Executive Summary

Project Title: *Salmonella* Levels in Bone Marrow and Neck Skin of Turkey that are utilized for Ground Turkey in Relation to *Salmonella* in Spleen as Predictor.

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Objectives:

1) Determine *Salmonella* levels (presence/absence and numbers) in drumstick bone marrow, spleen, and neck skin samples.
2) Determine the relationship between *Salmonella* levels in these sample types and *Salmonella*-contamination of ground turkey.

Conclusions:

Salmonella presence externally on turkey neck skin and internally in spleen and bone may contribute to *Salmonella* contamination of ground turkey. However, neck skin may pose a significantly higher risk to ground turkey contamination. *Salmonella* presence at quantifiable numbers internally in spleen and/or bone and in neck skin may indicate a highly contaminated flock that resulted in ground turkey contamination. This study provides a possible explanation of the higher *Salmonella* prevalence in ground turkey compared to that on turkey carcasses.

Deliverable:

1. Neck skin appears to be a more significant risk factor to ground turkey contamination compared to internalized *Salmonella* in spleen and bone.

2. Turkey flocks with *Salmonella*-positive bones and/or spleens are likely to produce contaminated ground turkey finish products.

Technical Abstract

The objective of this study was to determine *Salmonella* levels (presence and numbers) in turkey drumstick bone, spleen and neck skin samples in relation to *Salmonella* contamination levels in ground turkey at the flock level. A total of 300 samples of each turkey part (i.e., neck skin, spleen, drumstick) from 20 flocks were collected at a commercial turkey processing plant after evisceration and tested for the presence and number of *Salmonella* using most probable number (MPN) and enrichment methods. Ground turkey samples were collected and analyzed for *Salmonella* presence and numbers by the cooperating turkey company as part of the routine sampling and testing plans. The flocks were classified as targeted and non-targeted based on the farm/flock historical *Salmonella* contamination data in the ground product. The outside surface of bone and spleen were sterilized prior to *Salmonella* analysis. The overall *Salmonella* prevalence in bone, spleen, neck skin and ground turkey samples was 9.3%, 6.7%, 42.0%, 14.5%, respectively. *Salmonella* prevalence in neck skin, spleen, bone and ground turkey from the targeted flocks was significantly higher than those from non-targeted flocks ($P< 0.05$).Within the targeted flocks, *Salmonella* prevalence and numbers in neck skin samples from ground turkey *Salmonella*-positive flocks were significantly higher than those from ground turkey *Salmonella*-negative flocks ($P<0.05$). When *Salmonella* was present in spleen and/or bone (at MPN >1 log), and in neck skin (MPN>2 log), the ground turkey lot was *Salmonella*-positive. Our findings suggested *Salmonella* presence at higher levels in neck skin but lower in spleen and bone may indicate a highly contaminated flock that resulted in ground turkey contamination.

Objectives:

1) Determine *Salmonella* levels (presence/absence and numbers) in drumstick bone marrow, spleen, and neck skin samples.
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Materials and Methods:

In cooperation with a turkey production company, 15 drumsticks, 15 spleens, and 15 neck skins were collected per flock (with 3 parts per carcass) post evisceration and USDA inspection. The flocks sampled were either targeted or non-targeted selected. A flock originated from a turkey farm that has produced one or more flock with a high *Salmonella* prevalence (i.e., > 20%) in the ground turkey was labeled as a 'targeted flock'. Other turkey flocks/farms with a history of low or no *Salmonella*-positives in the ground turkey product were labeled as 'non-targeted flocks'. In this study, 13 flocks were targeted and 7 were non-targeted. All samples were tested for the number and presence of *Salmonella*. Neck skins were weighed and stomached and then tested for *Salmonella*. Bones were extracted from drumstick meat, sterilized from outside, crushed aseptically to release the bone marrow, and tested for *Salmonella.* Spleens were sterilized from outside and then test for *Salmonella*. *Salmonella* prevalence and numbers was determined according to selective enrichment and most probable number (MPN) method following USDA-FSIS protocols. Ground turkey samples were collected and tested internally by the cooperating turkey company.

Results:

The overall *Salmonella* prevalence and mean $\log_{10}$MPN/sample in turkey parts and ground turkey is shown in Table 1. *Salmonella* prevalence and mean $\log_{10}$ numbers in turkey parts and ground turkey by flock type is shown in Table 2. *Salmonella* prevalence in neck skin, spleen, bone and ground turkey from the targeted flocks were significantly higher than those from non-targeted flocks ($P< 0.05$). As for *Salmonella* numbers, there was no significant differences ($P> 0.05$) between those in neck skin samples in targeted flocks compared to the numbers in non-targeted flocks. *Salmonella* numbers were undetectable via the MPN method in turkey bone, spleen and ground product samples from the non-targeted flocks. The distribution of the mean $\log_{10}$ MPN of *Salmonella* in turkey parts by flock type is shown in Figure 1. Within *Salmonella* MPN positive samples, 91% of *Salmonella* MPN numbers of bone samples and 50% of that in spleen samples in the targeted flocks fell in the low number interval (i.e., 0.5-1.5 $\log_{10}$ MPN/sample). For skin samples, 39% of *Salmonella* numbers in targeted flocks and 41% of *Salmonella* number in non-targeted flocks fell in the 1.6-2.5 logs interval. The percentage of *Salmonella* numbers in skin samples from targeted flocks that fell in the high interval (3.6-3.9 logs) were10 times higher than that in skin samples from non-targeted flocks.

Salmonella prevalence and numbers within the targeted flocks by ground turkey *Salmonella*-status is shown in Table 3. Among the 13 targeted flocks collected, five flocks resulted into ground turkey lot being *Salmonella*-positive (i.e., at least one positive sample).*Salmonella* prevalence and numbers of neck skin samples in ground turkey *Salmonella*-positive flocks were significantly higher than those in ground turkey *Salmonella*-negative flocks ($P<0.05$). There was no significant difference between *Salmonella* prevalence of turkey bone and spleen samples in ground turkey *Salmonella*-positive and negative flocks ($P>0.05$). When *Salmonella* was present in spleen and/or bone (at MPN >1 $\log_{10}$), and in neck skin (MPN>2 $\log_{10}$), the ground turkey lot was *Salmonella*-positive (data not shown).

TABLE 1: *Overall* Salmonella *prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses*

Sample type	No. of samples	No. of Positive	Prevalence	Mean $\log_{10}$MPN/sample[a]	95% CI[a]
Bone	300	28	9.3%	1.3	1.2-1.4
Spleen	300	20	6.7%	1.5	1.0-2.0
Neck skin	300	126	42.0%	2.4	2.2-2.6
Ground turkey	117	17	14.5%	1.9	1.1-2.6

[a]Mean log most probable number (MPN) of *Salmonella* per sample and its 95% confidence interval (CI).

TABLE 2: Salmonella *prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses by flock type*[a]

Sample type	Targeted flocks (n=195 carcass)				Non-targeted flocks (n=105 carcass)			
	Prevalence	95% CI	Mean	95% CI	Prevalence	95% CI	Mean	95% CI
Bone	13.8%[A]	9.7%-19.4%	1.3	1.2-1.4	1.0%[B]	0.17%-5.19%	-[b]	
Spleen	10.3%[A]	6.7%-15.3%	1.5	1.0-2.0	0%[B]		-	
Neck skin	51.3%[A]	44.3%-58.2%	2.5	2.3-2.7	24.8%[B]	17.5%-33.8%	2.0	1.6-2.4
Ground turkey	18.8%[A]	11.9%-28.4%	1.9	1.1-2.6	3.0%[B]	0.5%-15.3%	-	

[a]Comparison of prevalence: Values within the same row followed by the same uppercase letter were not significantly different (P>0.05).Means of neck skin samples were not significantly different (P>0.05). Statistical comparisons were based on Chi-square (for prevalence data) and independent t-test (for mean data) using STATA statistical software version 10.1 (Stata Corp., College Station, TX).

[b]"-"*Salmonella* numbers were undetectable via MPN method.

TABLE 3: Salmonella *prevalence and numbers in drumstick bones, spleens, and neck skins from commercially processed turkey carcasses by ground turkey Salmonella-status in targeted flocks*[a]

Sample type	Ground turkey *Salmonella*-positive flocks (n=75carcasses)				Ground turkey *Salmonella*-negative flocks(n=120 carcass)			
	Prevalence	95% CI	Mean	95% CI	Prevalence	95% CI	Mean	95% CI
Bone	21.3%[A]	13.6%-31.9%	1.3	1.2-1.4	9.2%[A]	5.2%-15.7%	-[b]	-
Spleen	14.7%[A]	8.4%-24.4%	1.5	1.0-2.0	7.5%[A]	4.0%-13.6%	-	-
Neck skin	86.7%[A]	77.2%-92.6%	2.6	2.4-2.9	40.8%[B]	32.4%-49.8%	1.9	1.5-2.2

[a] Comparison of prevalence: Values within the same row followed by the same uppercase letter were not significantly different (P>0.05).Means of neck skin samples were significantly different (P <0.05). Statistical comparisons were based on Chi-square (for prevalence data) and independent t-test (for mean data) using STATA statistical software version 10.1 (Stata Corp., College Station, TX). .

[b] "-" *Salmonella* numbers were undetectable via MPN method.

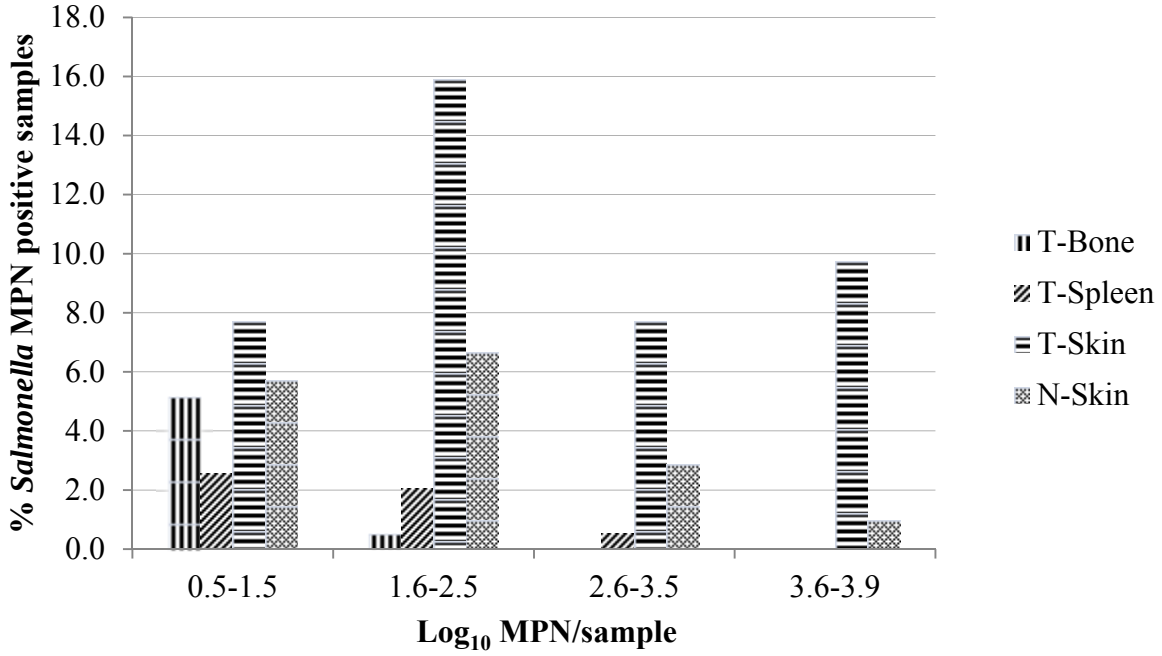

FIGURE 1. *Percentage bar chart illustrating the log Most Probable Number (MPN) distribution of* Salmonella *on turkey bone, spleen and neck skin in targeted and non-targeted flocks.* Salmonella *numbers were undetectable on turkey spleen and bone from non-targeted flocks. T: Targeted flocks, N: Non-targeted flocks*

Conclusion

Salmonella present in external surface of turkey neck skin and internalized sources of spleen and bone may contribute to *Salmonella* contamination of ground turkey. However, neck skin appears to be a more significant risk to ground turkey contamination. *Salmonella* presence at quantifiable numbers internally in spleen and bone and in neck skin may indicate a highly contaminated flock that resulted in ground turkey contamination. This study provides a possible explanation of the higher *Salmonella* prevalence in ground turkey compared to that on turkey carcass.

Recommendations for future research

Further research is needed to characterize the isolates from this study using serotyping and the molecular subtyping to better understand how *Salmonella* becomes internalized in turkey parts/organs and if the strains detected in the parts are similar to those in ground turkey. Additionally, the prevalence, numbers and serotype distribution on different skin parts is needed to be determined in relation to ground turkey contamination.

Presentation and publications

1. Cui, Y., W.Q. Alali, M.A. Harrison, C.L. Hofacre. *Salmonella* levels in turkey neck skin, bone marrow and spleens. International Poultry Scientific forum, Atlanta, 27 January 2014.
2. Alali et al. *Salmonella* levels in turkey neck skin, bone, and spleens in relation to ground turkey. American Meat Institute Foundation Show Case. Atlanta, Georgia. 30 January 2014 (Invited).
3. *Salmonella* in poultry internal parts and skin in relation to ground product. USDA-FSIS Science and Technology Seminar Series (via telephone), Washington, DC, February 24, 2014.
4. Cui, Y., W.Q. Alali, M.A. Harrison, C.L. Hofacre. *Salmonella* levels in turkey neck skin, bone marrow and spleens. The Twentieth Annual Meeting of Center for Food Safety-University of Georgia, Atlanta, 5 March 2014.
5. Alali, W. Q., D. Wu, Y. Cui, C. Celik, C. L. Hofacre. Neck skin more significant risk in ground poultry contamination. *WATT PoultryUSA*. June 2014. Pages: 32-36.
6. Cui, Y., W.Q. Alali, M.A. Harrison, C.L. Hofacre. *Salmonella* levels in turkey neck skin, bone, and spleens in relation to ground turkey production. International Association for Food Protection (IAFP), Indianapolis, Indiana, 28 July 2014.
7. Cui, Y., W.Q. Alali, M.A. Harrison, C.L. Hofacre. *Salmonella* levels in turkey neck skin, bone, and spleens in relation to ground turkey production. *In preparation* for submission to *Journal of Food Protection*.
8. Alali et al. External vs internal presence of *Salmonella* in chicken and turkey in relation to ground finish products. To be presented at the US Poultry and American Meat Institute Educational session at the International Production and Processing Expo. 28 January 2015.