TECHNICAL REPORT submitted to EFSA

Fate of *Salmonella* spp. on broiler carcasses before and after cutting and/or deboning[1]

Prepared by

Dr. Dean Burfoot, Julie Archer, Dr. Erika Horváth, Greg Hooper
Campden BRI, Station Road, Chipping Campden, Gloucestershire GL55 6LD UK

Dr. Vivien Allen, Dr. Michael Hutchison, Dawn Harrison
University of Bristol, Langford, North Somerset, BS40 5DU UK

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Campden Technology Limited, Station Road, Chipping Campden, Gloucestershire GL55 6LD, UK
Tel: +44 (0)1386 842000 Fax: +44 (0)1386 842100 www.campden.co.uk

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Summary

The tests described in this report were carried out following EFSA Tender CFT/EFSA/BIOHAZ/2008/01. The aim was to collect and report data on the presence /absence and numbers of *Salmonella* spp. on broiler carcasses and portions in slaughterhouses and cutting plants. Caeca samples were also taken at slaughterhouses. In all trials, except for one, sampling of carcasses and portions was carried out for birds that status testing at the holding had shown to be positive for *Salmonella*. In the one trial when status sampling was negative, the caeca samples where positive when taken at the slaughterhouse. Samples (caeca, carcass rinse, neck skins, skin-on and skin-off portions) were taken during a total of 6 trials at 2 slaughterhouses and cutting plants in Country A, and during 7 trials at 3 slaughterhouses/cutting plants in Country B. Portions consisted of breasts, crowns, drumstick and thigh, or thigh alone, depending on the ease of obtaining samples at the appropriate location on the production line at each plant. A total of 13 trials sampling skin-on and skin-off portions were completed as specified in the tender. Results from one other trial, when the cutting plant did not produce skin-on portions, are also reported.

All holdings were conventional production units with between 2 and 14 houses and, on the day of status sampling, held between 42400 and 220000 birds. *Salmonella* status, based on boot swab sampling, had been carried out when the birds were between 14 and 31 days-old. The number of positive houses, relative to the total number at the holding, varied between 0 and 100%,

The birds were delivered to the slaughterhouses in batches with between 11650 and 60000 birds in a batch which generally came from one house, but sometimes with birds being mixed from several (*Salmonella*-positive) houses. Birds were slaughtered between 37 and 47 days-old at mean weights between 1.8 and 2.8 kg. The six slaughterhouses had capacities between 6×10^6 and 60×10^6 birds per year and operated at line speeds between 3000 and 15000 birds per hour. Four plants used electric stunning, one plant used gas stunning, and one plant used gas or electric stunning: the target flock was gas stunned. Slaughterhouses used scalding baths with temperatures between 50 and 57ºC and residence times between 2.5 and 6 minutes. Rotary finger pluckers, automated blade-and-spoon eviscerators, and air chillers were used at all plants. Chillers were operated at -1 to 2.5ºC for between 85 and 120 minutes.

Trials were carried out at six cutting plants: four of them were on the same site as the slaughterhouse and two were remote. The cutting lines operated between 2200 and 6600 birds per hour: 50% of them were automated and the rest were manual operation. 40% of the de-skinning lines were automated and 50% of the deboning lines were automated or semi-automated. Automated and manual operations were used in both Country A and B.

The total number of birds being housed at the holdings at the times of status sampling was 1.8×10^6. The number of birds processed during the trials from parts of the batches of interest was 205000. The number of those birds used to produce portions was 164000 and of those 102000 produced skin-on portions and 106000 produced skin-off portions. Some carcasses were used to produce both types of portion.

Presence/absence and confirmation analyses for *Salmonella* were carried out according to EN/ISO 6579 and enumeration (Most Probable Number, MPN) was carried out according to a draft ISO Technical Specification that was given in the tender document and described in this report. 94 of the 560 caeca sampled (17%) were positive for *Salmonella*: all of these samples came from birds in Country B. Five of the 7 trials from Country B resulted in *Salmonella*-positive caeca. Overall, 65 of the 350 neck skin samples (19%), 80 of the 560 carcass rinses (14%), 70 of the 560 skin-on portions (13%), and 58 of the 600 skin-off portions (10%), tested positive for *Salmonella*.

Early trials in February and March 2009 showed very low prevalence of *Salmonella* on portions, with only 2 of 200 portions testing positive for *Salmonella*. The portions taken were breasts or crowns, depending on the particular practices of the cutting plant. In two trials in late March in Country B, combined drumstick and thigh portions or thigh portions alone were taken and these showed much greater prevalence of S*almonella*: 29 out of 80 portion samples (36%) were *Salmonella*-positive. Consequently, further trials tended to focus on this type of portion. Beyond March, there was considerable difficulty in locating *Salmonella*-positive flocks in Country A, and further trials were focussed on sampling drumstick/thigh portions in Country B where *Salmonella* was being detected at the holdings. Three trials, carried out at one plant on one day in August in Country B, showed much greater prevalence of *Salmonella* than other trials. 53 of the 70 *Salmonella*-positive skin-on portions (76%) detected in the entire study were found in those 3 trials and 43 of the 58 *Salmonella*-positive skin-off portions (74%) came from those 3 trials.

Using the ISO Draft Specification, 7 of the 80 positive carcass rinse samples (8.8%) produced numbers of *Salmonella* that could be detected. The values were between 1.6 and 8.9 MPN g^{-1}. 5 of those 7 positive carcass rinse results (71%) came from birds from the same flock.

Overall, within the limits of sampling, the trials in Country A used flocks that were *Salmonella*-positive at the holdings, but the caeca of the birds were not positive at the slaughterhouses. The higher prevalence of *Salmonella* on neck skins and carcass rinses tended to come from batches with high prevalence in caeca samples. Higher prevalence of *Salmonella* on portions was also associated with higher prevalence from caeca. Provided that the cutting plant is not transferring contamination onto the flesh, skin-off samples would be

expected to have lower prevalence, and lower counts, of *Salmonella* than skin-on portions and the results support this expectation. The trials indicated that prevalence of *Salmonella* was greater from neck skin and carcass rinse samples than from samples of portions. EFSA is to test whether the differences are statistically significant.

1. Background and Need

The research described in this report was carried out following EFSA Tender CFT/EFSA/BIOHAZ/2008/01.

A process hygiene criterion exists for broiler carcasses after chilling (Commission Regulation (EC) No 2073/2005). Improvements in hygiene and controls at the farm or at the processing plant are required if the criterion is not met. The need for a food safety criterion for fresh retail poultry needs to be assessed. As part of the research to support the assessment of the need for a criterion, there is a requirement to report on the *Salmonella* contamination on broiler carcasses after chilling post-slaughter, and before and after processing at the cutting plant. The purpose of this study was to provide such data from different processing facilities and at different times.

2.1 Overall Objective

To collect and report raw data on the presence/absence and presumptive numbers of *Salmonella* spp. on broiler carcasses and fresh poultry products originating from *Salmonella* positive-flocks in the slaughterhouse and before and after cutting and/or deboning in the cutting plants.

2.2 Specific Objectives

2.2.1 To collect and report on the presence/absence of *Salmonella* spp. on broiler carcasses originating from *Salmonella* positive flocks after chilling but before further processing in the slaughterhouse. The tests to be carried out according to the method described in Section 5 "Requirements" in the Tender Document.

2.2.2 To collect and report on the presence/absence and presumptive numbers of *Salmonella* spp. on whole broiler carcasses originating from the same batches as tested in 2.2.1 before cutting and/or deboning in the cutting plant, again using the methods described in Section 5 "Requirements" of the Tender Document.

2.2.3 To collect and report on the presence/absence and presumptive numbers of *Salmonella* spp. on fresh broiler meat originating from the same batches as tested in 2.2.1 and 2.2.2 after cutting and/or deboning, but before placing on the market, in the cutting plant, again according to the method described in Section 5 "Requirements" of the Tender Document.

2.2.4 To collect information on the possible factors affecting the occurrence of *Salmonella* spp. This will be based on data from this project and experience from other projects and contracts.

3. Methods

3.1 Selection process for *Salmonella*-positive flocks

The two countries where the trials were carried out were chosen because of the good contacts the researchers have with the industry in those countries and because one of the countries had a very high prevalence of *Salmonella*-positive broiler flocks. Poultry processors in both countries gave notification of flocks with *Salmonella*-positive status at the holdings and sampling trials at slaughterhouses and cutting plants were then arranged to coincide with the processing of those flocks. This information provided an increased level of confidence in selecting flocks that would provide *Salmonella*-positive samples at the slaughterhouses and cutting plants.

Originally, three trials were to be carried out at each of three slaughterhouses/cutting plants in Country A and two trials were to be carried out at each of two slaughter/cutting operations in Country B. In total, 13 trials were planned. About 4 months into the project, information from the poultry industry in Country A strongly indicated that the likelihood of sourcing *Salmonella*-positive flocks in that country was diminishing as the traditionally drier and warmer months were approaching. Consequently, the project plan was adjusted with permission from EFSA, to include three trials at each of two slaughter/cutting operations in Country A; two trials at each of two operations in Country B, and a further three trials at another operation in Country B. The total number of trials remained at 13 but the likelihood of sourcing *Salmonella*-positive flocks had been increased.

At the time of the slight change in plan, four trials had been carried out in Country A and four trials in Country B. However, in one of the trials in Country A, only skin-off portions had been collected due to changes in production scheduling at the plant whereas the experimental design required both skin-on and skin-off samples. The results from that work are included in this report in addition to results from the 13 main trials contracted in the tender.

3.2 Details of the holdings, slaughterhouses, and cutting plants

Tables 1a-c outline the specific details of the holdings, slaughterhouses and cutting plants included in the study. All of the holdings were conventional production units, not housing free-range or organic birds. The holdings housed between 42400 and 220000 birds on the day of status sampling. The holdings had between 2 and 14 houses. The *Salmonella* status of the birds was based on boot swab sampling and was carried out by laboratories chosen by the operator (in-house or independent). Status testing was carried out when the birds were between 14 and 31 days old. The number of houses identified as *Salmonella*-positive, relative to the total number of houses at the holding, varied between 0 and 100%. In only one case were birds sampled at the slaughterhouse from a house that had tested negative at the holding, and in that example the caeca of the birds were positive when tested at the slaughterhouse.

The birds were delivered to the slaughterhouses in batches of between 11650 and 60000 birds. These birds generally came from one house, but sometimes they were mixed from several *Salmonella*-positive houses. Birds were slaughtered between 37 and 47 days old at weights between 1.8 and 2.8 kg. These data are mean weights provided by the slaughterhouses but in some cases the weights of the birds in a batch clearly varied from the mean. Slaughter capacity varied between 6×10^6 and 60×10^6 birds per year and operated at line speeds between 3000 and 15000 birds per hour (Table 1b). One plant used gas stunning, one plant used gas or electric stunning (the target flock was gas stunned), and the other plants used electric stunning. Scald baths were operated between 50 to 57ºC with residence times of 2.5 to 6 minutes. All of the plants used pluckers with rubber fingers on discs, although plants tended to describe them slightly differently. Automatic blade and spoon machines were used for evisceration. All plants used air chillers operating at -1 to 2.5ºC with residence times between 85 and 120 minutes.

Four of the six cutting plants were co-located next to the slaughterhouse and two of the cutting plants, both in Country A, were sited away from the slaughterhouses. In Country A, the carcasses were generally processed to portions within 12 hours of slaughter. During one trial (A1), during a period of severe weather, the birds were processed about 58 hours after slaughter because the cutting plant was not on the same site as the slaughterhouse and transport was hampered by poor weather. In all plants in Country B, the slaughterline led directly onto the cutting plant line and the carcasses were processed directly after chilling. Processing lines operated between 2200 and 6600 birds per hour: 50% of the portioning lines were automatic; 50% of the de-skinning lines were automatic; 50% of the de-boning lines were automatic or semi-automatic.

The types of portion chosen for sampling depended on the type generally produced at the plant, the availability of skin-on and skin-off portions, which was dependent on customer demand, and the ease of reliably taking the samples from the line. In trials in Country A, breasts were removed from the carcass and obtained as skin-on and skin-off portions. At one plant in Country A, only skin-off portions were produced at the time of the trials due to customer demand. An additional trial at another plant was carried out to ensure that 13 trials with skin-on and skin-off portions were completed. In another trial in Country A, skin-on and skin-off portions of breasts and (separately) thighs were obtained. Cutting plant BC, in Country B, did not produce skin-on breast but it did produce skin-on and skin-off crowns and it was these that were taken for microbiological testing. At cutting plant DC, in Country B, skin-on and skin-off breasts were difficult to obtain and combined drumstick and thigh portions were obtained in one trial, and thighs were obtained in another trial. At Plant EC, combined drumstick and thigh portions were taken in all three trials.

3.2 Sampling at Slaughter and Cutting Plants

When sampling, the co-operation of plant staff was sought to ensure that a 2 to 3 minute gap of shackles without birds was created on the line prior to the start of hanging of the flock of interest. This gap in the processing could be followed through to evisceration ensuring that caeca were collected from the correct flock. Similarly, around 90 minutes after the caeca were collected, the same gap in the shackled birds was used to identify the carcasses of interest as they exited from the chiller.

3.2.1 Caeca sampling

Although not requested in the official EFSA tender, we proposed to take caecal samples and these were carried out in all of the trials. Both caecal appendices were collected into stomacher bags (Seward, Thetford, UK) from the viscera of carcasses immediately after the evisceration stage of processing. Care was taken to ensure the caeca were not ruptured whilst being removed from the intestines. The caeca were stored in cool boxes containing crushed ice and ice-packs prior to microbiological testing. 40 caecal samples, both appendages, were collected during each plant visit.

3.2.2 Excision sampling of neck skins

Excision sampling of neck skins was on moving lines during normal commercial processing. Samples were collected immediately after the chilling phase of processing by turning a sterile stomacher bag (304 by 177mm; Seward, Thetford, UK) inside-out over a gloved hand,

selecting carcasses with neck skins of an appropriate length, and excising a 10g sample with a pair of sterile scissors. Three samples from separate carcasses, giving at least 25g of neck skin in total, were placed in a stomacher bag and then into a cool box with crushed ice and ice-packs. Twenty-five pooled neck skin samples (75 skins in total) were obtained from each plant visit.

3.2.3 Whole carcass rinse sampling

Rinse samples were obtained from carcasses, with neck skins attached, immediately after the chilling phase of commercial processing. Carcasses were removed from the processing line by capturing them into large stomacher bags (380 by 508mm; Seward, Thetford, UK) and unshackling the carcass legs with gloves sanitized with alcohol wipes (Azowipes, Vernon Carus Ltd., Preston, UK). A freshly sanitised pair of gloves was used for each carcass. Carcasses were rinsed with 300 ml of sterile maximum recovery diluent (LabM, Bury, UK) and shaken vigorously for 3 minutes. Rinse water was poured into sterile 30 ml plastic universals (Ross Labs, Macclesfield, UK) that were placed in cool boxes with crushed ice and ice packs. Rinse samples were collected from forty carcasses at each plant visit.

3.2.4 Portion samples taken at the cutting plants

Care was taken to ensure that the portions collected (single breast, crown (double breast), thigh, drumstick and thigh) were matched to the same flock and house as the caecal and post-chill carcass-derived samples. In Country A, plant staff put carcasses for sampling into a specially-labelled dolav (bin) and these carcasses were monitored as they progressed through the cutting plant hall. A gap in the carcasses on the line was created immediately before the carcasses of interest were portioned to enable easy identification of the portions of interest. In Country B, the slaughter-lines ran directly into the portioning halls. The lower line speeds in that country and the gaps created by the removal of post-chill carcasses made it easy to follow the birds of interest into the portioning hall.

In plants with automated cutting equipment, portions with skin intact or removed were aseptically collected as soon as direct access to the portions could be safely achieved. The portions were removed from the cutting lines by capturing them into large stomacher bags (380 by 508mm; Seward) using gloves sanitized with alcohol wipes. In the plants in Country B with manual portioning, the samples were taken as soon as the finished portion was generated. The type of portions sampled, and the reason for that choice, has been described earlier. Forty portions with skin removed and 40 with skin intact were collected during each

portioning plant visit (except for Trial C1 when skin-on portions were not available, and Trial E3 when 40 skin-on and skin-off breasts were taken and 40 skin-on and skin-off thighs).

All samples of caeca, neck skins, carcass rinses, and portions were placed in cool boxes containing crushed ice and ice packs and transported to the microbiology testing laboratory.

3.3 Microbiological Testing

The time from slaughter to the start of microbiological testing was recorded for all trials. In all except two cases, microbiological testing of the samples began within 24 hours of slaughter of the birds. In the case of Trial A1, the analysis of the caeca, carcass rinses, and neck skins, began within 24 hours of slaughter of the birds. The production carcasses were transferred from the slaughterhouse to a cutting plant many miles away and, due to poor weather conditions, were only portioned two days after slaughter. The portions were collected as soon as they were ready and microbiological testing began within four hours thereafter. In the case of Trial B1, the samples were delayed by Customs on entry into the UK, and microbiological testing began 26 hours after slaughter. The temperatures of the all batches of samples were confirmed at the laboratory to be below 4ºC

3.3.1 Detection (Presence/Absence and Confirmation) of *Salmonella* spp.

The detection of *Salmonella* in the caeca (40 samples per trial), neck skins (25 x3 pooled samples), carcass rinses (40 samples), and portions (40 skin-on and 40 skin-off samples) was performed according to the procedure given in the standard method EN/ISO 6579 (Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp.). This method should enable the detection of 1 cfu of *Salmonella* in a 25 g sample.

For caecal samples, approximately 1g of caecal contents was taken from each of the forty paired caeca. The majority of the caecum contained only 1-2g of content. One gram was taken of the pooled content of the paired caeca from each bird for consistency. Each 1 g sample was transferred into 10 ml of buffered peptone water (BPW CM1049, Oxoid, Basingstoke, UK) to yield an approximate 1/10 dilution. For the other samples, 25 g of neck skin (comprising 3 pooled samples), carcass rinse, or broiler portion, was mixed with 225 ml of BPW to also yield a 1/10 dilution in this pre-enrichment medium. All samples were then homogenised for 60 seconds. The BPW was then incubated at 37°C ±1°C for 18h ± 2h after which 0.1ml was transferred into a tube containing 10ml Rappaport Vassiliadis medium with soya (RVS broth, LA86, LabM, Bury, UK) and 1ml into another tube containing 10ml Muller

Kauffmann tetrathionate/novobiocin broth (MKTTn, pre-prepared TV5065E, Oxoid). The RVS and MKTTn broths were incubated for 24 h ± 3h at 41.5°C ± 1°C and 37 °C ± 1°C, respectively. After incubation, the RVS and MKTTn broths were streaked onto two plates of xylose lysine deoxycholate agar (XLD, CM0469, Oxoid) and modified brilliant green agar (mBGA CM0329, Oxoid). After incubating the XLD and mBGA plates at 37°C ±1°C for 24h ± 3h, they were inspected for typical *Salmonella* colonies. Five suspect colonies were taken from each plate, or all suspect colonies were taken if there were less than 5. These suspect colonies were then streaked onto nutrient agar plates (NA, CM003, Oxoid), incubated at 37°C ±1°C for 24h ± 3h, and then confirmed using an oxidase reaction (Oxidase Test BBL Dry slide, Becton, Dickinson & Company, Maryland, USA) followed by serological confirmation of *Salmonella* somatic O antigens using polyvalent O antisera (M10345, Mast Diagnostics, Bootle, UK) and flagella H antigens using polyvalent H antisera (M143000, Mast Diagnostics). Biochemical identification was determined using a commercially available kit (API 20e, bioMérieux, Basingstoke, UK). No further tests were carried out on other isolates once a single isolate had been confirmed as *Salmonella*.

3.3.2 Enumeration of *Salmonella* spp. on whole carcasses and portions

Carcass rinse samples and skin-on and skin-off portion samples from each trial were analysed for the enumeration of *Salmonella*. Analysis was carried out according to the draft ISO Technical Specification - Microbiology of food and animal feeding stuffs, animal faeces and environmental samples from primary production stage - Horizontal method for the enumeration of *Salmonella* by a miniaturised MPN technique. This method has been developed for the enumeration of motile *Salmonella* spp.

The initial homogenised suspension of 25 g of portion or carcass rinse, with 225 ml of BPW, as described in Section 3.3.1, was used in the MPN method. A portion of this initial suspension (2.5ml) was added to each of the 3 wells in the first row of a 12-well microtitre plate. Two ml of BPW was put into each of the remaining wells and 0.5 ml of the initial suspension was transferred from each well of the first row into the wells in the subsequent row. This step of transferring 0.5 ml of suspension into the subsequent row was repeated across the remaining rows of the plate. Before each transfer the contents were mixed by gently sucking up and pipetting out the contents of each well. The microtitre plate was then incubated at 37°C ± 1°C for 18h ± 2h after which a 20µl drop from each well of the plate was added to each well of a second micro titre plate containing 2ml of semi solid Rappaport Vassiliadis agar (MSRV CM0910, Oxoid). This second microtitre plate was incubated at 41.5°C ± 1°C for 24h ± 3h and then inspected for positive reactions. The medium in any positive wells was typically grey-white with a turbid zone (characterised by a white halo and clearly defined edge) extending from the inoculated drop. If wells were negative, this plate

was incubated for a further 24h ± 3h and examined again. Using a 1 µl loop, medium from each positive well (of the highest dilutions) was streaked onto a plate containing XLD medium (XLD, CM0469, Oxoid). After incubating the plates of XLD at 37°C ± 1°C for 24h ± 3h, confirmation was performed on suitable isolated typical colonies using the same procedures outlined in EN/ISO 6579. Presumptive *Salmonella* isolates obtained using this MPN method were confirmed as *Salmonella* using conventional biochemical and serological techniques as described above (Section 3.3.1).

The number of positive wells and dilutions was used to determine the count in each sample. Each well in the first row of the microtitre plate contained 0.20 g of the original broiler sample (portion or rinse); wells in row 2 contained 0.04 g; wells in row 3 contained 0.008 g; and the wells in row 4 contained 0.002 g. The most probable number of *Salmonella* per gram of portion, or carcass rinse, was calculated using the number of positive wells per row of the mictotitre plate and the resulting 4 digit code was entered into the MPN calculator specified in the draft standard The calculator can be found at http://www.i2workout.com/ mcuriale/mpn/index.html).

4. Results

Table 2 shows the overall summary of the number of birds at the holdings, estimate of the number of birds in houses that tested positive for *Salmonella*, and the number birds in the batch of interest that were slaughtered, portioned and de-skinned. From a total number of birds in the batches of interest (1.8×10^6), 205000 were processed in the batches of interest, and 164000 of those were portioned. Table 3 shows the number of samples taken from the batches of interest (2630) and summaries the microbiological analyses. 94 of the 560 caeca samples (17%), 65 of the 350 pooled neck skin samples (19%), 80 of the 560 carcass rinses (14%); 70 of the 560 skin-on portions (13%), and 58 of the 600 skin-off portions (10%) were confirmed as *Salmonella*-positive.

Despite all birds, apart from those in one batch, coming from houses that testing at the holding had shown to be *Salmonella*-positive, 9 of the 14 batches of caeca (64%) did not contain *Salmonella*-positive samples. For the one batch that came from a house that had not tested *Salmonella*-positive, 12 of the 40 caeca samples (30%) tested positive at the slaughterhouse. That slaughterhouse had found that on average the percentage of flocks testing positive at the slaughterhouse, but coming from "negative flocks" at the holding, was approximately 40%. Disparities between the status of birds at the holding and at the slaughterhouse are not unusual and reflect some of the difficulties in sampling for *Salmonella*. The sampling or testing of the original boot samples by the organisation

carrying out the procedures might not have been effective. The re-emergence of *Salmonella* after sampling at the holding is unlikely.

All five batches of the caeca samples that included *Salmonella*-positive samples came from birds in Country B. Five of the 7 batches of 40 caeca from Country B (71%) were confirmed to include *Salmonella*-positive samples thereby strongly indicating that *Salmonellae* were entering the slaughterhouse, certainly in the caeca and possibly on the feathers and skin.

All of the slaughterhouses used automatic evisceration and air chilling, and the main difference was in the type of stunning. Slaughterhouses A, C, and E were in Country A. Slaughterhouse A used electric stunning and the cutting plant used automated (some semi-) operations; Slaughterhouse C used gas stunning and automated cutting operations; and Slaughterhouse E used gas stunning and manual cutting operations. Three trials were carried out at Slaughterhouses A and E but only one trial, when skin-on portions were not available, was carried out at Slaughterhouse C. Only one of the 145 samples taken from Slaughterhouse C was *Salmonella*-positive (a neck skin). From Slaughterhouse A, 0 of the 120 caeca, 9 out of 75 neck skins (12%), 6 out of 120 carcass rinses (5%), 2 out of 120 skin-on portions (1.7%), and 0 skin-off portions tested *Salmonella*-positive. From Slaughterhouse E, 0 of the 120 caeca, 8 out of 75 neck skins (11%), 0 of the 120 carcass rinses, 1 of the 160 skin-on portions (breast, thigh), and 0 of the 160 skin-off portions tested *Salmonella*-positive. Apart from the carcass rinse data, there was little difference between the results from slaughterhouses A and E. None of the trials in Country A provided samples with detectable levels of *Salmonella* using the MPN method.

Slaughterhouses B, D, and F were in Country B. All of these plants used electric stunning and two of the plants used manual cutting operations (Plants BC1 and DC1) and the other (Plant FC1) had manual de-skinning with the other operations being automated. For one of the 2 trials at Plant B, 28 out of 40 caeca (70%), 1 out of 25 neck skins (4%), 3 out of 40 carcass rinses (7.5%), and 0 skin-on and 0 skin-off portions tested *Salmonella*-positive. In the second trial at Plant B, none of the samples of any type tested *Salmonella*-positive. In the first trial at Plant D, 0 of the 40 caeca, 11 out of 25 neck skins (44%), 6 out of 40 carcass rinses (15%), 12 out of 40 skin-on portions (30%) and 5 out of 40 skin-off portions (13%) tested *Salmonella*-positive. These results show that despite finding no *Salmonella*-positive caeca, quite a high prevalence of *Salmonella* was found in the samples. In the second trial at Plant D, 4 out of 40 caeca (10%), 4 out of 25 neck skins (16%), 2 out of 40 carcass rinses (5%), 2 out of 40 skin-on portions (5%) and 10 out of 40 skin-off portions (25%) tested *Salmonella*-positive.

The trials at Plant F, all carried out on the same day but with different flocks of birds and cleaning of the processing line between flocks, produced the highest prevalence of *Salmonella*-positive samples of any plant. Caeca samples were positive from all three trials. 62 of the 120 caeca samples (52%), 31 of the 75 neck skins (41%), 63 of the 120 carcass rinses (53%), 53 of the 120 skin-on portions (44%) and 43 of the 120 skin-off portions (36%) from the batches on the day were found to be positive for *Salmonella*. Considering just the *Salmonella*-positive samples from the trials at all plants, 66% of all positive caeca, 48% of positive neck skins, 79% of positive carcass rinses, 76% of positive skin-on and 74% of positive skin-off portions came from trials at Plant F.

Overall, 125 of the 128 *Salmonella*-positive samples of broiler portion, 98% of those tested in the study, came from Country B.

Turning to the MPN results, 7 carcass rinses, all from Country B, gave numbers of *Salmonella* that could be detected using the MPN method. All of these samples came from batches of birds which had 22 or more caeca testing positive out of a batch of 40 samples. Numbers of *Salmonella* detected by the MPN method from carcass rinses were between 1.6 (lower 95% confidence value, LL = 0.22; upper confidence value, UL = 11) MPN g^{-1}, and 8.9 (LL = 2.9; UL = 27) MPN g^{-1}. Five samples of the skin-on portions gave detectable numbers of *Salmonellae*. All of these samples came from thigh or combined drumstick and thigh samples. Values ranged from 1.6 (LL = 0.22, UL = 11) MPN g^{-1} to 31 (LL = 10; UL = 99) MPN g^{-1}. Three samples of skin-off portions, all from thigh or combined drumstick and thigh, gave detectable numbers of *Salmonellae* between 1.6 (LL = 0.22; UL = 11) MPN g^{-1} and 8.1 (LL = 2.6; UL = 25) MPN g^{-1}.

Table 4 presents the results of the presence/absence and MPN tests carried out on carcass rinse samples and skin-on/skin-of portions in a form required for analysis by the BIOHAZ Working Group.

5. Discussion and Conclusions

Statistical analysis of the data is to be carried out by EFSA, nevertheless, without such analysis, the results presented above do indicate some factors that appear to influence the prevalence and numbers of *Salmonellae* along the production chain. Some factors relate to specific trials and factory operations, and other factors enable more general conclusions to be drawn. Specific points are:

5.1 In Trial B1, the number of *Salmonella*-positive caeca was high but no *Salmonella*-positive portions were detected. This result would be commensurate with the use of a higher than usual level of chlorination of the bore hole water at the plant. However, the plant management confirmed that the chlorine levels used at the time of the tests was within the legislative limits.

5.2 In the trials at Plant D, the numbers of *Salmonella*-positive caeca were low and the numbers of positive portions were relatively high. This may have been caused by cross-contamination. Bile was observed on some carcasses and this is an indication of poor evisceration that may have been caused by variation in the weights of the birds on the days of the trials.

5.3 Prevalence and numbers of *Salmonella* were greater from the trials on the one day of tests at slaughterhouse F, and cutting Plant FC, than found on other days at other plants. Carcass rinses and portions, in particular, generally showed much higher prevalence. There were no obvious differences in the operations at Plant F compared to those at the other plants other than it used potable mains water, whereas the others in Country B used chlorinated borehole water.

More general conclusions are:

5.4 In Country A (Trials A, C and E), within the limits of sampling, the flocks were *Salmonella*-positive at the holdings but the caeca of the birds were not positive at the slaughterhouses. The small number of *Salmonella*-positive portions (3 out of a total of 128 positive samples in all trials) was likely due to cross-contamination from the equipment or a very low prevalence in the flock.

5.5 Although none of the caeca were *Salmonella* positive in Country A, many of the caeca samples were positive from Country B.

5.6 The higher prevalence on neck skins and carcass rinses tended to be found on samples that came from batches of birds with high prevalence of *Salmonella* from caeca sampling, although this was not always the case.

5.7 The higher prevalence of *Salmonella* on skin-on and skin-off portions seemed to be associated with higher prevalence in the caeca samples.

5.8 Skin-off samples would be expected to have lower prevalence, and lower counts, of *Salmonella* than skin-on portions and the results do not disagree with this expectation. (Contamination is more likely on the skin unless the cutting plant is transferring contamination onto the exposed flesh.)

5.9 Average values for neck skins removed directly after chilling showed higher prevalence of *Salmonella* than carcass rinses, also taken after chilling, which showed higher prevalence than portions taken at the cutting plant. As noted above, no analysis was carried out to test for any levels of statistically significance differences between these prevalence values. This analysis is to be carried out by EFSA.

Table 1a Details of the holdings sending birds to the slaughterhouses

Trial Number	Holding	Country	Type of Holding	Number of houses at holding	Number of houses testing +ve for Salmonella	Number of houses providing birds to the batch of interest	Number of birds at the holding on day of sampling holding	Number of birds in the batch	Age of birds at Salmonella sampling, days	Date of slaughter	Age of birds at slaughter, days	Weight of birds at slaughter, kg
A1	AH1	A	Conventional	8	3	2	149500	19250	20 -22	02/03_Feb	37	1.8
A2	AH2	A	Conventional	8	2	1	147000	18400	22	19/20_Feb	40	2.3
A3	AH3	A	Conventional	2	1	1	76900	38400	21	01_May	37	2.1
B1	BH1	B	Conventional	8	6	6	160000	20000	28	24_Feb	40	1.9
B2	BH2	B	Conventional	14	10	4	220000	60000	31	30_Mar	41	2
C1	CH1	A	Conventional	9	1	1	109500	11700	21	06_Mar	39	2.1
D1	DH1	B	Conventional	10	2	2	138000	32300	25	30_Mar	38	2.2
D2	DH2	B	Conventional	12	12	1	195000	16000	21	31_Mar	42	2-2.2
E1	EH1	A	Conventional	12	4	4	77200	25600	21	19_Mar	44	2.7
E2	EH2	A	Conventional	2	1	1	42400	17550	21	22_Apr	41	2.2
E3	TBA	A	Conventional	6	1	1	71200	11850	21	20_May	47	2.8
F1	FH1	B	Conventional	3	0	1	84000	14000	24	10_Aug	38	2.1
F2	FH2	B	Conventional	7	3	1*	153800	11950	14	10_Aug	40	2.2
F3	FH2	B	Conventional	7	3	1	153800	11650	14	10_Aug	40	2.2

* In trials F1 and F2, birds came from the same holding (FH2) but different houses on the holding

Table 1b Details of the slaughterhouses where trials were carried out.

Slaughter Plant	Birds slaughtered per year	Line speed, birds per hour	Stun type	Scald type	Pluck type	Evisceration	Chiller
A	60×10^6	10000-15000	Electrical	Bath (54°C, 3.5-4 min)	Rubber finger and disc	Automatic blade and spoon	Air (2 to 4°C, 90 min)
B	12.6×10^6	6600	Electrical	Bath (50 to 53°C, 3 min)	Rubber finger and disc	Automatic blade and spoon	Air (-0.5 to 2.5°C, 90 min)
C	Unknown	7600	Gas	Bath (56 to 57°C, 3 min)	Rubber finger and disc	Automatic blade and spoon	Air (2 to 4°C, 90 min)
D	8.3×10^6	3000	Electric	Bath (52-53C, 2.5 min)	Rubber finger and disc	Automatic blade and spoon	Air (0 to 4°C, 90 min)
E	35×10^6	10000	Gas*	Bath (50-55C; 3 to 4min)	Rubber finger and disc	Automatic blade and spoon	Air (-1°C, 120 min)
F	6×10^6	6500	Electric	50 to 52°C, 5 to 6 min)	Rubber finger and disc	Automatic blade and spoon	Air (-0.5 to 2.5°C, 85 min)

* Birds could be stunned by electric or gas at this slaughterhouse. The batch of interest was stunned using gas.

Table 1c Details of the cutting plants where trials were carried out

Cutting Plant	Location	Birds processed per hour	Portioning	Deskinning	Deboning
AC1	Remote	5200	Automated	Automated	Semi-automated
BC1	At slaughter	6600	Manual	Manual	Manual
CC1	Remote	Unknown	Automated	Automated	Automated
DC1	At slaughter	2200	Manual	Manual	Manual
EC1	At slaughter	3125	Manual	Manual	Manual
FC1	At slaughter	6500	Automated	Manual	Automated

Table 2 Summary of the number of birds at the source holding and the numbers of birds processed and portioned produced

Trial Number	Country	No. of birds at holding on day of sampling	(No. of +ve houses /Total no. of houses) x No. of birds	Number of birds processed in the trial	Number of birds portioned in the trial	No. of carcasses for skin-on portions	No. of carcasses for skin-off portions
A1	A	149500	56000	12122	12086	604*	11482
A2	A	147000	36700	11858	11818	590*	11228
A3	A	76900	38400	5752	5712	286*	5426
B1	B	160000	120000	15222	14004	14004	14004
B2	B	220000	157100	47138	43367	43367	43367
C1	A	109500	12200	11700	11660	11660	0
D1	B	138000	27600	23955	23491	23491	3993
D2	B	195000	195000	9558	9222	9222	4721
E1	A	77200	25700	14179	2428	426*	2002
E2	A	42400	21200	17524	3420	600*	2820
E3	A	71200	11900	8883	2016	354*	1662
F1	B	84000	0	7975	7200	5616*	1584
F2	B	153800	65900	7990	7240	5647*	1593
F3	B	153800	65900	11441	10325	8053*	2272
TOTAL		1778300	833600	205297	163989	101744	106154

* The number of portions produced with skin-on from the batch had to be estimated based on annual production figures as the exact numbers were not known. However, the samples taken for sampling were definitely from the batch of interest.

Table 3 Summary of microbiological analyses

Country	Trial No.	Number of caeca samples	Number of Salmonella +ve caeca samples	Number of neck skin samples #	No. of Salmonella +ve neck skins	Number of carcass rinses	Number of Salmonella +ve carcass rinses	Carcass Rinse MPN g^{-1}	LL,UL for MPN on carcass rinse	Type of portion sampled	Number of skin-on portions	Number of Salmonella +ve skin-on portions	Skin-on portion MPN g^{-1}	LL,UL for MPN g^{-1} on skin-on portions	Number of skin-off portions	Number of Salmonella +ve skin-off portions	Skin-off portion MPN g^{-1}	LL,UL for MPN g^{-1} on skin-off portions
A	A1	40	0	25x3	7	40	3	<1.3	***	Breast	40	2	<1.3	***	40	0	<1.3	***
A	A2	40	0	25x3	1	40	0	<1.3	***	Breast	40	0	<1.3	***	40	0	<1.3	***
A	A3	40	0	25x3	1	40	3	<1.3	***	Breast	40	0	<1.3	***	40	0	<1.3	***
B	B1	40	28	25x3	1	40	3	4.2	1.1 , 16	Crown	40	0	<1.3	***	40	0	<1.3	***
B	B2	40	0	25x3	0	40	0	<1.3	***	Crown	40	0	<1.3	***	40	0	<1.3	***
A	C1	40	0	25x3	1	40	0	<1.3	***	Breast	0	N/A	<1.3	***	40	0	<1.3	***
B	D1	40	0	25x3	11	40	6	<1.3	***	Drum and thigh	40	12	<1.3	***	40	5	<1.3	***
B	D2	40	4	25x3	4	40	2	<1.3	***	Thigh	40	2	3.8	0.97 , 15	40	10	3.2	0.76 , 14
A	E1	40	0	25x3	5	40	0	<1.3	***	Breast	40	0	<1.3	***	40	0	<1.3	***
A	E2	40	0	25x3	1	40	0	<1.3	***	Breast	40	0	<1.3	***	40	0	<1.3	***
A	E3	40	0	25x3	2	40	0	<1.3	***	Breast	40	1	<1.3	***	40	0	<1.3	***
										Thigh	40	0	<1.3	***	40	0	<1.3	***
B	F1	40	12	25x3	13	40	23	<1.3	***	Drum and thigh	40	23	<1.3	***	40	5	<1.3	***
B	F2	40	28	25x3	6	40	15	1.4	0.18 , 11	Drum and thigh	40	17	<1.3	***	40	20	8.1	2.6 , 25
B	F3	40	22	25x3	12	40	25	8.9	2.9 , 27	Drum and thigh	40	13	31	10 , 99	40	18	1.6	0.22 , 11
								3.2	0.76 , 14				3.2	0.76, 14				
								3.8	0.97 , 15				1.6	0.22 , 11				
								1.6	0.22 , 11				13	4.3 , 39				
	TOTAL	560	94	350x3	65	560	80				560	70			600	58		

MPN is the most probable number of *Salmonella* determined using the draft standard. MPN values are reported per g of broiler portion or per g of carcass rinse. Using the MPN calculator (http://www.i2workout.com/mcuriale/mpn/index.html) with no positive wells (0,0,0,0) provides a value of <1.3 MPN g^{-1}. The latter value has been used in the above table when no *Salmonella* could be detected. Furthermore, the microtitre plate contains a total of 0.75 g of broiler portion or carcass rinse. One cfu in 0.75 g equates to 1.3 cells g^{-1}.

*** No confidence interval is appropriate when the MPN is < 1.3 MPN g^{-1}.

25 pooled samples of 3 neck skins

Table 4 Detailed results of positivity for tests done in carcass rinses and skin-on/skin-of portions

		Carcass rinses					Skin-on portions					Skin-off portions				
Country	Trial No.	Number of carcass rinses	No. of rinses +ve to P/A test only*	No. of rinses +ve to MPN test only**	No. of rinses +ve to both P/A and MPN***	No. of carcasses +ve Total****	No. of portions tested	No. of portions +ve to P/A test only*	No. of portions +ve to MPN test only**	Portions +ve to both P/A and MPN test ***	No. of portions +ve Total****	Number of portions tested	No. of portions +ve to P/A test only*	No. of portions +ve to MPN only**	No. of portions +ve to both P/A and MPN tests***	No. of portions +ve Total****
A	A1	40	3	0	0	3	40	2	0	0	2	40	0	0	0	0
A	A2	40	0	0	0	0	40	0	0	0	0	40	0	0	0	0
A	A3	40	3	0	0	3	40	0	0	0	0	40	0	0	0	0
B	B1	40	2	0	1	3	40	0	0	0	0	40	0	0	0	0
B	B2	40	0	0	0	0	40	0	0	0	0	40	0	0	0	0
A	C1	40	0	0	0	0	0	0	0	0	0	40	0	0	0	0
B	D1	40	6	0	0	6	40	12	0	0	12	40	5	0	0	5
B	D2	40	2	0	0	2	40	1	0	1	2	40	10	1	0	11
A	E1	40	0	0	0	0	40	0	0	0	0	40	0	0	0	0
A	E2	40	0	0	0	0	40	0	0	0	0	40	0	0	0	0
A	E3	40	0	0	0	0	80	1	0	0	1	80	0	0	0	0
B	F1	40	23	0	0	23	40	23	0	0	23	40	5	0	0	5
B	F2	40	14	0	1	15	40	17	0	0	17	40	19	0	1	20
B	F3	40	22	2	3	27	40	12	3	1	16	40	17	0	1	18
	Total	560	75	2	5	82	600	68	3	2	73	560	56	1	2	59

* Number of samples that tested for *Salmonella* in only the presence/absence test

** Number of samples that tested positive for *Salmonella* in only the MPN test

*** Number of samples that tested positive for Salmonella in both the P/A and MPN tests

**** Number of samples that tested positive in either the P/A or MPN tests. This value equals the number of samples that tested +ve in the P/A tests plus the number of samples that tested +ve in the MPN tests.

The analytical sensitivity of the *Salmonella* P/A test is 0.04 cfu g^{-1}, while the analytical sensitivity of the *Salmonella* MPN test is 1.3 cfu g^{-1}.