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Routes for salmonella contamination of poultry meat: epidemiological study from hatchery to slaughterhouse

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SUMMARY

Data were collected on the prevalence of salmonella at different stages during the life cycle of 18 broiler flocks on different farms as well as during slaughter in different poultry slaughterhouses. For the isolation of salmonella, the highest sensitivity (93·9%) was obtained by enrichment in the semi-solid agar Diasalm. The 'overshoe method' utilizing several pairs of overshoes provided the highest sensitivity for determining the salmonella status of the broilers during rearing. A clear decrease of the relative importance of the first production stages was demonstrated for the salmonella contamination of the end product, whereas horizontal transmission of salmonella to broilers during rearing and to broiler carcasses in the slaughterhouse was shown to be the main determinative factor. Ten of the 18 flocks received a salmonella positive status with the highest shedding occurring during the first 2 weeks of rearing. The shedding of the animals was significantly negatively influenced by the use of subtherapeutic or therapeutic doses of antibiotics. The intake of portable material in the broiler house was identified as the most important risk factor for horizontal transmission. Significant associations were found between the contamination level of a flock and hygiene of the broiler house, feed and water in the broiler house and both animal and non-animal material sampled in the environment. No correlation was found between contamination during the rearing period and contamination found after slaughtering. The presence of faecal material in the transport crates and predominantly the identity of the slaughterhouse seemed to be the determining factors for carcass quality. Improved hygiene management during transport of broilers and in some slaughterhouses could significantly reduce the risk of salmonella contamination of poultry meat.

INTRODUCTION

Salmonella is one of the major foodborne causes of gastroenteritis and is frequently associated with contaminated poultry meat [1]. Contamination of poultry or poultry meat may occur throughout the whole production chain and important risk factors for contamination at each stage of this process have been identified. For implementation of an efficient and cost-effective control programme in Belgium, quantification of the relative contributory effect of these risk

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factors on contamination during the poultry production process is necessary. The vertical transmission of *S*. Enteritidis and *S*. Typhimurium from the parent flock to the day-old chicken leaving the hatchery has often been reported and has been implemented as a main controlling factor in many eradication programmes [2–4].

Horizontal transmission in hatcheries and on the farm during the rearing period is, in certain cases, of greater importance and leads to the isolation of a greater variety of salmonella serovars [4, 5]. A number of risk factors for horizontal transmission have been identified and include inadequate cleaning and disinfection of broiler rearing houses leading to contamination of the following flock [6–10], a poor level of hygiene [11] and contamination of feed [12]. Other factors are: the size of the farm [13], rearing of flocks in the autumn [13] and the presence of litter-beetle in the house and rodents on the farm [14, 15]. Contamination of salmonella negative flocks during transport to and processing at the slaughter plant has been observed with contaminated crates and plant contamination as apparent sources [16–18].

It is clear from the literature that the contribution of different risk factors on contamination with salmonella has changed over time and differs according to the geographical location of poultry houses. This article describes an updated quantitative epidemiological study of risk factors contributing to poultry meat contamination in Belgium. For this purpose, 18 individual broiler flocks were intensively studied from hatchery to slaughterhouse.

MATERIALS AND METHODS

Sample collection

During the period April 1998 to March 2000, a total of 18 Belgian commercial broiler flocks, consisting of 16 independent and 2 successive flocks in the same house, were followed from the hatchery to the slaughterhouse. A wide range of samples was collected, as described in Table 1. Swabs were taken using sterile cotton wool moistened with sterile buffered peptone water (BPW) (Oxoid, London, England). The following samples were taken at commercial hatcheries during the collection of 1-day-old broiler chicks just before leaving the hatchery: 4 pools of broken eggshells from each tray, 4 pools of 20 pieces of paper tray liners when available, bowels and yolk sacs of about 20 diseased and dead chicks, wet and dry down from each incubator, swabs from the incubator walls, ventilation water in the incubators and swabs from cleaned transport boxes. Before arrival of the 1-day-old chicks (day 1), samples were taken inside the broiler house for hygiene control: feed and water supplies, swabs from feed boxes, air-inlets, ventilation and heating provisions, walls including chinks, insects and spiders, and movable material (as outlined in Table 1). After arrival of the 1-day-old chicks (day 1), 20 paper tray liners of the transport boxes were collected. During the rearing period (6 weeks), the farm was visited three times (days 14, 28 and 42). Samples were taken inside the broiler house: several pools of 10 caecal drops, several pairs of overshoes, feed and drinking water. The environment of the farm was sampled four times before and during the rearing period (days 1, 14, 28 and 42): in most cases, faeces from other animals (cattle, pigs, dogs, birds, sheep, deer, etc.), water from puddles and ditches and the containers with dead animals were sampled as well as other samples upon availability (Table 1). The farmer was asked to take samples from the bulk feeders (fresh feed) every 2 days. Footwear of the farmer (used outside the broiler house, called 'dirty'; exclusively used inside the broiler house, called 'clean') was rinsed with 250 ml of BPW in large sterile plastic bags. Birds were about 42 days old when slaughtered. At the slaughterhouse, the following samples were taken: 6 pools of faecal material from the transport containers or crates and neck skin of 30–60 carcasses after refrigeration. All samples were put into sterile plastic bags and boxes, cooled in an icebox and immediately transported to the laboratory for bacteriological culture.

Salmonella analysis

Eggshells (50 g), down (125 g), eggs (25 g), tray liners (20 pieces of different tray liners), 2–3 overshoes, faeces of different animals (25 g), water from the disinfection tray (25 ml), dung hill (25 g or ml), wet litter from the poultry house (25 g), faeces from transport crates (25 g), swabs (10 pieces) and neck skin (25 g) were incubated in 225 ml of BPW. Bowels and yolk sacs of 10 chicks, drinking and ventilation water (40 ml), a pool of about 10 caecal drops (about 16 g), feed (125 g), insects and spiders, water from the containers with dead chickens on the farm (40 ml) and water from puddles and ditches (40 ml) were incubated in 125 ml, 360 ml, 150 ml, 375 ml, 25 ml, 360 ml and 360 ml of BPW, respectively. After incubation of

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Table 1. *Types of samples taken per category*

Category	Samples taken
Hatchery	Incubators; crates with chicks; clean, empty crates; valve water; ventilation pipes of incubators; eggshells; down; wet down taken from ventilation pipes of incubators; addle eggs; paper tray liners; incubator gutters; formalin tray; overshoes; bowels and yolk sacs of diseased or dead chicks
Transport	Paper tray liners
Hygiene broiler house (day 1)	Ventilation system; heating system; walls; pipes; sockets; chinks in walls or floors; nipples or water tables; nipple water; feed in trays; feeders; feed trays; overshoes; wet straw or wood shavings
Feed and water in broiler house (days 14–42)	Nipple water; feed from trays; wet straw or wood shavings; mat; ventilation systems; walls
Animal material in broiler house	Invertebrate animals; rodents; dung; feathers
Movable material	Clean footwear; dirty footwear; wheelbarrows; carts; ladders; spades; cleaning material; radio
Animal material environment	Faecal material other domestic or wild animals; dung hills; spilled dung; overshoes or footwear in other stables; invertebrates; container with dead chickens; milk diseased cow; mussel shells; rat
Non-animal material environment	Disinfection tray at entrance stable; empty barrels; ditch water; pond water; puddles; mowed grass; household and garden refuse; kitchen sink; grass silage; maize silage; wet bedding other stables; drinking water other animals; overshoes; compost heap; spilled feed under bulk feeder; wood shavings; drain; drive to meadow
Fresh feed	Pooled fresh feed from bulk feeder
Gold standard	Caecal droppings; overshoes
Faecal material crates	Faecal material in transport crates for broilers
Carcasses after processing	Neck skin after processing in slaughterhouse

the samples in BPW at 37 °C for 20 h, 0·1 ml of pre-enrichment BPW broth was transferred to 9·9 ml of Rappaport-Vassiliadis broth (RV) (Oxoid), to diagnostic semi-solid salmonella agar (Diasalm) (LaB M, Bury, England) and to Modified Semi-Solid Rappaport-Vassiliadis (MSRV) agar (Oxoid). Alternatively, 0·2 ml of pre-enrichment BPW broth was added to 1·8 ml of RV in a 2 ml microcentrifuge tube (small volume RV). After 20–24 h at 42 °C, RV broth or suspected zones on Diasalm and MSRV were streaked onto xylose lysine desoxycholate agar (XLD) (Oxoid) and incubated at 37 °C for 24 h. Additionally, the RV enrichment broth of 10 ml was incubated for 48 h before transfer to XLD. Presumptive salmonella colonies on XLD were confirmed using PCR.

PCR confirmation of presumptive salmonella

The bacterial cells of suspected colonies were dissolved in 100 μl of sterile water and centrifuged for 2 min at 13000 ***g***. The pellet was resuspended in 100 μl of 0·05 M NaOH, 0·125% SDS and heated for 17 min at 90 °C. For PCR the salmonella specific primers ST11 (5′-AGCCAACCATTGCTAAATTGGCGCA-3′) and ST15 (5′-GGTAGAAATTCCCAGCGGGTAC-TG-3′) described by Aabo et al. [19] were used. PCR was performed in a final volume of 50 μl containing 50 mM KCl, 10 mM Tris–HCl (pH 8·3), 1·5 mM $MgCl_2$, 0·5% Tween-20, 0·01% gelatine, 200 μM of each dNTP, 1·5 U of AmpliTaq DNA polymerase (PerkinElmer, Norwalk, CT, USA), 50 pmol of each primer and 1 μl of crude cell lysate. The mixture was subjected to 30 cycles of amplification in a thermal cycler (Cetus 9600; PerkinElmer). The first cycle was preceeded by denaturation for 1 min at 95 °C. Each cycle consisted of denaturation for 15 sec at 95 °C, annealing for 15 sec at 57 °C, and elongation for 30 sec at 72 °C. A final elongation for 8 min at 72 °C followed the last cycle. The PCR products were analysed on a 1·5% (w/v) Seakem ME agarose gel (FMC Bioproducts, Rockland, ME, USA).

Sensitivity of the different salmonella enrichment methods

Each sample was tested by two or more of the selective enrichment methods for isolation of salmonella. A sample was determined positive when at least one of

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the enrichment methods yielded salmonella. The percentage sensitivity of a method was calculated as the number of positive samples, using the enrichment method concerned, divided by the overall number of positive samples.

Determination of salmonella status of a flock

The European directive 92/117/EEC [20] concerns measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications. The Zoonoses Directive mentions the number of sites from which separate faeces samples are to be taken in order to make a pooled sample as 60 faecal samples from a flock of 500 or more birds. In most countries including Belgium, however, it is generally accepted that the use of overshoes is equally efficient. Therefore, the salmonella status of a flock was determined by the results of the analyses performed on overshoes for all 18 flocks, with supplementary analysis of caecal droppings in 17 of these flocks. These samples were taken inside the broiler house at days 14, 28 and 42. For each flock, a minimum of 21 samples (overshoes and caecal drops) were analysed. If at least one of the enrichment methods yielded isolation of salmonella in the caecal drops or the overshoes at one of the sampling times, the flock was considered positive for salmonella.

Analysis of potential risk factors for the flock status and contamination level

The different kinds of samples taken in the broiler flocks, their housing facilities and environment were grouped in nine categories (Table 1), to allow analysis of their significance in relation to the status of the flock and the contamination level of the flock: (1) hatchery, (2) transport, (3) hygiene broiler house (day 1), (4) feed and water in broiler house (days 14–42), (5) animal material (other than poultry) in broiler house, (6) movable material, (7) animal material in the environment, (8) non-animal material in the environment, and (9) fresh feed. Overshoes and caecal droppings were categorized as the gold standard. A gold standard is an independent, valid criterion, by which an animal's true disease status can be defined. The influence of the number of houses and the use of antibiotics on the flock's status was also examined.

Analysis was performed using a univariate logistic regression, with 'status' as the dependent variable. The following independent variables were continuous: the number of houses, and the nine categories mentioned above, consisting of the proportion of samples of a specific category from which salmonella had been isolated. The remaining independent variable, i.e. the use of antibiotics, was dichotomous. The effect of the use of antibiotic therapy on the contamination level, defined as the proportion of salmonella positive overshoes and caecal droppings, was tested using analysis of variance (ANOVA). Results were considered as significant at P-values $\leqslant 0{\cdot}05$. Correlation between environmental samples and samples from animal origin within the house on the one hand, and caecal drops and overshoes on the other, was assessed using a bootstrapped correlation, since the variables did not meet with the assumptions necessary to perform a Pearson's correlation [21]. To assess the simultaneous influence of the different categories, antibiotics and the number of broiler houses on the contamination level, the best general linear model was determined. The software used was SPSS 8.0 for Windows. Bootstrapping was done with S-Plus 4.0 for Windows.

Analysis of potential risk factors for carcass quality at the slaughter plant

Carcass quality was defined as the proportion of salmonella contaminated carcasses after processing. The significance of salmonella isolation from faecal material of transport crates in relation to carcass quality was also tested using a bootstrapped correlation as described above. The influence of the flock's status, the gut evisceration method, the order in which the flocks were slaughtered on a slaughtering day (first or not), and the identity of the slaughter plant on carcass quality, was tested in an ANOVA. The software used was as described above.

RESULTS

Sensitivity of different salmonella selective enrichment methods

A total of 3150 samples were analysed for salmonella: 3150 with Diasalm, 342 with MSRV, 2848 with RV (0·1 ml in 10 ml RV, incubation for 24 h), 583 with RV (0·1 ml in 10 ml RV, incubation for 48 h) and 2945

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Table 2. *Efficiency of different selective salmonella enrichment methods*

Enrichment method	No. tests	Sensitivity (%)	95% CI (lower level–upper level)
Diasalm	3150	0·939	0·922–0·956
MSRV	342	0·792	0·677–0·907
RV			
10 ml 24 h	2848	0·613	0·575–0·650
10 ml 48 h	583	0·462	0·326–0·597
2 ml	2945	0·533	0·494–0·571
Diasalm			
+ MSRV + RV 10 ml 24 h	1059	1·000	1·000–1·000
+ RV 2 ml + RV 10 ml 24 h	2897	0·996	0·992–1·000
+ MSRV	1044	0·988	0·980–0·996
+ RV 10 ml 24 h	2974	0·979	0·970–0·989

Table 3. *Efficiency of sampling methods for determination of salmonella status in a flock*

Sample	No. flocks tested	Sensitivity (%)	95% CI (lower level–upper level)
Overshoes	18	1·000	1·000–1·000
Clean footwear	14	0·750	0·450–1·000
Caecal drops	17	0·667	0·359–0·975
Feed in poultry house	17	0·500	0·231–0·880
Drinking water	17	0·222	0·000–0·494

with small volume RV (0·2 ml in 1·8 ml RV) (Table 2). The highest sensitivity (93·9%) was obtained with culture in Diasalm. MSRV gave a sensitivity of 79·2% and the selective enrichments for 24 h and 48 h in 10 ml RV (Oxoid) 61·3% and 46·2%, respectively. The small volume RV enrichment gave a sensitivity of 53·3%. A sensitivity of 100% was obtained with a combination of Diasalm, MSRV and RV (0·1 ml in 10 ml RV, incubation for 24 h) as selective enrichment.

Salmonella status of the broiler flocks during rearing

The salmonella status during rearing of the broilers was determined most sensitively by the 'overshoe method' (Table 3). No extra positive flocks were found by testing caecal drops, clean footwear of the farmer, feed or drinking water in the broiler house.

Salmonella prevalence in the production chain

A total of 18 broiler flocks, 16 independent and 2 successive flocks in the same house (flocks 6 and 7), were sampled from hatchery to slaughterhouse (Table 4), inclusive of the broiler house and the farm environment of each flock (Table 5). The study included a total of 7 different hatcheries, 17 different poultry houses on 17 different farms and 9 different slaughterhouses. In the hatchery and after transport of the 1-day-old chicks, salmonella was isolated from only one and two flocks, respectively. In four broiler houses, salmonella was found by the hygiene control, i.e. after cleaning and disinfection and before arrival of the one-day-old chicks. In 3 of these 4 broiler houses, the ventilation or heating system was positive for salmonella (Table 5). However, although this was the case in flock 1, no salmonella was subsequently isolated from the caecal drops or overshoes in this flock. In the broiler house of flock 10, the highest salmonella contamination of all flocks was observed at the hygiene control with the feed trays and silo, the feed in the trays, chinks in the wall and a dead mouse all found positive (Table 5). In two flocks, including one additional flock than the four already mentioned, the clean footwear in the hygiene gate of the broiler

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Table 4. *Prevalence of salmonella in the production chain of 18 flocks. The number of salmonella positive samples out of the total number of samples is indicated*

Flock/ no. houses*	Hatchery	Transport	Rearing period on farm					Slaughtering phase		
			Antibiotics†	Hygiene‡	Environment§	Fresh feed	Status animals¶	Crates**	Carcasses††	Slaughter house‡‡
1/1	−/20	−/4	—	1+/16	−/5	−/10	−/28	−/6	60+/60	A[a]
2/1	−/19	−/4	1×	−/11	−/6	−/4	1+/31	−/6	1+/60	B
3/3	−/19	1+/3	2×	−/11	1+/11	−/6	−/29	5+/6	60+/60	A[a]
4/1	−/16	−/5	2×	−/9	−/2	1+/13	−/29	2+/6	−/60	B
5/1	−/20	−/4	1×	−/9	−/31	−/2	−/32	2+/6	58+/60	A
6/3§§	−/16	−/4	—	−/15	20+/34	−/4	22+/25	6+/6	30+/30	A
7/3§§	−/9	−/2	—	1+/12	19+/24	ND¶¶	11/25	5+/6	28+/30	A
8/3	−/19	2+/2	3×	1+/8	4+/23	−/5	10+/21	1+/6	3+/60	B[a]
9/3	1/19	−/2	—	−/13	5+/17	−/1	7+/23	−/6	11+/60	B
10/8	−/18	−/2	1×	5+/15	14+/25	1+/12	28/28	6+/6	47+/47	C
11/1	−/15	−/2	1×	−/9	−/30	1+/16	−/27	ND	19/60	D
12/1	−/19	−/2	—	−/6	−/31	−/8	−/24	4+/6	14+/60	E
13/3	−/16	−/2	—	−/10	4+/21	−/6	−/30	3+/6	44+/60	F
14/2	−/14	−/2	1×	−/9	1+/28	−/6	−/21	ND	10+/60	G[a]
15/5	−/11	−/2	4×	−/11	7+/32	−/3	1+/27	3+/6	19+/60	G
16/1	−/23	−/3	2×	−/13	−/27	−/13	5+/48	3+/6	20+/30	H[a]
17/4	−/25	−/2	1×	−/10	1+/43	−/3	1+/34	−/6	2+/30	I
18/1	−/15	−/2	1×	−/9	1+/36	1+/3	6+/31	1+/6	3+/30	D
Total+	1+	2+	12+	4+	11+	4+/17	10+	12+/16	17+	
Total−	17−	16−	6−	14−	7−	3−/17	8−	4−/16	1−	

*, Number of the flock/number of poultry houses on the rearing farm; †, number of times antibiotic treatment given during rearing; ‡, hygiene broiler house on day 1; §, animal material and non-animal material environment and movable material; ¶, overshoes and caecal drops; **, faecal material from transport crates; ††, neck skin after processing; ‡‡, 'a' after slaughterhouse identity code means flock not slaughtered as first on the day; §§, two successive flocks in same broiler house; ¶¶, not determined.

Table 5. *Distribution of salmonella positive samples* (*indicated in bold*) *taken inside the broiler houses, in the hygiene gates and in the environment outside the broiler houses from negative and positive flocks*

	Flock number with negative status				Flock number with positive status							
Sample	1	3	13	14	6	7	8	9	10	15	17	18
In broiler house												
Ventilation and heating d 1	**1/4***	0/3	0/2	0/4	0/3	**1/2**	**1/3**	0/4	0/3	0/3	0/2	0/2
Walls, silo, trays, nipples d 1	0/3	0/2	0/3	0/3	0/6	0/5	0/2	0/3	**3/3**	0/2	0/3	0/2
Nipple water	0/5	0/7	0/4	0/3	0/4	0/4	**1/4**	0/4	**3/4**	0/4	0/4	0/4
Feed from trays d 1‡	0/1	0/1	0/1	0/1	0/2	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1
Feed from trays d 14–42§	0/3	**2/10**	0/3	0/2	**2/3**	**1/3**	**1/3**	**1/3**	**3/3**	0/3	0/3	0/3
Dead mouse d 1	—†	0/1	—	—	—	—	—	—	**1/1**	—	—	—
Hygiene gate												
Clean footwear d 1	—	—	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/2**	0/1	0/1	0/1
Clean footwear d 14–42	—	0/2	0/3 0/2¶	0/3 0/2¶	**1/3**	0/3 **1/2¶**	**1/2**	**3/3** **1/2¶**	**2/2** **7/10¶**	**1/3** **3/9¶**	**1/3** **0/5¶**	**1/3**
Outside broiler house												
Dirty footwear d 1	—	—	—	—	**1/1**	—	—	—	—	0/1	0/1	0/1
Dirty footwear d 14–42	—	—	0/1	—	**2/2**	—	0/1	0/1	**1/1**	0/1	—	0/3
Wheelbarrow, ladder, bucket**	—	—	0/5	0/2	**1/2**	**1/3**	—	0/4	0/2	0/2	0/6	0/3
Faecal material†† d 1	—	—	**1/2‡‡**	0/1	—	0/1	0/1	0/1	0/1	0/2	0/1	0/4
Faecal material†† d 14–42	0/3	**1/4‡‡**	0/2	**1/3‡‡**	**2/3** **1/2¶**	0/1 **9/18¶**	0/10	0/4	**2/2‡‡**	**1/2**	0/12	0/6
Dung hill d 1	—	—	**1/1**	—	**1/2**	—	—	—	—	—	—	0/2
Dung hill d 14–42	—	—	—	0/1	**3/5**	**3/4**	—	0/1	—	—	0/1	0/5
Container dead chickens d 1	—	—	0/1	0/1	**1/1**	—	—	—	—	—	—	—
Container dead chickens d 14–42	—	—	0/1	—	**4/4**	**3/3**	0/2	—	—	**1/2**	—	—
Puddles d 1	—	—	**1/1**	0/1	0/1	—	0/1	0/1	—	0/1	—	0/2
Puddles d 14–42	—	—	**1/1**	0/3	**2/3**	0/3	**1/4**	0/2	—	—	0/3	0/3
Disinfection tray	—	0/1	—	0/2	—	—	—	—	**1/5**	—	0/3	0/2
Compost heap	—	—	—	0/3	—	—	—	—	—	**1/3**	0/1	—
Ditch water d 14–42	—	—	—	0/1	**1/1**	**1/2**	**2/2**	—	—	0/1	0/3	0/1
Woodshavings	—	—	—	—	—	**1/1**	—	—	—	—	0/1	—

*, Number of positive samples/total number of samples taken; †, sample not taken or not available in this flock; ‡, sample taken at day 1 before arrival of 1-day-old chicks; §, sample taken during rearing period; ¶, taken at other poultry houses on the same farm; **, movable material may also be present inside broiler house or in hygiene gate; ††, faecal material from other domestic and wild animals (dog, cattle, sheep, horse, swine, deer, birds) and/or from other broiler houses on the same farm; ‡‡, faeces from dogs.

house was already positive for salmonella before arrival of the 1-day-old chicks (Table 5). In the case of the successive flocks 6 and 7, salmonella was isolated at the hygiene control before rearing of flock 7.

Ten of the 18 flocks received a salmonella positive status (i.e. salmonella positive overshoes and/or caecal drops) (Table 4). Nine of these positive flocks were already positive after 14 days rearing, the remaining flock becoming positive for the first time after 28 days. The number of positive flocks dropped to 7 and 6 after 28 days and 42 days rearing, respectively (data not shown). The highest contamination level (i.e. proportion of positive overshoes and caecal drops) was found after 14 days rearing for 7 flocks and after 28 days for 3 flocks (data not shown).

In 12 flocks, subtherapeutic or therapeutic doses of antibiotic were given during rearing. The agents were quinolones and/or fluoroquinolones in five flocks and

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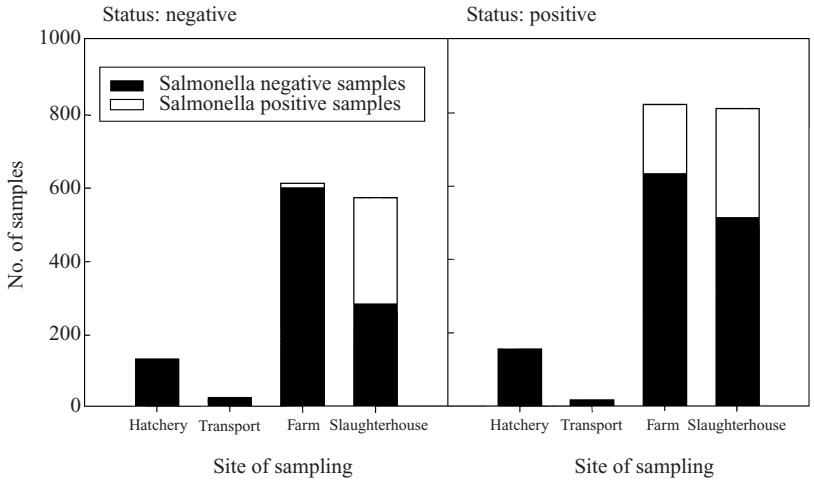

Fig. 1. The isolation of salmonella in the broiler production chain as function of the contamination status of the animals during rearing.

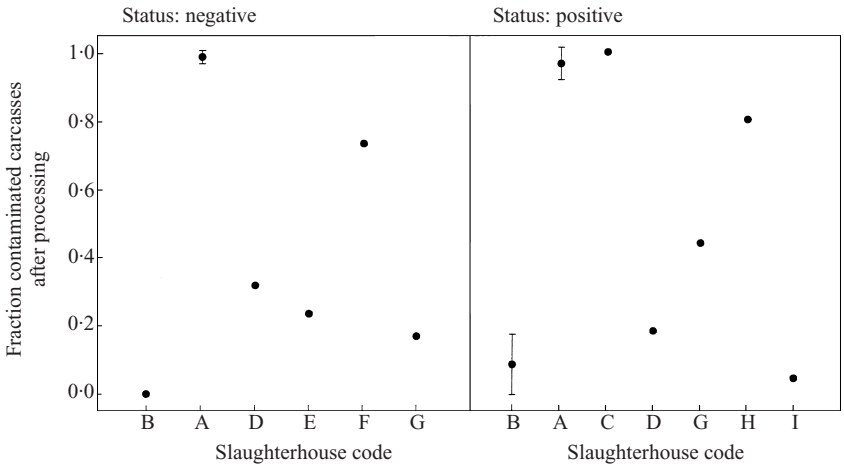

Fig. 2. The contamination by salmonella of broiler carcasses as function of the status of the flock and the identity of the slaughterhouse.

in single flocks as follows: ampicillin, a sulphonamide, a fluoroquinolone and trimethroprim + sulphonamide, a fluoroquinolone, sulphonamide and tetracycline, a macrolide, lincosamide and polypeptide antibiotic, a lincosamide with an aminoglycoside and a quinolone, a lincosamide with an aminoglycoside.

In the farm environment of the broiler house (i.e. clean footwear in the hygiene gate and samples taken outside and in other broiler houses), 11 of 18 flocks were positive for salmonella (Table 4). It is noteworthy that for 3 of these 11 flocks the bacteria were found only in the environment on clean footwear in the hygiene gate (Table 5). There was a moderate correlation between the salmonella contamination in the environment of the broiler house and the status of the flock (Table 4). For 5 of the 8 negative flocks, the environment was also negative, whereas for 8 of the 10 positive flocks, the environment also tested positive. There was a similar correlation between environmental contamination and the contamination level of positive flocks (Table 4). Here, for 4 of the 5 heavily contaminated flocks ($> 25\%$ of the samples positive), the environment was also heavily contaminated, but with 3 of the 4 slightly contaminated flocks ($\leqslant 10\%$ of the samples positive), the environment was either negative or yielded few salmonella.

The samples from the broiler houses, the hygiene gates and the environment which were found most frequently positive for salmonella can be deduced from Table 5. In the broiler houses, feed from the trays was most frequently contaminated (6 flocks). In the hygiene gate, the clean footwear was frequently contaminated (8 flocks). However, in the environment outside the broiler houses, dirty footwear, dung hills,

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Table 6. *Summary of the results obtained through univariate analyses to determine risk factors and correlations for the salmonella status and contamination level of a broiler flock and the carcass quality in the slaughterhouse*

Dependent variable	Independent variable	Type of analysis	Correlation	95% CI	*P*-value
Contamination level (% overshoes/caecal drops positive for salmonella)	Hygiene broiler house	Bootstrapped correlation	0·61	0·06–0·96	—
	Feed and water in broiler house d 14–42	Bootstrapped correlation	0·88	0·39–0·92	—
	Animal material environment	Bootstrapped correlation	0·64	0·09–0·89	—
	Non-animal material environment	Bootstrapped correlation	0·56	0·11–0·85	—
	Use of antibiotics	ANOVA	—	—	0·02
Flock status	Feed and water in broiler house d 14–42	Logistic regression	—	—	0·02
	Feed broiler house d 14–42	Logistic regression	—	—	0·02
	Water broiler house d 14–42	Logistic regression	—	—	0·10
	Movable material	Logistic regression	—	—	0·06
	Footwear	Logistic regression	—	—	0·08
	Identity slaughterhouse	ANOVA	—	—	< 0·01
Carcass quality (% carcasses contaminated)	% contamination of transport crates	Bootstrapped correlation	0·62	0·037–0·89	—

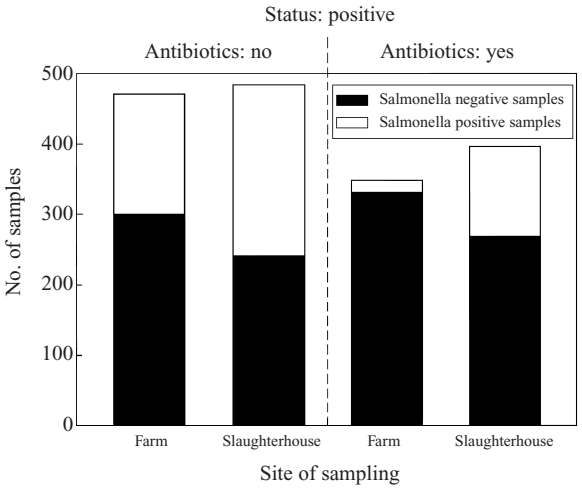

Fig. 3. The isolation of salmonella in status positive flocks in the farm and slaughterhouse as function of subtherapeutic and therapeutic antibiotic treatment during rearing.

containers with dead chickens, puddles and ditch water were regularly found to be contaminated (2–3 flocks). The same was true for faecal material from domestic and wild animals and from other broiler houses on the same farm (7 flocks). In four of these latter flocks, faeces of dogs was contaminated with salmonella, as well as cattle faeces from another flock.

For 16 of the 18 flocks, the faeces of the animals in the crates after transport to the slaughterhouse were sampled (Table 4). Twelve of these yielded salmonella, which reflects an increase in the number of positive flocks after transport. For five negative flocks during rearing, positive faeces was found in the transport crates, but conversely for three flocks which were positive during rearing, no salmonella was found in the faeces from the crates. For two of these (flocks 2 and 17), this corresponded with slight shedding of salmonella by the animals which was only observed after 2 weeks rearing and not just before transport (data not shown). For 12 of the 18 flocks, it could be arranged to have the flock slaughtered as first flock on the slaughtering day. In all but 1 of the 18 flocks, salmonella positive carcasses were found after slaughter (Table 4). No relation was found between the flock status during rearing and the contamination of the carcasses (Fig. 1).

The identity of the slaughterhouse appeared to be the determining factor for the carcass quality (Fig. 2). Two slaughterhouses (A and B in Table 4) were included several times in this study. Slaughterhouse A consistently delivered the highest contamination level of the carcasses for all nine slaughterhouses investigated, ranging from 93–100% of the carcasses being

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Table 7. *Statistical properties of the best regression model to determine the influence of risk factors on the contamination level of a broiler flock*

	Standardized regression coefficients	Partial correlation	*P*
Hatchery	0·27	0·65	< 0·01
Non-animal material environment	0·26	0·58	0·02
Feedstuff and water in stable (day 14–42)	0·75	0·91	< 0·01
Number of houses	0·25	0·62	0·02

salmonella positive, irrespective of the flock status or the time of slaughter. Slaughterhouse C also delivered 100 % positive carcasses, but from a flock with already all animals positive during rearing. On the other hand, slaughterhouse B consistently had a very low level of contamination (ranging from 2–18 %). In one case no contaminated carcasses were found where the flock was also negative during rearing.

Determining factors for salmonella contamination in the broiler production chain

Table 6 shows a summary of the results obtained by univariate analyses. Using the bootstrapped correlation technique, significant relations were found between the percentage of salmonella positive overshoes and caecal drops, and hygiene of the broiler house, feed and water in the broiler house, and both animal and non-animal material sampled in the environment. Using one-way ANOVA, a significant influence was seen of the use of subtherapeutic and therapeutic antibiotics on the proportion of positive overshoes and caecal drops during rearing of the animals ($P = 0{\cdot}02$). This is also evident from Figure 3, which illustrates the lower number of positive samples when antibiotics were given during rearing. Of the nine categories of samples investigated using univariate logistic regression, only the contamination of feed and water in the broiler house proved to be significantly related ($P = 0{\cdot}02$) to the status of the flock. Further analyses showed that it was the subgroup of feed samples taken from the trays within the broiler houses that made this relation significant ($P = 0{\cdot}02$). Water samples from the nipples in the broiler housed did not reveal a significant influence ($P = 0{\cdot}10$). Furthermore, a potential risk factor for horizontal transmission of salmonella during rearing was the introduction of movable material into the broiler house after cleaning and disinfection ($P = 0{\cdot}059$, which is just above significance). When this group was further subdivided into footwear versus other material, footwear showed a trend to influence the flock status ($P = 0{\cdot}08$). Contamination of the 1-day-old chicks ('hatchery'), their transport, and the contamination of the fresh feed did not appear to have a significant influence. Contrary to the results obtained with the bootstrapped correlation, the contamination of the broiler houses ('hygiene'), other animals on the farm including domestic animals and wild animals such as insects, spiders, rodents and birds ('animal material environment') or within the broiler house ('animal material broiler house'), and ditch water, puddles, compost heap, etc. ('non-animal material environment') did not show a significant effect on the flock status. Moreover, a one-way ANOVA with 'status' as factor, 'animal material environment' and 'animal material broiler house' did not reveal either to be significant. The number of houses present on each broiler farm (see Table 4) did not have a significant influence on the flock's status in the univariate logistic regression.

The identity of the slaughterhouse (ANOVA, $P < 0{\cdot}01$) and the contamination of the broilers during transport (salmonella positive faeces from the transport crates, bootstrapped $r = 0{\cdot}62$, with 95 % CI (0·037–0·89) were found to be the determining factors for the contamination of the end product (Table 6). Furthermore, the salmonella status of the broilers during rearing, the gut evisceration method used and the time of slaughter during the day (flock slaughtered first or not) also did not have a significant influence on the carcass quality. The bootstrapped correlation and the ANOVA analysis yield useful information with respect to the individual linear relationships between the dependent and the independent variables. Multivariate regression can help to capture possible redundancies between the independent variables themselves. All the possible general linear regression models using proportion of overshoes and caecal drops positive for salmonella as the dependent

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variable, and the different categories, the number of houses and the use of antibiotics as independent variables were analysed and the best model was selected based on Mallow's C_p, a statistic that takes into account the model bias and the complexity [22]. The resulting regression model fitted the data well (adjusted $R^2 = 0{\cdot}89$), while the regression coefficients were statistically significant (Table 7). The partial correlations can be interpreted as the remaining correlation after controlling for the other variables [23]. They show that 'feed and water in the broiler house (days 14–42)' is clearly most influential on the dependent variable, while 'hatchery', the number of houses and 'non-animal material environment' are more or less equally influential.

DISCUSSION

Different methods were compared for the isolation of salmonella. These were two methods with a liquid enrichment step in RV and two methods based on motility enrichment in the semisolid media Diasalm and MSRV. It is clear that in our study, based on more than 3000 broiler faecal and environmental samples, the highest number of positives was obtained by motility enrichment. This is in agreement with Voogt et al. [24] who found a significantly higher performance of Diasalm and MSRV compared with RV for 892 broiler faecal samples, and with Zdragas et al. [25] who found a higher sensitivity of MSRV compared with RV for 180 broiler internal organ and intestinal samples. Although the combination of these two semisolid media yielded a detection rate of 98·8%, it is debatable if this can be generalized. De Zutter et al. [26] showed salmonella isolation rates from naturally contaminated food to be 96% for MSRV compared to 90% for RV. On the other hand, Wiberg and Norberg [27] found a higher sensitivity for the isolation of salmonella from naturally contaminated food and feed with RV (99%) compared to MSRV (87%). By only using motility enrichment, it is possible to miss less or non-motile serovars (e.g. *S.* Panama) or strains which form part of about 0·1% of the clinical human isolates [28]. It has also been reported that MSRV partially inhibits *S.* Typhimurium [25]. Also, the highly selective environment of the semisolid media (supplemented with 20 mg/l or 10 mg/l novobiocin in MSRV and Diasalm, respectively) could suppress some injured salmonella strains [27]. Therefore, a combination of a semisolid medium with RV for the isolation of both motile and less or non-motile salmonella strains in broiler samples can be recommended.

Other important aspects in salmonella surveillance and monitoring programs are the type of sample and the time of sampling to determine a flock's status with the highest sensitivity. In this study overshoes proved to be the best type of sample for this purpose and the highest shedding of salmonella occurred after 2–4 weeks rearing. Even after 4 weeks of rearing shedding may already cease in some flocks. This can partially be explained by the use of subtherapeutic or therapeutic antibiotics during rearing which was shown here to have a dramatic effect on the number of positive faecal samples. This effect, which has been reported previously [29], has to be considered in controlling programmes, based on analysis of faecal connected samples (i.e. overshoes, caecal drops). The use of antibiotics during rearing had no direct effect on the contamination status of the animals in this study due to the large number of samples taken. It can thus be concluded that several overshoe samples should be taken preferably during the first half of the rearing period.

Horizontal transmission of salmonella to broilers and broiler carcasses was demonstrated here as the main determinative factor for contamination of the end product in the 18 investigated Belgian flocks. Vertical transmission is of lower importance probably due to the many years' efforts of controlling the breeding level of the production chain. Also, the importance of the different risk factors for contamination has changed over time as shown by a comparison between the data of this study and those of from earlier literature (see introduction). The limited number of data here allowed only for a univariate logistic regression, but its results were corroborated by the results obtained through a multivariate regression analysis. It is evident from these data that there is a clear decrease of the relative influence of the first stages in production (hatcheries, transport of 1-day-old chicks, contamination originating from the broiler house). Nevertheless, these production stages remain important to control by continuous monitoring of parent animals and by effective cleaning and disinfection procedures in hatcheries and broiler houses, including ventilation and heating systems. On the other hand, the last stages in production (transport of broilers and slaughter) become increasingly important. Furthermore, contamination during rearing is still extensive and is easily transferred from the environment to the

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animals within the broiler house and vice versa by the footwear of the farmer. Correct use of the hygiene gate is therefore of great importance.

Contamination by small animals like insects, rodents and birds as well as contamination by fresh feed have been reported as important factors during rearing [12]. This was not statistically confirmed by this study, even though a clear correlation was found between the proportion of salmonella positive overshoes and caecal drops and material from animal origin in the environment, which included faecal material from other domestic and wild animals as well as from other broiler houses on the same farm, dung hills and containers with dead chickens. This correlation was also found for non-animal material in the environment, such as puddles and ditches.

It has to be noted that some risk factors for contamination of a flock are difficult to prove statistically because of scarcity of samples; they may nevertheless be important in particular cases as shown here for rodents in the broiler house of one flock. Four fresh feed samples tested positive from a total of 115 samples. This proportion of 3·5% is in agreement with results found in different European countries (3·4% of the analysed poultry feed) [30] but lower than the 8·7% reported by Oggel et al. [31]. The significant relation found here between the flock's status and the high contamination level of the feed in the trays within the broiler house could be as much a result of the flock's shedding of salmonella, as it could be its cause. As a consequence of the low contamination level of fresh feed, the former is more likely. Feed in the trays of the broiler house, and to a lesser extent drinking water, substantially contribute to a further spreading of the contamination within a flock.

Skov et al. [13] commented that the number of hen houses is only influential on the status when there are more than five houses present. Our results indicate that an increasing number of houses could have a slight effect on the contamination level of a flock, even when there are less than five houses present. This influence is not automatically true for the status because this value is deduced from the proportion of positive overshoes and caecal droppings by a non-linear transformation. The logistic regression did not point to a significant influence on the status, which is in agreement with Skov et al. [13].

We did not find that contamination during rearing was significant in relation to the contamination of the end product. In contrast, a moderate correlation was found with the presence of salmonella in faecal material in the transport crates. Forty-three percent of the transport crates for broilers proved to be contaminated with salmonella compared with 86·6% reported earlier [17]. Overall, 47% of the carcasses were contaminated with salmonella. The identity of the slaughter plant was the most significant factor for the carcass quality obtained, whereas the gut evisceration method, the status of the flock at departure from the rearing farm and the time of slaughter were all not significant. Some slaughterhouses were found to consistently produce salmonella positive carcasses and/or to increase the contamination level of the carcasses, whereas other slaughterhouses performed much better by delivering salmonella free or only slightly contaminated carcasses. Improved hygiene management during transport of broilers and in some slaughterhouses can significantly reduce the risk of salmonella contamination of poultry meat. Further research is needed to identify accurately the contamination sources in broiler slaughterhouses.

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