bird washer—1 element | 1 |
| Three shackles | 3 | Cooling line[1] | |
| Two wheels and 25 cm transport rail | 3 | Construction of the chilling baths | 3 |
| Doors (400 $cm^2$)[1] | 3 | Water of the chilling baths | 4 |
| Roof (400 $cm^2$)[1] | 3 | Cutting line[1] | |
| Side surface just above the water level (100 $cm^2$) | 3 | Three shackles of line | 3 |
| 25 ml scalding water | 4 | Two wheels and 25 cm of transport rail | 3 |
| Plucking machine | | Knives to cut tail | 1 |
| Three shackles | 3 | Knives to cut wings | 1-3 |
| Two wheels and 25 cm transport rail | 3 | Knives to cut back | 1 |
| Plucking fingers—1 element (including behind the disc) | 3 | Knives to cut breast | 1 |
| Plastic bands between fingers (400 $cm^2$) | 3 | Knives to cut piece of back of the thigh | 1 |
| Construction (400 $cm^2$) | 3 | Knives to cleave the thighs in two | 1 |
| Evisceration line | | Conveyer belts for transport of wings | 2 |
| Three shackles | 3 | Conveyer belts for transport of breasts | 2 |
| Two wheels and 25 cm transport rail | 3 | Conveyer belts for transport of thighs | 2 |
| Rectum bore—1 element | 1 | Conveyer belts where the whole carcasses are sorted in accordance with weight | 2 |
| Vent cutter—1 element | 1 | | |
| During slaughter of the first flock | | | |
| Four transport crates after use # (4 × 400 $cm^2$) | 6 | Neck skins after evisceration (25g) | 30 |
| Swabs of feathers before scalding | 3 | Pooled sample of 10 duodena | 6 |
| Swabs of feathers after scalding | 3 | Pooled sample of 10 ceca | 6 |
| Feathers from the plucking machine (25g) | 3 | 25 ml of chilling water[1] | 6 |
| 25 ml scalding water | 6 | Neck skins after chilling (25g) | 30 |
| Neck skins after plucking (25g) | 30 | Thighs (25 g skin)[1] | 30 |

[1]If present; #: In total 24 transport crates were sampled; 4 swab samples were pooled together to obtain 6 samples in the laboratory.

During each sampling day, 90 pooled neck skin samples were collected: 30 immediately after plucking, 30 after evisceration, and 30 after chilling. Each pooled sample contained neck skins from different carcasses (approx. 5) to obtain a sample of at least 25 g. During cutting of the first flock, 30 thigh samples were collected.

Logistic slaughter was applied in all slaughterhouses. All sampled flocks had an official negative *Salmonella* status. To check this status at the moment of slaughter, 60 whole gastrointestinal tracts samples from each flock were collected just after evisceration. All samples were transported to laboratory under cooled condition and processed the same day.

## *Salmonella* Analysis

Per cotton swab sample, 40 mL of buffered peptone water (BPW) (Oxoid, Basingstoke, UK) was added. In total, 90 mL of BPW was added to each sponge swab from transport crates. Before incubation, these 24 samples were pooled into 6 samples. To 25 g of each feather, neck skin and thigh skin sample 225 mL of BPW was added. From each of the 60 gastrointestinal tracts, 1 g of duodenum content and 1g of cecal content were collected aseptically. Ten samples were pooled to create 6 pooled samples of 10 g cecal content and 6 pooled samples of 10 g duodenal content. To each of these pooled samples, 90 mL of BPW was added. In total, 25 mL of double-strength BPW was mixed with 25 mL of each water sample.

Bacterial isolation was performed based on ISO 6579-1:2017. In summary, after homogenizing in a stomacher blender at normal speed for 1 min, samples were pre-enriched in BPW at 37°C for 16-20 h. Then 0.1 mL of each enriched broth was transferred to 3 spots on Modified Semi-solid Rappaport-Vassiliadis (Oxoid, Basingstoke, UK) plates and incubated at 41.5 ± 0.5°C for 24 and 48 h. After 24 h of incubation, a loopful of migration zone of suspect Modified Semi-solid Rappaport-Vassiliadis plates were transferred to Xylose Lysine Deoxycholate (Oxoid, Basingstoke, UK) agar plates for further detection and isolation while negative plates were incubated for an additional 48 h, followed by the same procedure. From each Xylose Lysine Deoxycholate plate, maximum 2 suspect colonies were further purified and stored at −80°C for further confirmation and typing.

**Table 2.** The number of infected samples, serotype, and pulsotype of *Salmonella* in broiler slaughterhouses A, B, and C.

| Slaughterhouse | A | | | | B | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st sampling day | | 2nd sampling day | | 1st sampling day | | 2nd sampling day | | 1st sampling day | | 2nd sampling day | |
| Samples[a] | No. | Serotype (N)—pulsotype | No. | Serotype (N)—pulsotype | No. | Serotype—pulsotype | No. | Serotype—pulsotype | No. | Serotype—pulsotype | No. | Serotype (N)—pulsotype |
| Before slaughter | | | | | | | | | | | | |
| Hanging area | | | | | | | | | | | | |
| Three shackles before the hanging area | 1/1 | Infantis—F | | | | | | | | | | |
| Two wheels and 25 cm transport rail before the hanging area | 1/1 | Infantis—F | | | | | | | | | | |
| Two wheels and 25 cm transport rail after stunning | | | 1/1 | Infantis—F | | | | | | | | |
| Scalding tank | | | | | | | | | | | | |
| Three shackles | | | 1/3 | Infantis—F | | | | | | | 2/3 | Infantis—F'[1] |
| Two wheels and 25 cm transport rail | 2/4 | Infantis—F | 3/3 | Infantis—F | | | | | | | | |
| Plucking machine | | | | | | | | | | | | |
| Three shackles | 1/3 | Infantis—F | 1/3 | Infantis—F | | | | | | | 1/3 | Infantis—F′ |
| Two wheels and 25 cm transport rail | 1/3 | Paratyphi B var Java—E | 3/3 | Infantis (2)—F Paratyphi B var Java (1)—E | | | | | | | | |
| Plucking fingers −1 element | | | 2/3 | Infantis—F | 1/3 | Minnesota—B | 1/3 | Rissen—P | | | 2/3 | Infantis—F′ |
| Plastic bands between fingers (400 $cm^2$) | | | 3/3 | Infantis—F | | | 1/3 | Typhimurium—V'[1] | | | 1/3 | Infantis—F′ |
| Construction (400 $cm^2$) | | | 1/3 | Infantis—F | 1/3 | Typhimurium—V | | | | | 1/3 | Infantis—F′ |
| Evisceration line | | | | | | | | | | | | |
| Head puller | | | 1/1 | Infantis—F | | | | | | | | |
| Lung remover—1 element | | | | | | | | | | | 1/1 | Infantis—F′ |
| Cutting line | | | | | | | | | | | | |
| Two wheels and 25 cm of transport rail | | | 1/3 | Livingstone—C | | | | | | | | |
| Knife to cleave the thighs in two | | | 1/1 | Paratyphi B var Java—E | | | | | | | | |
| Samples during slaughter of the first flock | | | | | | | | | | | | |
| Four crates of 6 transport container after use (4 × 400 $cm^2$) | | | 2/6 | Infantis—F | | | | | 2/6 | Infantis—F | 1/6 | Infantis—I |
| Swabs of feathers before scalding | 1/3 | Infantis—F | 2/3 | Infantis—F | | | | | | | 2/3 | Infantis—I/H |
| Swabs of feathers after scalding | | | | | | | | | | | 2/3 | Infantis—I |
| Feathers from the plucking machine (25g) | 2/3 | Infantis—F | 3/3 | Livingstone—C | | | | | 1/3 | Infantis—I | 3/3 | Infantis—I |
| 25 ml scalding water | | | 5/6 | Livingstone—C | 2/6 | Typhimurium—V | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Neck skins after plucking (25g) | 5/30 | Infantis (1)—F′<br>Paratyphi B var Java (4)—E | 25/29 | Infantis (21)—F<br>Livingstone (6)—C | 1/30 | Rissen—P | | 9/30 | Infantis—I/F′ |
| Neck skins after evisceration (25g) | 5/30 | Infantis (4)—F<br>Paratyphi B var Java (2)—E | 23/30 | Infantis(13)—F<br>Livingstone (10)—C | | | | 6/30 | Infantis—I |
| Neck skins after chilling (25g) | 7/30 | Infantis (6)—F<br>Paratyphi B var Java (2)—E | 27/30 | Infantis (20)—F<br>Paratyphi B var Java (1)—E<br>Livingstone (6)—C | 1/30 | Infantis—F°[2] | 6/30 | Infantis—I/F | |
| Thighs (25 g skin) | 1/30 | Paratyphi B var Java—E | 20/30 | Infantis(17)—F<br>Livingstone (4)—C | | | 3/30 | Infantis (2)—I<br>Enteritidis (1)—M′[1] | |

N: a: An overview of all samples collected can be seen in Table 1. Only samples that were *Salmonella* positive on one of the sampling occasions, are shown here.
[1]The symbol "′" after the letter means that up to 2 bands were different between 2 isolates when digested with *Xba*I.
[2]the symbol "°" after the letter means that the fingerprints were the same when digested with *Xba*I but a difference of one band was noticed when digested with *Not*I.

All collected *Salmonella* isolates were confirmed at genus level by multiplex polymerase chain reaction (**PCR**) using primers described by Aabo et al. (1993). To identify isolates belonging to *S.* Typhimurium serotype the primers described by Lin et al. (1999) were used.

### Characterization of Salmonella Isolates

The non-Typhimurium *Salmonella* isolates were characterized by repetitive element palindromic-polymerase chain reaction (**rep-PCR**) using the ERIC primers set to limit the number of isolates that had to be serotyped. As described by Rasschaert et al. (2005), isolates belonging to the same cluster are all of the same serotype. At least one isolate per cluster was subsequently serotyped by the Belgian *Salmonella* reference laboratory (Sciensano, Brussels, Belgium). Based on the rep-PCR results, for each slaughterhouse per positive sample type, 1 to 5 isolates per cluster were selected randomly for characterization by pulsed-field gel electrophoresis in accordance with the protocol of PulseNet (CDC PulseNet, 2017). Enzyme digestion was performed with 50 U *Xba*I (New England Biolabs, Hitchin, UK) and 30 U *Not*I (Thermo Fisher Scientific, MA). Electrophoresis was performed in a Chef Mapper (Bio-Rad, CA). The obtained bands were analyzed with BioNumerics software V.7.6 (Applied Maths, Sint-Martens-Latem, Belgium) using Pearson correlation with 2% optimization. Pulsotypes were assigned to clusters based on visual comparison. Each cluster based on the fingerprints obtained with both restriction enzymes was assigned a letter. The symbol ' after the letter means that up to 2 bands were different between 2 isolates when digested with *Xba*I. The symbol ° after the letter means that the fingerprint was the same when digested with *Xba*I but a difference of one band was noticed when digested with *Not*I.

At each visit, slaughterhouse staff were asked if *Salmonella*-positive flocks—based on the official *Salmonella* status papers that are mandatory for each flock—had been slaughtered in the days before sampling. If so, those *Salmonella* isolates were obtained from laboratories that determined the *Salmonella* status. These isolates were also characterized by rep-PCR and pulsed-field gel electrophoresis as described above.

## RESULTS

In total, 43 of the 680 samples taken from the cleaned and disinfected slaughter line were *Salmonella* positive: 17.6% (24/136) in slaughterhouse A, 3.0% (4/134) in slaughterhouse B, 6.2% (8/130) in slaughterhouse C, 2.1% (3/142) in slaughterhouse D, and 2.9% (4/138) in slaughterhouse E. During slaughter of the first flock, 70 of 300 neck skin samples after chilling and 24 of 240 thigh samples were *Salmonella* positive: 56.7% (34/60) for neck skin samples and 35.0% for thigh samples (21/60) in slaughterhouse A; 11.7% (7/60) for neck skin samples and 5.0% for thigh samples (3/60) in slaughterhouse C; 48.3% (29/60) for neck skin samples

**Table 3.** The number of infected samples, serotype, and pulsotype of *Salmonella* in spent hen slaughterhouses.

| Slaughterhouse | D | | | | E | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st sampling day | | 2nd sampling day | | 1st sampling day | | 2nd sampling day | |
| Samples[a] | N | Serotype—pulsotype | N | Serotype—pulsotype | N | Serotype—pulsotype | N | Serotype—pulsotype |
| Before slaughter | | | | | | | | |
| Scalding tank | | | | | | | | |
| Two wheels and 25 cm transport rail | | | 1/3 | Enteritidis—K′[1] | | | | |
| Plucking machine | | | | | | | | |
| Plucking fingers—1 element (including behind the disc) | 2/3 | Tennessee— J | | | | | 1/3 | Livingstone—O |
| Plastic bands between fingers (400 $cm^2$) | | | | | 1/3 | Mbandaka— A | | |
| Construction (400 $cm^2$) | | | | | | | 1/3 | Livingstone— O |
| Cooling line | | | | | | | | |
| Two wheels and 25 cm transport rail | | | | | | | 1/3 | Livingstone - O |
| Samples during slaughter of the first flock | | | | | | | | |
| 24 transport containers after use (4 × 400 $cm^2$) | | | | | 2/6 | Enteritidis—M | | |
| Swabs of feathers before scalding | | | | | 1/3 | Enteritidis—M | | |
| Feathers from the plucking machine (25g) | 1/3 | Tennessee—J | | | 2/3 | Enteritidis—M | | |
| Neck skins after plucking (25g) | | | | | 4/30 | Enteritidis—M | 4/30 | Livingstone—O |
| Neck skins after evisceration (25g) | 1/30 | Enteritidis— K | | | 9/30 | Enteritidis—M | 4/30 | Livingstone—O |
| Pooled sample of 10 ceca | | | | | 2/6 | Enteritidis—M | | |
| 25 mL of chilling water | | | | | 4/6 | Enteritidis—M | | |
| Neck skins after chilling (25g) | | | | | 29/30 | Enteritidis—M | | |
| Thighs (25 g skin) | | | | | NA | | NA | |

a: An overview of all samples collected can be seen in Table 1. Only samples that were *Salmonella* positive on one of the sampling occasions are shown here.
[1]The symbol ' after the letter means that up to 2 bands were different between 2 isolates when digested with *Xba*I; NA: thigh samples were not available.

in slaughterhouse E, and no *Salmonella* was found from the neck skin samples after chilling and thigh samples in slaughterhouses B and D.

All flocks slaughtered first had an official *Salmonella*-negative status. This was confirmed by our *Salmonella* analyses on samples taken from the ceca and duodenum except for the second sampling day in slaughterhouse E. That flock was colonized with *S.* Enteritidis pulsotype M.

## Slaughterhouse A

One day before the first sampling day, *S.* Infantis pulsotype F and *Salmonella* Rissen pulsotype P positive flocks were slaughtered in slaughterhouse A.

Before slaughter activities, 6 of 68 samples (8.8%) from the cleaned and disinfected slaughter line were *Salmonella* positive (Table 2). Positive samples were all collected in the first part of the slaughter line: from shackles and wheels in the hanging area, and from the shackles and wheels in the scalding tank and plucking machine. The same *S.* Infantis pulsotype F as from the positive flock slaughtered 1 d before sampling was recovered from this line. *Salmonella* Paratyphi B var Java pulsotype E was isolated as well.

During slaughter of the first flock, 21 of 153 samples (13.7%) were *Salmonella* positive. *Salmonella* Infantis pulsotype F was isolated from the feathers before scalding, feathers collected from the plucking machine and neck skins after evisceration and after chilling. In addition, *S.* Paratyphi B pulsotype E was detected on the carcasses and thighs collected during slaughter and cutting as well.

Two flocks with a *Salmonella*-positive status were slaughtered on the day before the second sampling day: one flock was colonized with *S.* Infantis pulsotype F and another flock colonized with *S.* Coeln pulsotype D. On the second sampling day, before starting slaughter activities, 18 of 68 samples (26.5%) from the cleaned and disinfected equipment were found *Salmonella* positive. *Salmonella* was detected at all stages of the slaughter line. Shackles and wheels (10 samples) were found to be the most contaminated transport parts of the slaughter line, while the plucking machine (10 samples) was the most contaminated processing machine. The head puller was also found to be contaminated. In the cutting line, wheels from the transport rail and the knife used to cleave thighs were contaminated with *Salmonella*. All these isolates were identified as *S.* Infantis strain F positive except one isolate identified as *S.* Paratyphi B pulsotype E were recovered from wheels and transport rail in the plucking area and the knife used to cleave thighs.

During slaughter of the first flock, up to 70.4% (107/152) of the samples were found to be *Salmonella* positive. *Salmonella* Infantis pulsotype F was recovered from used transport containers and feathers before scalding, but also from neck skins after scalding, after evisceration, after chilling and from the thighs. *Salmonella* Livingstone pulsotype C was isolated from scalding water, feathers collected during plucking, and neck skins and thighs on all sampled places. In addition, *S.* Paratyphi B pulsotype E, which was also isolated from the cleaned and disinfected slaughter and cutting line, was also isolated from one carcass after chilling.

## Slaughterhouse B

On the day before both sampling days, only flocks with official *Salmonella* free status were slaughtered. On the first sampling day, *S.* Minnesota pulsotype B and *S.* Typhimurium pulsotype V were recovered from the plucking machine before slaughter (Table 2). During processing the first flock, *S.* Typhimurium pulsotype V was also isolated from scalding water.

On the second sampling day, the plucking machine was contaminated with *Salmonella* before onset of the activities. *Salmonella* Rissen pulsotype P was isolated from plucking fingers and *S.* Typhimurium pulsotype V′ was found on the plastic bands of the equipment. During the slaughter of the first flock, again, *S.* Rissen pulsotype P was isolated from one neck skin sample after plucking. This *S.* Rissen pulsotype P was the same as isolated from the positive flock slaughtered 1 d before sampling in slaughterhouse A.

## Slaughterhouse C

On the day before both sampling occasions, only flocks with an official *Salmonella*-free status were slaughtered. No *Salmonella* was isolated from the cleaned and disinfected slaughter line before slaughter on the first sampling day (Table 2). During the slaughter of the first flock, *S.* Infantis pulsotype F was isolated from the transport crates. A different *S.* Infantis strain (pulsotype I) was isolated from the feathers collected during plucking. Also, one neck skin sample collected after chilling was contaminated with *S.* Infantis F°.

On the second sampling day, 8 of 65 samples (12.3%) from the cleaned and disinfected slaughter line were found to be *Salmonella* contaminated: 2 samples from shackles in the scalding tank, 5 samples from the plucking machine and one sample from the evisceration line. They were all typed as *S.* Infantis pulsotype F'. During slaughtering the first flock, 32 of 153 samples (20.9%) were found to be *Salmonella* positive: transport crates; feathers; and neck skins after plucking, evisceration, and chilling; and the thighs. *Salmonella* Infantis pulsotype I was most frequently isolated. In addition, *S.* Infantis pulsotype H was isolated from feathers before scalding, *S.* Infantis pulsotype F' (same as before slaughter) was isolated from neck skins after plucking and *S.* Infantis pulsotype F from neck skins after chilling. The *S.* Infantis pulsotype F and F′ strains found in slaughterhouse C were the same as strains isolated in slaughterhouse A.

## Slaughterhouse D

The day before the first sampling day, a flock with a *S.* Tennessee pulsotype J positive status was slaughtered.

The same strain was isolated from cleaned and disinfected plucking fingers (Table 3). During processing, this strain was also isolated from feathers collected during plucking. In addition, *S.* Enteritidis pulsotype K was isolated from one neck skin sample after evisceration.

On the second sampling day, only one sample of 230 collected samples (0.4%); the wheels and transport rail in the scalding tank, was contaminated with *S.* Enteritidis pulsotype K′ before slaughter. During the slaughter of the first flock, no collected samples were *Salmonella* positive.

### *Slaughterhouse E*

Before onset of the slaughter activities, *S.* Mbandaka pulsotype A was isolated from the plastic bands in the plucking machine. *Salmonella* Enteritidis pulsotype M was isolated from the ceca of the first slaughtered flock, although this flock had an official *Salmonella*-negative status (Table 3). During the slaughter of this flock, 53 of the 129 samples (41.1%) were *Salmonella* positive. *Salmonella* was isolated from transport containers, feathers before scalding and feathers from the plucking machine, and also from neck skins after plucking, after evisceration, after chilling and from the chilling water. They were all contaminated with the same Enteritidis pulsotype M as isolated from the cecal samples.

On the second sampling day, 3 of 69 samples (5.0%) before slaughter were contaminated with *S.* Livingstone pulsotype O; 2 types of samples from the plucking machine and one sample from the water-chilling tank. During slaughter, 8 of 129 samples (6.2%) were contaminated with the same *Salmonella* pulsotype: 4 neck skin samples after plucking and 4 neck skin samples after evisceration.

## DISCUSSION

In total 43 of 680 (6.3%) samples from the cleaned and disinfected slaughter lines were *Salmonella* positive before slaughter started. Positive samples before slaughter ranged from 0 to 26.5% per sampling occasion and between 2.1 and 17.7% per slaughterhouse. Compared with a previous study in Belgian broiler slaughterhouses 15 yr ago (Rasschaert et al., 2007), the *Salmonella* contamination rate decreased clearly. In 2005, at average 21% of the samples were contaminated with *Salmonella* before onset of the activities with a maximum of 43%. A potential reason of this decrease may be the evolution of the cleaning and disinfection strategies. In the study of Rasschaert et al. (2007), cleaning and disinfection was most often performed by the slaughterhouse staff while in the present study thoroughly cleaning and disinfection was performed by external professional cleaning firms in all slaughterhouses. Other possible reasons are a lower infection pressure from the primary production, increased food safety awareness, use of more hygienic designed slaughter equipment, and better adaption of slaughter equipment to the size of the animals as this led to a less contaminated line before cleaning and disinfection. For example, broken gastrointestinal tracts were rarely observed during the present study.

The area of hanging, scalding and plucking (10.4%) was the most contaminated zone before commencement of slaughter. The plucking machine (17.3%) was the most contaminated equipment with *Salmonella* contamination rates ranging between 6.7 and 40.0% between slaughterhouses. Specifically, the discs with plucking fingers were identified as the most contaminated parts of the plucking machine (30.0%). In other studies, the plucking machine was also identified as one of the most contaminated equipment in broiler slaughterhouses (Olsen et al., 2003; Rasschaert et al., 2007). The shape of the fingers not only ensures that they are difficult to clean, but as the plucking fingers wear out their surface becomes rougher which allows bacteria to colonize cracks in the surface (Fries, 2002). The zone behind the discs on which the fingers are mounted is also difficult to clean. The slaughterhouse staff is aware of this issue and despite extra cleaning on these places, plucking fingers remains a very critical point. Further improvements by poultry processing constructors on the hygienic design of the plucking machine are needed.

If cleaning and disinfection is not able to eliminate *Salmonella*, the pathogen can be transferred to carcasses via the equipment the next slaughter day (Fries, 2002). Indeed, on all but one sampling day, the first flock slaughtered became contaminated with *Salmonella* during slaughter. In eight of ten cases, the carcasses (neck skins) were found to be contaminated after plucking, after evisceration or chilling. Similar results were found in the study of Rasschaert et al. (2007), where the carcasses (neck skins) were highly, more than in the present study, contaminated with *Salmonella* after plucking and evisceration in two of three of the investigated broiler slaughterhouses. In the study of Olsen et al. (2003), results also showed that the same *Salmonella* Virchow strain found from neck skins before packaging was also isolated from the slaughter line, and the cleaning procedure in place did not remove all *Salmonella* from the contaminated areas.

In 7 cases, the same *Salmonella* strains were isolated from carcasses of the first slaughtered flock as previously isolated from the cleaned and disinfected line or used crates. This clearly shows that contaminated slaughter material can lead to contamination of carcasses from flocks with an official *Salmonella*-free status. Samples of the end product (chilled carcasses or thighs) revealed that 5 of the ten tested flocks were contaminated with *Salmonella*, although to a lesser extent.

Contamination of carcasses during the slaughter of the flock on the second sampling day in slaughterhouse E was caused due to an incorrect *Salmonella* official status or a changed status between sampling and slaughter. The impact of this incorrect status was enormous as 29 of 30 cooled neck skin samples were contaminated with *S.* Enteritidis. This is in important issue, which was also observed in the previous Belgian study (Rasschaert et al., 2007). When a *Salmonella* colonized flock with an official *Salmonella*-negative status is

slaughtered before *Salmonella*-negative flocks, this leads to contamination of the slaughterline, for example, cooling water as demonstrated in this case, and following carcasses. This hampers the principle of logistic slaughter. At the moment, the official Belgian *Salmonella* status is determined two-three weeks before slaughter. This time interval is needed to determine the *Salmonella* status and to identify the *Salmonella* serovar. This indicates that the *Salmonella* status was either changed after the official *Salmonella* control or the *Salmonella* analysis yielded a false negative result. It is known that the *Salmonella* status may change during the further growth or production of the hens because of the catching, loading, and transport pressure (Mulder, 1995). Therefore, it is recommended to focus on this issue in future research and to determine the *Salmonella* status closer to the slaughter age. This should be possible as more recently faster techniques for *Salmonella* determination and serovar identification became available. These should be validated and implemented in the official regulation.

When *Salmonella*-positive flocks are slaughtered the day before, it seems difficult to successfully clean and disinfect the line, particularly certain serovars are difficult to eradicate. This was demonstrated in slaughterhouses A and D, where after cleaning and disinfection, the same strain of the *Salmonella*-positive flock slaughtered the day before was still recovered from the slaughter line and the carcasses of the first slaughtered flock the next day. However, after the slaughter of a *Salmonella*-positive flock, most slaughterhouses communicate this to the cleaning and disinfection staff to clean even more thoroughly and take additional samples after the cleaning and disinfection process to look for the presence of *Salmonella*. In personal communication, they said that in most cases, cleaning and disinfection is sufficient to remove *Salmonella* during cleaning and disinfection with the exception of the serotypes *S.* Infantis and *S.* Paratyphi B var Java. These serovars can be considered to be highly persistent. This was confirmed in our study with the recovery of these 2 genotypes. *S.* Paratyphi B var Java had been reported to be persistent in the broiler supply chain, and it seems that hygiene measures are less efficient against this serovar (Dorn et al., 2001; van Pelt et al., 2003; van de Giessen et al., 2006; Van Asselt et al., 2009). The last decade, *S.* Infantis clones have successfully emerged in the broiler production chain worldwide (Asai et al., 2007; Nógrády et al., 2008, 2012; Pate et al., 2019; Vinueza-Burgos et al., 2019). This clone seems difficult to eradicate at primary level and thus also at slaughterhouse level. In 2018, *S.* Infantis was the most reported serovar in poultry in the EU; both from broilers (36.5% of all serotyped isolates) and broiler meat (56.7% of all serotyped isolates) (EFSA and ECDC, 2019).

Cleaning and disinfection of transport crates is important for *Salmonella* control in the poultry chain in the EU. They are cleaned and disinfected after each use. The used crates were sampled for 2 reasons; first, to assess to *Salmonella* status of the transported flock and second, to assess if the crates harbored other *Salmonella* strains not present in the transported flock to evaluate the cleaning and disinfection process of the crates. Besides the flock studied on the first sampling day in slaughterhouse E, all flocks were *Salmonella* free as confirmed by the samples of the gastrointestinal tract. Nevertheless, *S.* Infantis was isolated from the crates in slaughterhouse A on the second sampling day and on both sampling days in slaughterhouse C. In addition, on the first sampling day of slaughterhouse C, the slaughter line was free from *Salmonella* before slaughter but even so, feathers and carcasses of the first slaughtered flock were contaminated with the same *S.* Infantis strain as isolated from the crates. This indicates that the crates may be an additional contamination source and may contaminate the birds before slaughter. If crates are inadequately cleaned and disinfected, this may even cause cross-contamination back to farm level (Rigby et al., 1980; Corry et al., 2002).

In summary, notwithstanding the dedicated efforts of the sector to control *Salmonella* contamination, this bacterium is still difficult to control in the slaughterhouses. Clearly, cleaning and disinfection of equipment and environment is challenging with the plucking machine as most critical point. Further optimization of cleaning and disinfection protocols and a more hygienic equipment design are needed, as cross-contamination can occur. In addition, the study shows that an accurate *Salmonella* status of each flock is needed to prevent contamination of the poultry carcasses. The individual results and sharing of results and experiences among the slaughterhouses provided also useful insights for each individual slaughterhouse resulting in a further improvement of their *Salmonella* control.

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## DISCLOSURES

None.

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