Project Title: A Systematic Review of Literature on Pork Chain Epidemiology
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Objectives: The objective of the review was to describe, based on all available studies identified, the points of introduction and amplification of *Salmonella* spp. in the pork production chain. The pork production chain was divided into slaughter-to-cooler, and post-cooler to-finished raw product prior to retail. Studies relevant to the review were those that reported measuring *Salmonella* spp. at multiple points on the same processing chain.

Conclusions: The slaughter-to-cooler review identified 15 publications describing 40 studies that evaluated the prevalence of *Salmonella* spp. on carcasses. The review provides little evidence that *Salmonella* is introduced into the pork product as it moves along the processing chain to the cooler. On the contrary, the aggregated data across the studies suggest that the processes employed from slaughter to the cooler are associated with steady decreases in *Salmonella* spp. prevalence.
The post-cooler to finished raw product review was unable to identify sufficient studies for an aggregate analysis, similar to that conducted for the slaughter-to-cooler review. From 713 references identified during the literature search, only four manuscripts describing 12 studies reported evaluating *Salmonella* spp. prevalence at multiple points in the post-cooler production process. This paucity of publically available information has previously been noted. Berands et al (1998) noted that information about cutting plants was rare and "practically all of which is published in confidential reports in Dutch or in specialized books of limited circulation"[1].

Deliverable: There are two potential impacts of this project on the meat industry. First, the slaughter-to-cooler review provides publically available empirical evidence for the efficacy of the procedures employed in modern pork production systems to control *Salmonella* spp (Figure 1). The finding that *Salmonella* spp. prevalence consistently decreases during processing, even in a variety of settings, provides evidence that the processes are robust. This information could be used to convey to the consumer, the efficacy of the measures taken to control *Salmonella* spp. from slaughter to the cooler.

The results of the post-cooler to finished raw product review indicate a paucity of publically available information about this aspect of pork production. The reviewers acknowledge that it is possible this information is available but subject to confidentiality agreements. The impact of the paucity of available data is that empirical evidence of efficacy of control programs in the post-cooler side of pork processing is not available.

If the industry or consumers are interested in a public document that summarizes the ecology of *Salmonella* spp. post-chilling in pork then more data are needed. If the reviewers are correct and much of this data is available but confidential, then it may be efficient to develop a mechanism, by which, data from already completed confidential studies which have evaluated post-cooling *Salmonella* spp. prevalence could be available for an aggregate analysis, similar to that conducted in the slaughter-to-cooler review. If the reviewers are incorrect and the studies have not been conducted, then primary research in this area could be needed.

Technical Abstract (501 words)

A systematic review was conducted to identify and summarize primary research studies that describe the introduction and amplification of *Salmonella* spp. in pork from slaughter to the finished raw product (i.e., prior to retail) produced in the European Union (EU), the United Kingdom (UK), Scandinavian or developed nations on the Pacific Rim. Relevant studies documented Salmonella *spp* prevalence at more than one processing point using the same cohort of pigs or the same production line for the post-cooler component. The review was conducted as two studies: slaughter-to-cooler and post-cooler to raw finished product.

Searches for the literature were conducted from February 2007 to 2008 in the following sources Agricola, CAB Abstract, AGRIS, MEDLINE, BIOSIS, Food Science Technology Abstracts (FSTA) Retrospective, Biological abstracts, Biological & Agricultural Index and FSTA. The tables of contents from the following conferences were also searched; The International Symposium on Epidemiology and Control of Salmonella in Pork (1996-2005), International Pig Veterinary Society (1969-2006), American Association of Swine Veterinarians/ Practitioners (1970- 2006) and The Annual Reciprocal Meat Conference (1999-2006). The reference lists of the final relevant manuscripts were also hand searched for relevant citations. Two reviewers independently evaluated each citation to identify relevant studies for the reviews. After identifying relevant studies data from the studies were extracted and summarized.

For the slaughter-to-cooler review, 5116 citations were retrieved by the searches. 15 publications describing 40 studies evaluated the presence of *Salmonella* spp. on pork carcasses to the point of cooling. The carcass sampling points evaluated were; after bleeding, after stunning, after scalding, after dehairing, after singeing, after polishing, after evisceration, after washing and after cooling. Forty-eight (48) unique comparisons of *Salmonella* spp. prevalence between points on the processing line were reported. Forty of the 48 point-to-point comparisons were associated with either no change or a decrease in *Salmonella* prevalence. Of the 8 times *Salmonella* spp. prevalence increased as the carcasses moved closer to the cooler, only 4 times was a 10% or greater increase in *Salmonella* spp. prevalence observed. The median prevalence of *Salmonella* spp. positive carcasses evaluated in the cooler was 0%, and the mean was 4%. This compares favorably to the median prevalence of *Salmonella* spp. after bleeding of 37% and mean of 58%. This suggests that generally the processing procedures in place resulted in decreased carcass contamination as the carcass moved toward the cooler.

716 citations were identified by the searches for the post-cooler to finished raw product review. Only two manuscripts were available in English that described sampling of pork after chilling at more than one location post chilling. Two studies also reported the prevalence of *Salmonella* spp. on carcasses and then at one sampling point in the processing chain after the cooler. None of these studies described the stages of processing employed by the study plants. Several potentially relevant articles could not be located and a large number of articles that appeared to be relevant were excluded because they were not published in English. It was not possible to aggregate the data for the review.

Goals and Objectives:

Salmonella spp. is one of the most important food-borne pathogens causing gastroenteritis. One of the most efficient places for food safety interventions is during carcass processing. However, despite the prevalence of information describing effective interventions during processing, the combined efficacy of the system is poorly described. Describing the system from slaughter to the final shipped product requires incorporation of multiple sites and processes, as studies based on a single site are not reflective of the variety observed in multiple plants. Therefore, the objective of this review was to describe the *Salmonella* spp. prevalence reported from multiple studies that have evaluated *Salmonella* spp. in slaughter plants and to quantitatively describe patterns of prevalence that might otherwise not be observable in single site studies.

Materials and Methods

The review aim was to describe the points of introduction, amplification or reduction of *Salmonella* spp. prevalence from slaughter to the cooler and from the cooler to the shipped product. The population of interest was pork during the production process from slaughter to the shipped product (i.e., prior to retail) produced in the European Union (EU), the United Kingdom (UK), Scandinavian or developed nations on the Pacific Rim. The outcome of interest was the change in *Salmonella* spp. prevalence in pork during the production process. Studies relevant to the review documented *Salmonella* spp. prevalence at more than one processing point using the same cohort of pigs or same production line for the post-cooler component. As the purpose of the review was to assess the ecology of the organism during production, interventions studies conducted in production plants or artificial production settings were not relevant to the review.

The review was divided into two reviews to allow different search terms for different products and easier screening; slaughter to cooler and post-cooler to finished raw product.

Searches for the slaughter-to-cooler review were conducted from inception to February 2007 on the following databases, PubMed (1956- Feb. 2007), Agricola (1970- Feb. 2007), CAB Abstract (1910- Feb. 2007), AGRIS (1975- Feb. 2007), MEDLINE (1950- Feb. 2007), BIOSIS (1926-Feb. 2007), Food Science Technology Abstracts (FSTA) Retrospective (1969-1989), Biological abstract (1980-1989), Biological & Agricultural Index and FSTA (1989 – 2007). The tables of contents from the Proceedings of The International Symposium on Epidemiology and Control of Salmonella in Pork (1996-2005), International Pig Veterinary Society (1969-2006), American Association of Swine Veterinarians/ Practitioners (1970-2006) and The Annual Reciprocal Meat Conference (1999-2006) were hand searched for relevant citations. The reference lists of the final relevant manuscripts were also hand searched for relevant citations. The same sources were searched for the post-cooler to finished raw product review in February 2008.

After identifying the citations for screening, relevance screenings were employed to remove citations not relevant to the review. Two reviewers evaluated each citation independently. The 1st and 2nd level of screening was conducted by staff in the principle investigators lab based on the title and abstract. For subsequent screening levels based on the full manuscript,

the reviewers were either masters or doctoral level students in the epidemiology of food safety or the principle investigator.

For the slaughter-to-cooler review, the 1st level screening questions were;

- Does the abstract and/or title report primary research?
- Does the abstract and /or title report isolation of *Salmonella* spp. from pork at slaughter?

For the post-cooler to finished product review, the 1st level screening questions were;

- Does the abstract and/or title report primary research?
- Does the abstract and/or title report isolation of *Salmonella* spp. from pork after cooling?

Citations for which both reviewers responded no to either question were excluded from further consideration. The 2nd level of screening removed citations for which the full text was not available in English, as no funds were available for translation.

For the cooler-to-slaughter review, the 3rd and final level of relevance screening was based on the full manuscript and the questions were:

- Does the manuscript report the evaluation of *Salmonella* spp. prevalence on carcasses?
- Was the study conducted in the EU, UK, Scandinavian or developed nations on the Pacific Rim?
- Does the manuscript describe sampling the same cohort of pigs at more than one processing point from slaughter to cooler?
- Does the manuscript describe a prevalence study (i.e. not an assessment of an intervention)?

For the post-cooler to finished raw product review, the 3rd and final level of relevance screening was based on the full manuscript and the questions were:

- Does the manuscript report the evaluation of *Salmonella* spp. prevalence post carcass chilling?
- Was the study conducted in the EU, UK, Scandinavian or developed nations on the Pacific Rim?
- Does the manuscript describe sampling the processing line at more than one processing point?
- Does the manuscript describe a prevalence study (i.e. not an assessment of an intervention)?

Again citations for which both reviewers responded no to any question were excluded as not relevant. If there was disagreement between reviewers, the study was retained in the review and evaluated further at the next screening level. Data were extracted from the remaining studies.

For all relevant studies, data were extracted and the outcome was described as occurring after the processing point. For example, samples described as bleeding refer to samples collected after bleeding but prior to the next step in processing, and samples described as transportation samples, refer to after transportation but prior to fabrication. For manuscripts relevant to the slaughter-to-cooler review the data extracted were the number and proportion

of Salmonella samples at the each of the following processing points; stun, bleed, kill, scald, dehair, singe, polish, bung removal, evisceration, split, stamp, final wash, immediately after chill and 18-24 hours after chilling. The description of the plant system, carcass sampling methods and the method of organism detection as described by the authors of the primary research paper were also extracted and tabulated. Wherever possible data are extracted as plant or site specific i.e. if a study reported multiple visits separately or data from two plants the data from both visits are treated as separate.

For manuscripts relevant to the post-cooler to shipped raw product review, the data extracted were the number and proportion of Salmonella samples at the each of the following processing points; transportation, storage, fabrication (primal cut/ sub-primal cut), mechanical tenderization, final raw product prior to shipping to retail. Data about the prevalence of *Salmonella* spp. in seasonings, cooked products or retail product at the point of sale were not extracted. The description of the processing system, sampling methods and the method of organism detection as described by the authors of the primary research paper were also extracted and tabulated. Again, wherever possible data are extracted as plant or site specific i.e. if a study reported multiple visits separately or data from two plants the data from both visits are treated as separate.

Estimates of the exact proportion of *Salmonella* spp. positive carcasses and a 95% exact binomial confidence limit were determined for each carcass processing location using the freeware statistical package R. Exact confidence limits were used; as many point estimates were zero. After describing the prevalence for all studies, the data were summarized for processing points with at least three observations. Descriptive data evaluated was the minimum, 1st quartile, 3rd quartile, mean, median and maximum prevalence of observed for each processing point. Scatter line plots and box-and-whisker plots were used to describe the data. For the box-and-whisker plots, the box represents 50 % of observations i.e. the ends of the boxes are the 25th and 75th quartiles. The whiskers represent the 95% confidence limits, and the dots represent outliers. When the whickers are missing this means the range is the same as the 75th or 25th quartile.

Results:

Slaughter-to-cooler review

5116 citations (available upon request) were identified by the searches for the slaughter-to-cooler review. Fifteen manuscripts described sampling of carcasses at more than one location in a swine abattoir from the same cohort of pigs. The 15 manuscripts reported data from 40 studies. Only one study reported continuous *Salmonella* spp. data (number of *Salmonella* on the carcass) but these were not extracted, as there was no comparison to make with other studies. Tables describing the 40 studies included in the review, including the number of *Salmonella* positive carcasses, the number of carcasses sampled and the carcass sampling points during processing are included in the appendix.

The processing points with more than three observations were bleeding, scalding, dehairing, singeing, polishing, evisceration, washing, and chilling. For each of these processing points

the distribution of positive samples was determined i.e. the mean proportion, the median proportion, the quartiles and maximum and minimum are presented in Table 1.

The extracted data contained 48 possible point-to-point changes for the prevalence of *Salmonella* spp. on the carcass. For example, a study that sampled at bleeding, singeing and chilling would have two point-to point-changes, the change in prevalence from bleed to singe and the change in prevalence from singe to chill. Figure 1 presents a scatter line plot with connecting data points for all 40 studies. The majority of studies show a downward trend in *Salmonella* spp. prevalence as the carcass moves toward the cooler. Only eight point-to-point changes showed an increase in *Salmonella* prevalence as the carcass moved toward the cooler and of these only four showed a greater than 10% increase in *Salmonella* prevalence (Figure 1). Figure 2 shows a box and whisker plot describing the distribution of estimates of *Salmonella* positive carcasses at each processing point.

Cooler to shipped raw product review

716 citations (available upon request) were identified by the searches. Only two manuscripts were available in English that described sampling of pork after chilling at more than one location in a pork processing facility after the cooler. One study was reported in 1983 , and therefore its relevance to modern production systems is doubtful. Two studies also reported the prevalence of *Salmonella* spp. on carcasses and then at one sampling point in the processing chain after the cooler. The information provided by these four studies is documented in Table 2. None of these studies described the stages of processing employed by the study plants. Several potentially relevant articles could not be located and a large number of articles that appeared to be relevant were excluded because they were not published in English.

Conclusions

The slaughter-to-cooler review identified 15 publications describing 40 studies that evaluated the prevalence of *Salmonella* spp. on carcasses. The review provides little evidence that *Salmonella* is introduced into the pork product as it moves along the processing chain to the cooler. On the contrary, the aggregated data across the studies suggest that the processes employed from slaughter to the cooler are associated with steady decreases in *Salmonella* spp. prevalence. The slaughter-to-cooler review provides publically available empirical evidence for the efficacy of the procedures employed in modern pork production systems to control *Salmonella* spp. (Figure 1). The finding that *Salmonella* spp. prevalence consistently decreases during processing, even in a variety of settings, provides evidence that the control processes are robust. This information could be used to convey to the consumer, the efficacy of the measures taken to control *Salmonella* spp. from slaughter to the cooler.

The post-cooler to finished raw product review was unable to identify sufficient studies for an aggregate analysis, similar to that conducted for the slaughter-to-cooler review. From 713 references identified during the literature search, only four manuscripts describing 12 studies reported evaluating *Salmonella* spp. prevalence at multiple points in the post-cooler production process. The results of the post-cooler to finished raw product review indicate a paucity of publically available information about this aspect of pork production. The reviewers

acknowledge that it is possible this information is available but subject to confidentiality agreements. The impact of the paucity of available data is that empirical evidence of efficacy of control programs in the post-cooler side of pork processing is not available.

This paucity of publically available information has previously been noted. Berands et al (1998) noted that information about cutting plants was rare and "practically all of which is published in confidential reports in Dutch or in specialized books of limited circulation"[1].

If the industry or consumers are interested in a public document that summarizes the ecology of *Salmonella* spp. post-chilling in pork then more data are needed. If the reviewers are correct and much of this data is available but confidential, it may be efficient to develop a mechanism, by which, data from already completed confidential studies which have evaluated post-cooling *Salmonella* spp. prevalence could be available for an aggregate analysis, similar to that conducted in the slaughter-to-cooler review. If the reviewers are incorrect and the studies have not been conducted, then primary research in this area could be needed.

It is important to note several potential biases in the review. First it was noted that this review compared to other reviews conducted by our group had a larger number of potentially relevant publications were excluded because of the inability to either obtain a full copy of the manuscript. In this review at least 16 publications that could have been potentially reported to the slaughter-to-cooler review could not be found. To illustrate this issue, these are reported in Table 3. Similarly in the cooler-to-slaughter review 999 articles were identified as potentially relevant after the 1st level screening, 461 (46%) were excluded because the manuscript was not available in English. Unfortunately it is not possible to conclude that the non-English papers were truly relevant to the review. However, we note that Berands et al (1998) did use two Dutch language articles that may have been relevant.

Recommendations for Future Research:

The major data gap identified by the current project was the information about *Salmonella* spp. prevalence after the cooler. As discussed, it was not clear why so little data was identified but the reviewers suspect that it is more likely that this aspect of the ecology is not published rather than not studied. If companies are able to conduct this private research and there is no call from the industry or consumers for this information to be publically available then this is of little concern. Alternatively if it is of interest to have more of this information in the public domain then two approaches could be pursued. As previously discussed, it may be more efficient to identify **a** mechanism, by which, data from already completed confidential studies which have evaluated post-cooling *Salmonella* spp. prevalence could be available for an aggregate analysis, similar to that conducted in the slaughter-to-cooler review. If the reviewers are incorrect and the studies have not been conducted, then primary research in this area could be needed. The focus of those studies however should be to include a design that assesses multiple points in the processing chain. Many studies publish just one point, i.e., retail, or carcass, and this does not provide the type of data required to document changes in *Salmonella* spp. By providing multiple data points within the same study it is easier to observe patterns, control between plant differences, and to draw inference from the aggregated data. We believe this is documented by plotting the point-to-point changes in the slaughter-to-cooler review (Figure 1).

Presentations and Publications

Presentations:
The ecology of Salmonella in pork production: A systematic review. O'Connor AM, McKean JD, Dickson J, Presented to the meeting of the Pre-Harvest Food safety Committee of the National Pork Board Dec 2008

Investigating the epidemiology of Salmonella in pork. A systematic review. O'Connor AM, McKean JD, Dickson J, To be presented to the 8th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork September

Publication:
Investigating the epidemiology of Salmonella in pork. A systematic review. Being prepared for submission

Table 1. Descriptive statistics for the proportion of *Salmonella* positive samples at carcass sampling locations

Carcass Sampling Point	Number of studies reporting	Minimum	1st Quartile.	Median	Mean	3rd Quartile	Maximum.
Bleed	19	0.10	0.26	0.37	0.58	1.00	1.00
Scald	10	0.06	0.095	0. 13	0.23	0.27	0.64
Dehair	10	0.00	0.08	0.17	0.21	0.20	0.88
Singe	9	0.003	0.03	0.20	0.17	0.28	0.40
Polish	8	0.00	0.01	0.06	0.15	0.21	0.48
Eviscerate	8	0.01	0.04	0.05	0.08	0.07	0.32
Wash	11	0.00	0.02	0.04	0.1	0.09	0.55
18-24 hours chilling	21	0.00	0.00	0.00	0.04	0.073	0.30

Figure 1. Line and scatter plot describing the proportion of *Salmonella* positive samples for 40 studies and the median and mean of the studies at each carcass sampling location.

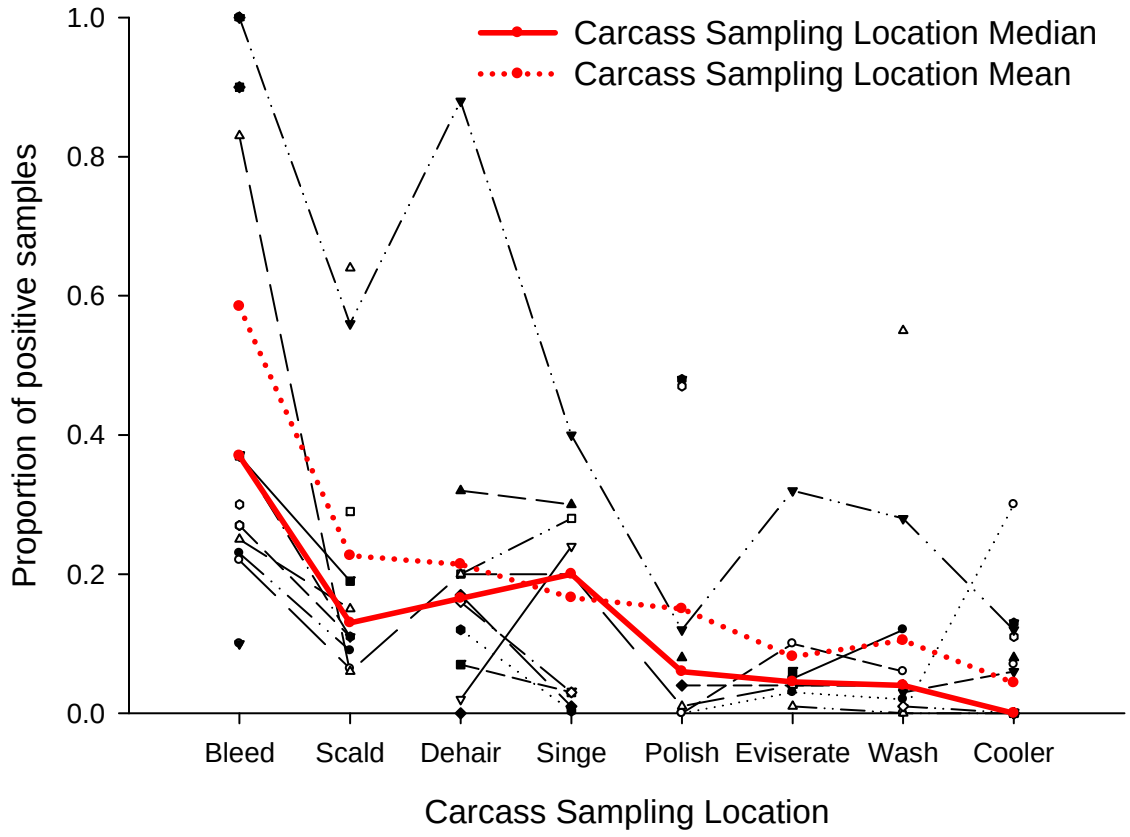

Figure 2. Box and whisker plot describing the distribution of *Salmonella* positive samples for 40 studies and mean of the studied at each carcass sampling location. (N=The number of studies).

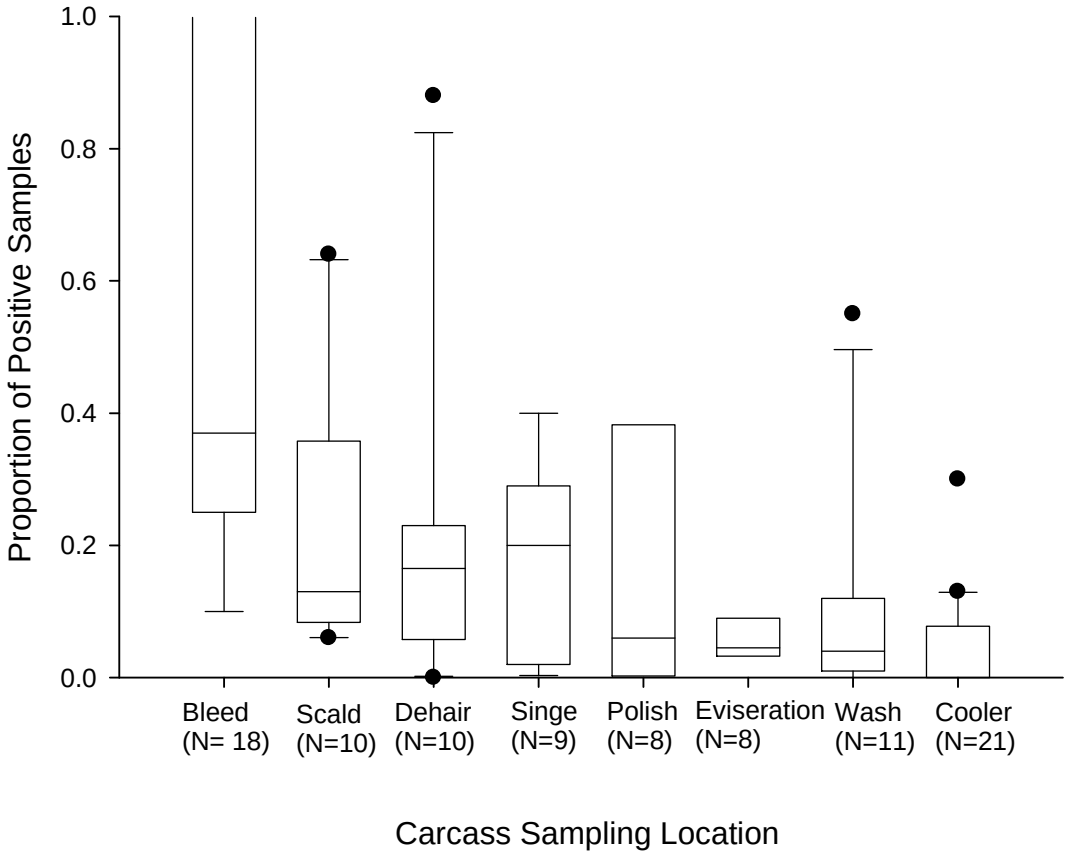

Table 2: Multiple sample point studies post- cooler to finished raw product

Study	Study plants	Year	Country	Sample	Specimen size	Number of samples	Number of replicates per sample	Salmonella spp. positive samples
[2]	1	1980-1981	UK	Linked sausages	1 kg	20	5 replicates each of 60 grams	13 (65%)
				Lean pork	1 kg	15	5 replicates each of 60 grams	6 (40%)
				Belly meat	1 kg	20	5 replicates each of 60 grams	7 (35%)
				Head Meat	1 kg	20	5 replicates each of 60 grams	2 (10%)
				Semi-lean meats	1 kg	20	5 replicates each of 60 grams	7 (35%)
				Rinds	1 kg	20	5 replicates each of 60 grams	6 (30%)
				Back fat	1 kg	20?	5 replicates each of 60 grams	0
[3]	2 plants (600 and 800 pigs per hour)	ND	France	Carcass after 1st chilling	8 pooled samples		1 Cotton swabs for 5 carcasses	7/8
				Carcass during refrigeration before cutting	8 pooled samples		1 Cotton swabs for 5 carcasses	6/8
				Raw ham	2.5 cm^{2} per unit	8 samples (pooled samples)	5 samples of 0.5mm squares for each unit and 10 units	4/8
				Deboned	25 cm^{2}	8 samples		2/8

				and defatted shoulders		(pooled samples)		
				Bellies	25 cm ^{2}	8 samples (pooled samples)		1/8
[4]	6 plants	ND	Korea	Carcass in cooler	swabs	ND	ND	0
				Cuts meats	swabs	ND	ND	0
[5]	3 plants (~1000 per hour)	ND		Carcass in cooler	Swabs of dorsal size of ham and midpoint of loin	15 swabs	Not applicable	0.4% of 270
				Boneless loins	Ventral side, prior to packaging	15 swabs	Not applicable	0.7% of 135 loin samples
				Boneless loins	36 days of storage at 2C	15 swabs	Not applicable	0% of 45 loin samples

ND= not described

Table 3: Slaughter to cooler papers that were potentially relevant but could not be identified

Examples of papers that could not be located
1. Bouvet,J., C.Bavai, R.Rossel, A.Le-Roux, M.P.Montet, C.Mazuy, and C.Vernozy-Rozand. 2003. Evolution of pig carcass and slaughterhouse environment contamination by Salmonella. Revue de Medecine Veterinaire 154:775-779. Reference was unavailable online or at university library, attempted to request through ILL but no results were returned.
2. Canteras,A.C., andJ.C.Bernardo. 1996. Incidence of Salmonella contaminations among slaughtered pigs in selected abattoirs of Metro Manila [Philippines]. Araneta Research Journal 34:71-74. Was said to be located in the Araneta Research Journal however this journal was not published at time this research paper was said to be published
3. Chung,G.T. 1977. Comparison of various sites of slaughtered pigs for the isolation of salmonella organisms. Journal of Veterinary Science Seoul University 2:38-42. Unable to locate: attempted to locate in the Journal of Veterinary Science Seoul University 1977, volume 2 issue 2 pages 38-42 however Journal of Veterinary Science is only recorded to exist from the year 2000 to present. When the publishers website was located it was discovered to be only in Korean.
4. Donahue,J.M., andS.J.Locke. 1985. Salmonellosis in swine in Kentucky. Progress report Kentucky Agricultural Experiment Station 51-52. Was discovered to be in the Kentucky Progress Report in may of 1985 number 284 pages 51-52. This report was not available to us although it was requested through Interlibrary loan.
5. Fuchs,J. 1983. Prevalence of salmonellae of healthy slaughter pigs in Austria. Unable to locate: insufficient information, unable to locate through general internet searches of title and author, more information could not be located.
6. Holst,S. 1993. Salmonella infection in Danish slaughter pigs. Dansk Veterinaertidsskrift 76:645-652. Unable to locate, journal was located for correct date but article could not be found
7. Huisman,W. 1950. The occurrence of Salmonella in healthy pigs. Utrecht. Unable to locate, found information through a general internet search that this was a thesis published in Utretcht but

could not find the publication or any further information, requested article through interlibrary loan with no response.
8. Korsak,N., B.Groven, B.Jacob, G.Daube, and E.Flament. 2002. Prevalence of Salmonella along a meat pork production system. Wageningen, Netherlands: Wageningen Academic Publishers.. Emailed Wageningen Academic Publishers September 18, 2007 received no reply. Article was looked up and referenced to be in the Food Safety Assurance in the Pre-Harvest Phase proceedings but when ordered and looked up abstract was missing.
9. Morgan,I.R., F.L.Krautil, and J.A.Craven. Reduction of salmonella contamination on pig carcases. Insufficient information provided, could not locate attempted general internet search of the authors and title with no results returned.
10. Pless, P. and Koefer, J. Prevalence of Salmonella in Styrian slaughter pigs. 136-137. Proceedings with the Program. Zbornik s programom. Ljubljana (Slovenia, 1998. p. 136-137. Slovene Microbiological Socitey, Ljubljana (Slovenia). Bole-Hribovsek, V., Ocepek, M., and Klun, N. Slovene Microbiological Socitey. Unable to locate: found through general internet search that this reference should be on pages 126 and 137 of the Proceedings that came with the program from the Slovene Microbiological Society but Interlibrary Loan could not locate.
11. Riza,B.F., O.L.Ariza, V, M.F.Bustos, and B.-N.E.Pena. 1983. Prevalence of Salmonella sp. In pigs at 2 summary slaughterhouses in Bogota Columbia. Revista del Instituto Colombiano Agropecuario 18:501-506. Unable to locate, found volume 18, but special issue is not available that this article is assumed to be part of.
12. Schutz,G. 1958. Occurrence of rare salmonella types in bile and faeces of healthy slaughtered cattle and pigs. Unable to locate: insufficient information attempted to gather more information by searching both internet search databases and general internet search for title and author but found no further information
13. Sisak,F.m.s., H.Havlickova, R.Karpiskova, and I.Rychlik. Prevalence of salmonellae and their resistance to antibiotics in slaughtered pigs in the Czech Republic. n.d. was found to be located in the Czech Journal of Food Sciences, however this journal was not located and could not be located by Interlibrary loan.
14. Stern,H. 1938. The Incidence of Salmonella in Abattoir Pigs at Zagreb. Insufficient information available, article title and author names were used in a general internet search and on internet

databases with no results.
15. Tiecco,G. 1965. A search of healthy carriers of Salmonella among regularly slaughtered pigs. Unable to locate, searched databases and a general internet search for author's name and title along with the year of publication but returned no results.
16. Wahlstroem, H., Wierup, M., Olsson, E., and Engvall, A. Prevalence of Salmonella in swine, cattle and broilers after slaughter in Sweden. 141-150. International course on Salmonella control in animal production and products arranged by the National Veterinary Institute of Sweden and the World Health Organization August 1993. A presentation of the Swedish Salmonella Programme. Proceedings. Uppsala (Sweden, Statens Veterinaermedicinska Anstalt. Apr 1994. p. 141-150. #. Oeijeberg-Bengtson, S. Proceedings from Oeijeberg-Bengtson, when a general internet search was conducted it was discovered that this reference should be on pages 141-150 of the journal, however the journal was requested through Inter Library Loan and no journal was found.

APPENDIX

Appendix 1 Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample Location	[6]	[7]	[7]	[8]	[8]
Stun					
Bleed	31% of 24 samples (7.7 carcasses)				
Kill					
Scald	1% of 24 samples (0.24 carcasses)				
Dehair	7 % of 53 samples (3.71 carcasses)				
Singe	0 of 29 samples				
Polish	0 of 48 samples				
Bung removal		6% of 50	0 of 50		
Eviscerate	7% of 20 (1.4 carcasses)			1.6% of 210 pigs (3.36 carcasses)	12% of 209 pigs
Split					
Stamp					
Final wash		12% of 50	0 of 50	2.9% of 210 pigs	0% of 209 pigs
Immediate After Chill	? of 20 . Studied but result not reported	6% of 50	6% of 50		
18 to 24 hours after Chilling		30% of 50 t	6% of 50	0.5% of 210 pigs	0% of 209 pigs

Appendix 2 Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[9]	[9]	[9]	[9]	[9]
Stun Bleed	90% of 100	90% of 100	100% of 100	100% of 100	100% of 100
Kill Scald					
Dehair					
Singe					
Polish					
Bung removal					
Eviscerate					
Split					
Stamp					
Final wash					
Immediate After Chill					
18 to 24 hours after Chilling	0% of 122	0% of 122	0% of 122	0% of 122	0% of 122

Appendix 3: Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[9]	[9]	[9]	[9]	[9]
Stun Bleed	100% of 100	30% of 100	100% of 100	10% of 100	10% of 10
Kill Scald Dehair Singe Polish Bung removal Eviscerate Split Stamp Final wash					
Immediate After Chill 18 to 24 hours after Chilling	0% of 122	0% of 122	0% of 122	7% of 122	0% of 122

Appendix 4. Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[10]	[11]	[11]	[11]	[11]
Stun					
Bleed					
Kill	16 of 17				
Scald	14 of 22				
Dehair		8 of 120	19 of 93	21 of 124	11 of 67
Singe		3 of 120	26 of 93	1 of 129	2 of 67
Polish					
Bung removal					
Eviscerate					
Split					
Stamp					
Final wash	58 of 105				
Immediate After Chill					
18 to 24 hours after Chilling					

Appendix 5 Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[11]	[11]	[11]	[11]	[11]
Stun Bleed				29 of 108	29 of 108
Kill Scald				12 of 108	10 of 108
Dehair	32 of 100	2 of 82	63 of 526		
Singe	30 of 100	2 of 82	2 of 526		
Polish					
Bung removal					
Eviscerate					
Split					
Stamp					
Final wash					
Immediate After Chill 18 to 24 hours after Chilling					

Appendix 6: Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[11]	[11]	[11]	[11]	[11]
Stun Bleed	25 of 108	24 of 108	37 of 108	28 of 108	37 of 108
Kill Scald	7 of 108	12 of 108	16 of 108	21 of 108	31 of 108
Dehair					
Singe					
Polish					
Bung removal					
Eviscerate					
Split					
Stamp					
Final wash					
Immediate After Chill					
18 to 24 hours after Chilling					

Appendix 7: Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[12]	[5]	[4]	[13]	[13]
Stun Bleed	0 from 192				
Kill Scald					
Dehair	0 from 192				
Singe					
Polish	4.4% of 270			12 of 143	69 of 144
Bung removal					
Eviscerate	4.16% from 192		0 from unspecified number of samples		
Split					
Stamp					
Final wash	4.16% from 192	1.1% of 270			
Immediate After Chill					
18 to 24 hours after chilling	0% from 192	0.4% of 270	0 from unspecified number of samples	12 of 142 (9.5%)	18 of 144

Appendix 8. Frequency of Salmonella positive samples at carcass sampling point. Percentages are given if the authors did not report frequency data

Sample location	[13]	[14]	[14]	[15]	[15]
Stun Bleed				25 of 25	58 of 70
Kill Scald				14 of 25	4 of 70
Dehair				22 of 25 (1^{st} dehair) 8 of 25 (2^{nd} dehair)	14 of 70 (1^{st} dehair) 17 of 40 (2^{nd} dehair)
Singe				10 of 25	0 of 50
Polish	68 of 144	0 of 120	0 of 120	3 of 25	1 of 70
Bung removal Eviscerate		3 of 120	12 of 120	8 of 25	2 of 50
Split				8 of 25	7 of 50
Stamp				77 of 300	25 of 200
Final wash		2 of 120	7 of 120	7 of 25	2 of 54
Immediate After Chill 18 to 24 hours after chilling	46 of 430			3 of 25	

Appendix 9: Detailed description of the plant used in the slaughter to cooler review

Paper number	Description of plant system
6	Animals were held in lairage, stunned using carbon dioxide, transferred into the ‘‘wet’’ room, and immediately exsanguinated by severing of the carotid arteries and jugular vein. Exsanguinated animals were scalded for approximately 8 min using a linear ‘‘scald tank’’ (61F1 ^{0}C). Scalded carcasses were dehaired using a rotating drum with scrapers that flailed the carcass surface, dislodging hair and skin debris. Dehaired carcasses were secured to an overhead conveyor rail by insertion of a gambrel hook into the hind leg tendons. Carcasses were then passed through a singer operating at approximately 1200 ^{0}C for 15 s. Singed carcasses were polished by passage through a series of horizontal and vertical flails in a process that lasted approximately 5 min. Polished carcasses were moved into a separate evisceration area. Carcasses were ‘‘debunged’’ by cutting around the rectum with a knife, which had been immersed in water heated to 82 ^{0}C before use. The detached rectum was sealed with a plastic bag to prevent fecal contamination of carcasses during subsequent processing. The belly was opened, and the diaphragm, heart, lungs, trachea, and the digestive tract, were removed. Carcasses were manually split along the midline, from the hind to the fore using a splitting saw, the heads were removed, and the spinal cord excised. Carcasses were then trimmed, weighed and graded, before spray washing for approximately 10 s with cold potable water containing between 0.8 and 1.2 ppm chlorine (to remove bone dust and blood clots). Washed carcasses were chilled to between 2 and 4 ^{0}C overnight.
7	Experiments were carried out in a Dutch pig slaughterhouse. Slaughter techniques and procedures were stunning, bleeding, scalding, flaming, singeing, polishing, bung removal, evisceration, pluck removal, splitting of carcass, veterinary inspection, classification, cleaning up carcasses. At the slaughterhouse approximately 500 pigs per hour on one slaughter line. In this slaughterhouse, carcasses were flamed at 600 8C, followed by polishing with rubber beaters and rotating brushes. Scalding took place by complete immersion of the pigs in a scalding tank containing water of 60 8C, and the bung dropper used sucked up faeces from the rectum, so that leakage of faeces on the carcass was avoided. Splitting the carcasses was done by automatic carcass splitters two splitters in the line, which one by one split the next carcass. (,Swanenburg, M., Urlings, H.A.P., Snijders, J.M.A., Keuzenkamp, D.A., Van Knapen, F., 2001b. *Salmonella* in slaughter pigs: prevalence serotypes and critical control points during slaughter in two slaughterhouses. Int. J. Food Micro. 70, 245–256.)
8	3 abattoirs, no processing information provided

9	Medium size plant, approximately 7,000 swine/day. The general processing steps in the plant included animal holding, electrical stunning, exsanguination, scalding, dehairing, washing, evisceration, and overnight chilling prior to deboning and further fabrications.
3	Samples were collected from two pork slaughter and cutting plants in France. The slaughterhouses process 600 and 800 pigs per hour, respectively. Processed included lairage, killing, scalding, dehairing, flaming, polishing, evisceration, splitting, first chilling, transportation by trucks, storage at 4^0C, cutting.
10	1970's abattoir with automatic conveyance system 90% of pigs slaughtered imported from China (up to 1 week transport) Electric stun, suspended by one leg on conveyor, stick and bleed, exsanguination, rinsing with overhead sprinklers for several seconds, scalding tank for 5 mins at 60 ^{0}C, dehair, ventral splitting evisceration, inspection, rinsing with overhead sprinklers, market
11	6 total slaughterhouses 4 with scraping machine 2 with hand scrapers In all plants: Bled Scalding tub (62ºC) Dehairing/Scraping with machine or hand scrapers, Final cleaning, Singed. No further details provided.
11	Two slaughter plants with a singe oven after scalding tub and scraping machine. Pigs passed though the cylindrical singe oven at 1200-1400ºC for 10-12 seconds. Then passed under a cold-water sprinkler followed by a black-shaving machine designed to scrapes off what was burned in the scald oven
11	Same as study I
12	Processing included, stick, scald/dehair, wash, evisceration, wash before transport, transport before chilling, chilling. No further details provided
5	Three plants located in Midwest, had comparable line speed approximately 960 to 1000 heads per hour.
4	6 different swine farms and slaughterhouses
13	Not described
14	Two commercial pig slaughterhouses. Slaughterhouse A slaughtered 350 pigs per hours and slaughterhouse B slaughtered 650 pigs per hours. Both used the same undescribe procedure except the scald tank. In slaughterhouse A carcasses were scalded with water vapor. IN slaughter house B a scald tank was used (60 ± 1^0C)
15	Small older style abattoir for one visit an one large modern abattoir for 8 separate visits
16	Not described

17	Not described

Appendix 10: Carcass sampling methods as described by the authors in the slaughter to cooler review

Paper number	Carcass sampling methods
6	Carcasses were tagged and tracked through the dressing process. At each sample stage, the entire outside surface of each tagged half carcass was swabbed using an individual swab prepared as previously described (reference in original manuscript).
7	The swab method was described by Van den Elzen and Snijders Ž1993. and was validated for *Salmonella* isolation by Swanenburg Ž2000. Swabs were made of disposable diapers with no plastics nor preservatives. The disposable diaper was cut into two equal pieces and sealed with sterilisation tape. These swabs were packed into aluminum foil and sterilized during 15 min at 121 8C. After sterilization, the swabs were packed separately in stomacher bags under sterile conditions. Shortly before sampling, the swabs were moistened with 50-ml buffered peptone water (BPW, pH 7.0).with 0.1% Tween. With a sterile glove, the swab was taken out of the bag and the sampling area was swabbed. After replacing the swab in the stomacher bag, another 50-ml BPW– Tween was added. The swab was massaged in a stomacher for 2 min, then the swab was squeezed out and removed. A 50-ml of BPW–Tween was added to the swab samples from carcasses Then the swabs were put in a stomacher for 2 min, whereafter they were squeezed out and removed from the stomacher bag.
8	$0.1m^2$ area running 10 cm up and across from the midline, starting at the level of the elbow., followed by sampling of the jowl area. Sterile moppet sponges moistened with buffered peptone water. All samples were kept at ambient tempterature
9	Carcass sponge following FSIS sponge protocol (see original paper for reference page 1306). Briefly, 10 ml of 0.1% peptone water was added to Whirlpack bags containing a single sponge. A single sponge was used to sample three areas (approximately 1000 cm^2 per area), including the side of the neck, belly and the ham. This process was repeated with a second area in the same approximate area. The sponges were returned to the Whirlpak bags and transported back to the lab on ice, within 30 minutes. Microbiological analysis began with 18 hours.
3	Most samples were collected by a swabbing technique performed with sterile cheese-cloth (CC) cut into 20 X 20 cm squares, pre-moistened with buffered peptone water (BPW), and placed in sterile boxes. Sterile gloves were used and changed between two different sampling sites. Two CC were used for each kind of pork sample, e.g. pigs and carcasses at various stages. A single CC was then used to sample ®ve units (pigs or carcasses). Carcasses on slaughter lines, from killing to the end of evisceration, were swabbed over the largest possible area, with particular attention to section lines and any areas soiled by intestinal and gastric contents. For each series, ®ve intestinal tracts from the carcass group were kept for collection of mesenteric lymph nodes (one per carcass) using sterile scalpels. Hanging half-carcasses from the ®rst chilling to the step before cutting were sampled according to the procedure described by Emborg et al. (1996), i.e., swabbing of the pelvic cavity and medial surface of the ham, together with the sternum and abdomen along the incision line. According to the procedure used here, sampling of half-carcasses was completed by swabbing of the necks. Pork cuts (hams, shoulders and bellies) were sampled by both swabbing and excision techniques. Excision was performed on the rind, near the anus for ham, on the ventral section line for bellies and on an external site for shoulders For each kind of pork cut, excision was performed with sterile scalpels by cutting ®ve 0á5 mm thick squares measuring approximately 25 cm2 on 10 different units, which were dispatched into two stomacher bags for the two analyses. Swabbing was then carried out using one CC to sample the total surface of ®ve units. The samples were immediately placed in refrigerated boxes and transferred to the food laboratory where they were processed the same day.

10	Body surface swabbed. No further description of surface area, location or sample collection device.
11	Skin scraping from behind ears , the two lateral surfaces of the crass and the flexures of the groins. No further details provided
11	Skin scrapings. No description area, location or sample collection methods provided. Further the entire pig was then taken to the laboratory in a sterilized paper bag. In the laboratory, 22 different prices of skin the size of the palm of the hand were ground to powder and tested for *Salmonella* presence.
11	Skin scrapings, of the abdomen and flanks, the area behind ears, inguinal region were scraped off with a sharp instrument
12	Swabs from 4 locations per sample point at jowl, rump, back and brisket. Sterile swabs (3 cm long and 1 cm in diameter) moistened with 0.1% peptone water, used. A 100 sq. cm area was marked with a sterile metal frame having dimensions of 10x 10 cm for each site of the carcass. The swabs were rubbed on sites continuously for 30 sec and transferred to a screw-capped-test tube containing 10mls of sterile maintenance medium (0.85% saline and 0.1% peptone)
5	On the dorsal side of the ham and midpoint of the loin. Sterile cotton swabs were first aseptically dipped into a 9 ml screw cap test containing 0.1% phosphate buffer saline solution adjusted to a pH of 7.0 into 7.2. While held at approximate 30^0 angle the swabs were stroked across the meat surface firmly and uniformly 12 to 15 times inside a 100 cm^2 template. The swabs were then rotated and stroked 12- to 15 times perpendicularly to the 1^{st} swabbing direction. After swabbing the swabs were placed in a screw-cap tubes containing PBS solution (volume not described). Transported on ice to lab.
4	Swabbed at belly, ham and jowl using one sterile swine and template in accordance with the U.S. Department of Agriculture manual (reference in original manuscript) Cut meat swab
13	Carcasses were swabbed following the USDA protocol (see manuscript for original reference) with one modification: the order of swabbing was belly, jowls then ham.
14	Carcasses were sampled by vigorous rubbing of ham, belly jowl and back.
15	$0.1m^2$ carcass surface as a 10 cm band running up from the neck along the ventral incision line. The carcass were vigorously swabbed with a sterile surgical gauze swab.
16	Not described
17	Not described

Appendix 11: Culture methods used to detect *Salmonella* as described by the authors in the slaughter to cooler review

Paper number	Culture methods
6	Swabs were stomached with 100 ml of buffered peptone water (BPW, Oxoid) for 1 min and incubated at 37 ^{0}C for 24 h. After incubation, a 0.1-ml aliquot of the enriched culture was transferred into 10 ml of Rappaport Vassiliadis (RV) medium (Oxoid) and incubated at 42 ^{0}C for a further 24 h. Enrichment cultures from swabs and from scald tank water were streaked out onto Brilliant Green Agar (BGA, Oxoid), incubated at 37 ^{0}C for 24 h, and examined for red colonies (presumptive Salmonella). The enrichment cultures were also streaked out onto Mannitol Lysine Crystal Violet Brilliant Green (MLCB, Oxoid) Agar, incubated at 37 ^{0}C for 24 h, and examined for large black colonies (presumptive Salmonella). Both types of presumptive Salmonella were recovered, purified and cultured on non-selective media (TSA, Oxoid) at 37 ^{0}C.
7	All samples were incubated at 37 8C for 18–24 h. *2.2.2.* The pre-enrichment broth was mixed and 0.1 ml was transferred to 9.9-ml pre-warmed Rappaport– Vassiliadis enrichment broth ŽRV, Oxoid CM 669, Oxoid, Haarlem, The Netherlands.. This was incubated in a water bath of 42 8C for 24–48 h. *2.2.3.* A loop of material from the RV broth was transferred and spread onto the surface of a brilliant green agar plate ŽBGA modified, Oxoid CM 329., so that isolated colonies could develop. The plates were incubated in inverted position at 37 8C for 18–24 h. After incubation, the plates were checked for growth of typical *Salmonella* colonies Slightly transparent, reddish color... When no typical colonies were found after 24 h of incubation, a loop of RV Ž48 h incubated.
8	Entire carcass swab pre-enriched in BPW and incubated or 16-24 hours at 37^0 C . This was followed by selective enrichment in both Rappaport Vassiliadis broth and semi-solid Rappaport Vassiliadis medium at 41.5 ^{0} C for 18 to 24 hours and subculture onto brilliant green agar and mannitol lysine crystal violet brilliant green agar at 37 0 C for 20 to 24 hours. 5 colonies selected were cultured and incubated on McConkey agar and incubated at 37^0 C for 18-24 hours . Colonies confirmed using inoculation of triple sugar iron agar slopes and lysine decarboxyylase broths, followed by serotyping.
9	The Vidas methods (see original paper for reference page 1306). Approximate 25 ml of lactose broth added to each Whirlpak bag., then bags massaged, then pre-enriched fro 18 hours at 35^{0}C . Next 1.0 ml of incubated lactose broth was transferred to a test tube containing 10 ml of selentine cysteine broth and another 10 ml transferred to a test tube containing 10 ml tetrathionate broth. The tubes were incubated for 6 to 8 hours at 35 and 42 ^{0} C respectively. After selective enrichment, 1.0 ml of each broth was transferred to separate tube of M-broth and incubated for 18 hours at 42^{0}C.

The selenite cystein and tetrathionate broths were reincubated for 24 hours at 35 and 42 ^{0}C respectively. In addition, 1ml portions transferred to M-broth. One ml of each M-broth was transferred to a separate tube and incubated at in a water bath at 100^{0}C for 15 min. The boiled both samples were analyzed using the Vidas instrument. Samples with a value ≥ 0.23 absorbance were considered positive. When a positive Vidas reaction occurred, stored M-broth was streaked to enteric agar, bismuth sulfite agar and xylose-lysoine-deoxychocolat agar and incubated for 16-18 hours at 37^{0}C. *Salmonella*-like colonies were stabbed into triple sugar iron and lysine iron agar slants. If positive the isolates were tested with *Salmonella* o- antiserum poly A-I and Vi and with a mixture of 7 H antisera. Isolates that agglutinated by *Salmonella* polyvalent A-I and Vi were considered presumptive *Salmonella* spp.

3 The Salmonella detection method was based on the AFNOR V08-52 method (see original paper for reference), slightly modifed as shown in Fig. 2. Each CC was transferred into a stomacher bag with 100 ml of buffered peptone water (BPW) (Merck). BPW (100 ml) was added to each sample consisting of +ve excision units. The mesenteric lymph nodes from a group of pigs were aseptically lacerated with scalpels and placed together in a stomacher bag with 100 ml BPW. All samples were homogenized for 2 min with a stomacher (AES Laboratoires, Combourg, France). The pre-enrichment broths were then incubated for 18±20 h at 37°C, after which three enrichment media were used. Pre-enrichment culture (2 ml) was transferred to 20 ml Muller-Kaufmann tetrathionate broth (AES Laboratoires) supplemented with 0á1% brilliant green (Merck Clevenot) and novobiocin (AES Laboratoires), and 1 ml pre-enrichment culture was transferred to 9 ml KIMAN broth (Blivet et al. 1998), a Whitley Impedance Broth basal medium (Shipley, UK) supplemented with novobiocin, malachite green and potassium iodide. These enrichment broths were both incubated for 24 h at 37°C. In parallel, three drops of 50 ll of the pre-enrichment culture were transferred to the surface of modifed semi-solid Rapapport-Vassiliadis agar (MSRV; Oxo) (De Smedt et al. 1986). The plates were incubated for 24±48 h at 42°C. Muller-Kauffmann tetrathionate and KIMAN enrichment broths were streaked onto XLT4 (Difco) and were subsequently incubated for 24 h or 48 h at 37°C. MSRV plates showing migration zones were further investigated by streaking material from the edge of the zone onto Rambach Agar (Merck) (Rambach 1990). Up to three suspect colonies from XLT4 and Rambach media were streaked onto nutrient agar and subsequently tested for identifcation. Several confirmation tests were performed, including biochemical characterization on Kligler Hajna medium (AES Laboratoires). Up to nine isolates per sample were therefore isolated and preserved for serotyping and genotyping. Salmonella isolates were stored at ±70°C in brain heart infusion (BHI, Difco) supplemented with 15% glycerol (v/v).

10 All specimens collected in G.N. broth were incubated at 37^{0}C at 6-8 hours before subculture into U shaped tubes containing a semi-solid enrichment- indicator medium (see original paper for reference). Enrichment medium contained magnesium chloride, brilliant green and novobiocin as the main selective substance while the indicators medium layer on the former was a H_2S production medium selective for *Salmonella*. After incubation at 41 ^{0}C for 42- 48 hours, bacterial

	growth of *Salmonella* at the un-inoculated end of the semi-solid medium was sub cultured on McConkey agar and colonies picked for serotyping.
11	Specimens concentrated in tetrathionate broth to which brilliant green and ox bile were added using the Muller Kauffman method (see original paper for reference). Smears from the enrichment medium were spread out across brilliant green-phenol red agar plates, 14 cm in diameter with 18- 24 hours an a second time within 64 to 72 hours. After incubation at 37)C for 18 to 24 hours suspected colonies were inoculated into triple sugar iron agar and studies biochemically and serologically if required.
11	As above.
11	Used method described in reference in paper, with modification of two enrichments instead of one with the MULLLER KAUFFMAN medium and selenite brilliant green broth.
12	Test-tube containing swabs were shaken on Remi cyclomixer for 30 sec. Sample swabs were pre-enriched in buffered peptone water ,enriched in selenite cystein broth and inoculated on brilliant green sulpha agar and bismuth sulphide agar.
5	Isolation methods from reference listed in original text. Samples were pre-enriched in lactose broth followed by enrichment in tetrathionate broth. Isolation was done by plating on brilliant green and *Salmonella shigella* agar. Typical colonies were identified by triple sugar iron and lysine iron agar test.
4	Original manuscript contains reference to methods. Samples were enriched with selenite F broth and plated on MacConkey agar. Typical colonies were selected and streaked to Rambach agar. Red colonies were selected and finally confirmed with Viteck.
13	Entire swab enriched for 24 hours in buffered peptone water. 1ml of the broth was transferred to tetrathionate broth and 0.1 ml to Rapport-Vassisiadis broth. The TB and RV were incubated at 35^0C and 42^0C respectively. 150 micro l of TB and RVB were combined and screened for *Salmonella* using real-time PCR. R-PCR positive samples were cultured. An isolated from carcass swab was analysed using PFGE using Pulsenet methods (see manuscript for original reference).
14	Pre-enrichment step with buffered peptone water (37^0C, 18 h), selective enrichment in Rappaport-Vassiladis broth (42^0C, 48 h) and final plating on XLT4 agar. Suspect colonies were identified by means of API20E and *Salmonella* isolated further serotypes according to Kauffman-White-Leminor scheme.
15	Pre-enriched in buffered peptone water at 37^0C for 18 hours. O.2 ml was then inoculated into a Petri dish containing 20 ml of DIASSALM medium. After 24 and 48 hours inoculation at 41.5^0C were streaked onto Rambach agar using a 1 microL disposable loop. The Rambach agar plates were incubated for 24 hours at 41.5^0C and suspected *Salmonella* colonies were confirmed by serotyping. Semiqualitive estimates of *Salmonella* were carried out by vigorous shaking and decimal dilution of sample in BPW immediately after sampling and culturing each dilution of *Salmonella* as above.

16	No description provided
17	No description provided

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