

# Survival of *Salmonella* in dried chicken meat residues on the surface of packaging materials

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**Abstract**— *Salmonella* contamination in raw chicken products may be transferred on materials used for meat packaging or re-packaging, which could then potentially serve as sources of cross-contamination of surfaces or foods in the home. This study evaluated survival of *Salmonella* in chicken meat residues on stored food packaging materials. Samples (5×5 cm) of aluminium foil, butcher paper, cardboard, PVC overwrap film, and vacuum bags were spot-inoculated (0.5 ml; 2-3 log CFU/cm<sup>2</sup>) with a 7-strain mixture of *Salmonella* suspended in a non-sterile raw chicken meat/water homogenate (10% wt/wt), simulating chicken meat purge. Materials were stored aerobically in petri dishes at 4 or 25°C, and were periodically analyzed (two replicates with three samples per material each time) for survivors on tryptic soy agar and XLD agar. Initial levels (2-3 log CFU/cm<sup>2</sup>) of *Salmonella* increased to 4-5 log CFU/cm<sup>2</sup> within 4 days of storage at 25°C on all tested materials, except cardboard. After 123 days at 25°C, *Salmonella* was recovered from all the tested materials, with counts ranging from <0.82±1.09 (cardboard) to 4.79±0.70 (butcher paper) log CFU/cm<sup>2</sup>. Counts decreased on all materials stored at 4°C and reached non-detectable levels (<0.40 log CFU/cm<sup>2</sup>) on cardboard and PVC overwrap film by day-39 and -53, respectively. Survivors of not more than -0.32±0.19 and -0.20±0.49 log CFU/cm<sup>2</sup> were recovered from butcher paper and vacuum bags, respectively, after 88 days at 4°C. *Salmonella* can survive in food residues present on packaging materials for long periods of time. Thus, cross-contamination should be considered when handling or storing soiled packaging materials.

**Keywords**— *Salmonella*, chicken meat residues, packaging materials

## I. INTRODUCTION

A total of 5,550 foodborne outbreaks were reported in the European Union in 2009, resulting in 48,964 cases of human illness, 4,356 hospitalizations and 46

deaths. The causative agent of most (31.0%) of these outbreaks was *Salmonella* [1]. Historically, the food most commonly associated with salmonellosis cases is raw or undercooked poultry. *Salmonella* contamination associated with raw chicken products may be transferred onto the materials in which they are packaged or stored. A limited number of studies [2, 3] have investigated the prevalence of this pathogen on the packaging materials of raw poultry meat. Specifically, Harrison *et al.* [3] reported that 11% of packaging materials (n=300) associated with raw poultry meat obtained from supermarkets and butcher shops were positive for *Salmonella*. Within the home, handling of the raw chicken packaging may lead to cross-contamination of utensils, kitchen work surfaces, refrigerator surfaces, and ready-to-eat foods. Studies have shown that the packaging material is often not removed from the preparation area during food preparation [4]. Literature data on the survival of *Salmonella* in chicken meat residues on the external surface of packaging materials are not available. Therefore, the aim of this study was to evaluate survival of *Salmonella* in chicken meat residues on various food packaging materials stored for up to 123 days at 25 or 4°C.

## II. MATERIALS AND METHODS

### A. Bacterial strains and inoculum preparation

The *Salmonella* inoculum used in the study was comprised of a mixture of seven strains of chicken or turkey origin. The strains were individually cultured twice in 10 ml tryptic soy broth (35°C, 22 h), following which, the cells were harvested by centrifugation (4,629×g, 15 min, 4°C), washed with 10 ml phosphate-buffered saline (pH 7.4) and then centrifuged again. Washed cell pellets of individual strains were resuspended in 10 ml sterile raw chicken

meat/water homogenate and habituated at 4°C for 72 h. The chicken meat/water homogenate was prepared by pummelling (2 min) fresh chicken breast meat with distilled water to yield a 10% (wt/wt) suspension. This suspension was passed through cheesecloth twice and then filter-sterilized (0.22 µm) before use. Following habituation, the seven *Salmonella* cultures were combined and serially diluted in a non-sterile raw chicken meat/water homogenate (10% wt/wt; simulating chicken meat purge) to a concentration of 4 log CFU/ml.

#### B. Inoculation and storage of packaging materials

Five materials were evaluated in this study, namely, vacuum bags, PVC overwrap film, aluminium foil, cardboard, and butcher paper. New (i.e., unused) samples of these materials were cut into 5×5 cm pieces, placed into sterile petri dishes, and spot-inoculated, on one side, with 0.5 ml of the *Salmonella* inoculum to a target level of 2-3 log CFU/cm<sup>2</sup>. The inoculated materials were stored aerobically, in the petri dishes, at 25 or 4°C for up to 123 days.

#### C. Microbiological analysis

The packaging materials were periodically analyzed during the 123 day storage period for *Salmonella* and total bacterial counts. Samples were placed into WhirlPak bags containing 10 ml of diluent (0.85% NaCl and 0.1% peptone), pummelled for 2 min, and diluted in 0.1% buffered peptone water. Appropriate dilutions were then surface-plated onto xylose lysine desoxycholate (XLD) agar (for *Salmonella* counts) and tryptic soy agar (TSA; for total bacterial counts). Plates were incubated at 35°C for 24 h (XLD) or 25°C for 72 h (TSA) before enumeration of colonies. The detection limit of the analysis was -0.40 log CFU/cm<sup>2</sup>. The study was conducted twice with three samples analyzed per material each time.

### III. RESULTS

Initial levels (2-3 log CFU/cm<sup>2</sup>) of *Salmonella* increased to 4-5 log CFU/cm<sup>2</sup> within 4 days of storage at 25°C on all tested materials, except cardboard (Table 1). After 123 days at 25°C, *Salmonella* was recovered from all the tested materials with counts

ranging from <0.82±1.09 (cardboard) to 4.79±0.70 (butcher paper) log CFU/cm<sup>2</sup>. *Salmonella* counts decreased on all materials stored at 4°C and reached non-detectable levels (<-0.40 log CFU/cm<sup>2</sup>) on cardboard, PVC overwrap film and butcher paper by day-39, -53 and -123 respectively (Table 2). Survivors of not more than -0.30 log CFU/cm<sup>2</sup> were recovered from vacuum bags and aluminium foil after 123 days at 4°C. In general, irrespective of storage temperature, packaging materials most and least conducive to *Salmonella* survival in dried chicken meat residues were butcher paper and cardboard material, respectively (Tables 1 and 2).

Table 1 *Salmonella* counts (mean ± standard deviation; log CFU/cm<sup>2</sup>) on packaging materials (A=vacuum bag; B=PVC overwrap film; C=aluminium foil; D=cardboard; E=butcher paper) stored at 25°C for 123 days

Day	Packaging Material				
	A	B	C	D	E
0	2.95 ± 0.32	2.79 ± 0.26	2.80 ± 0.16	2.27 ± 0.14	2.48 ± 0.18
4	4.48 ± 0.48	3.69 ± 2.05	5.04 ± 0.94	1.91 ± 1.94	5.10 ± 0.93
11	4.20 ± 0.08	4.70 ± 0.57	5.67 ± 0.25	1.55 ± 1.26	5.39 ± 0.73
18	4.64 ± 1.39	4.64 ± 0.70	5.24 ± 0.51	2.16 ± 1.67	3.33 ± 2.74
25	4.11 ± 2.31	3.51 ± 1.94	4.86 ± 0.26	1.73 ± 1.56	5.74 ± 0.53
32	2.93 ± 2.60	2.57 ± 2.32	5.00 ± 0.75	2.66 ± 1.80	5.96 ± 0.49
39	3.08 ± 2.31	4.33 ± 0.88	4.53 ± 0.16	2.20 ± 1.69	5.86 ± 0.76
46	2.84 ± 1.66	4.09 ± 1.13	4.70 ± 0.78	<1.07 ± 1.51	4.40 ± 2.40
53	3.50 ± 1.99	2.38 ± 2.23	4.61 ± 0.62	1.81 ± 1.69	5.65 ± 0.68
60	3.19 ± 1.65	4.18 ± 0.40	4.99 ± 0.55	1.74 ± 0.99	5.37 ± 0.67
67	2.98 ± 2.05	2.98 ± 1.31	4.64 ± 0.45	1.68 ± 0.26	5.63 ± 0.42
74	3.69 ± 0.62	2.89 ± 0.68	4.94 ± 0.50	1.74 ± 0.99	5.83 ± 0.58
81	4.66 ± 0.65	3.89 ± 0.55	4.38 ± 0.65	1.68 ± 1.03	5.22 ± 0.82
88	4.18 ± 0.94	3.32 ± 0.64	4.20 ± 0.70	0.91 ± 1.29	5.18 ± 0.32
123	2.44 ± 1.69	2.23 ± 2.07	3.99 ± 0.55	<0.82 ± 1.09	4.79 ± 0.70

Table 2 *Salmonella* counts (mean  $\pm$  standard deviation; log CFU/cm<sup>2</sup>) on packaging materials (A=vacuum bag; B=PVC overwrap film; C=aluminium foil; D=cardboard; E=butcher paper) stored at 4°C for 123 days

Day	Packaging Material				
	A	B	C	D	E
0	2.95 $\pm$ 0.32	2.79 $\pm$ 0.26	2.80 $\pm$ 0.16	2.27 $\pm$ 0.14	2.48 $\pm$ 0.18
4	1.73 $\pm$ 0.65	2.06 $\pm$ 0.62	2.17 $\pm$ 0.50	<-0.35 $\pm$ 0.12	2.28 $\pm$ 0.27
11	0.10 $\pm$ 0.41	<-0.19 $\pm$ 0.24	0.14 $\pm$ 0.42	<-0.40	0.12 $\pm$ 0.17
18	<-0.35 $\pm$ 0.12	<-0.17 $\pm$ 0.38	<-0.40	<-0.40	<-0.09 $\pm$ 0.26
25	<-0.25 $\pm$ 0.37	<-0.27 $\pm$ 0.21	<-0.12 $\pm$ 0.25	<-0.35 $\pm$ 0.12	<-0.17 $\pm$ 0.38
32	0.78 $\pm$ 0.84	<-0.40	<-0.40	1.54 $\pm$ 2.13	<-0.40
39	<-0.40	<-0.40	<-0.40	<-0.40	<-0.40
46	<-0.40	0.36 $\pm$ 1.22	<-0.27 $\pm$ 0.21	<-0.40	0.20 $\pm$ 0.48
53	<-0.40	<-0.40	<-0.40	<-0.40	0.10 $\pm$ 0.25
60	<-0.40	<-0.40	<-0.40	<-0.40	<-0.30 $\pm$ 0.25
67	<-0.40	<-0.40	<-0.30 $\pm$ 0.16	<-0.40	0.13 $\pm$ 0.67
74	<-0.40	<-0.40	<-0.40	<-0.40	0.14 $\pm$ 0.45
81	<-0.40	<-0.40	<-0.40	<-0.40	<-0.32 $\pm$ 0.19
88	<-0.20 $\pm$ 0.49	<-0.40	<-0.40	<-0.40	<-0.32 $\pm$ 0.19
123	<-0.30 $\pm$ 0.25	<-0.40	<-0.30 $\pm$ 0.16	<-0.40	<-0.40

#### IV. CONCLUSIONS

Although researchers have investigated the prevalence of *Salmonella* in poultry, there are few studies on the occurrence of this pathogen on packaging materials of raw chicken [2]. This is the first study providing information on the survival of *Salmonella* in dried chicken meat residues on the surface of packaging materials. The results of this study demonstrate that *Salmonella* can survive in food residues present on various packaging materials for long periods of time. *Salmonella* counts on aluminium foil and butcher paper after 123 days at 25°C were

approximately 1-2 log CFU/cm<sup>2</sup> higher than initial levels (2-3 log CFU/cm<sup>2</sup>), whereas pathogen counts on vacuum bags, PVC overwrap film and cardboard were 0.5 to 1.5 log CFU/cm<sup>2</sup> lower than initial levels. In contrast, *Salmonella* populations rapidly decreased on materials stored at 4°C, reaching non-detectable levels on cardboard (day-39), PVC overwrap film (day-53) and butcher paper (day-123); however, low levels of survivors were still detected on vacuum bags and aluminium foil after 123 days of storage.

The survival of *Salmonella* on the packaging materials for poultry raises concern because consumers may not expect pathogen contamination on the package and could consequently do nothing to avoid cross-contamination in the home environment. Although the risk relative to cross-contamination may be small, this study highlights that packaging materials may be implicated in the transmission of *Salmonella*. It is important to treat raw meat packaging materials as a potential source of contamination in domestic, catering and retail premises, and appropriate hand washing should be encouraged to minimize the risk of foodborne illness. Consideration of cross-contamination from packaging materials should be investigated further to reduce the risk of foodborne illness.

#### ACKNOWLEDGMENT

This work was funded in part by the American Meat Institute Foundation, and the Colorado State University Agricultural Experiment Station.

#### REFERENCES

1. Scientific Report of European Food Safety Authority and European Centre for Disease Prevention and Control (2011) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA Journal 9(3):2090. [378 pp] doi:10.2903/j.efsa.2011.2090.
2. Burgess F, Little CL, Allen G, Williamson K, Mitchell RT (2005) Prevalence of *Campylobacter*, *Salmonella*, and *Escherichia coli* on the external packaging of raw meat. J Food Prot 68:469-475
3. Harrison WA, Griffith CJ, Tennant D, Peters AC (2001) Incidence of *Campylobacter* and *Salmonella* isolated

from retail chicken and associated packaging in South Wales. *Lett Appl Microbiol* 33:450-454

4. Griffith C, Davidson C, Peters A, Lewis A (2000) The use of notational analysis to assess cross-contamination during domestic food preparation. In Proceedings of the

87th Annual Meeting of the International Association for Food Protection, Atlanta, Georgia, USA, 6–9 August 2000. Iowa, USA: International Association for Food Protection.