Comparison of Four Sampling Methods for the Detection of *Salmonella* in Broiler Litter[1]

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ABSTRACT Experiments were conducted to compare litter sampling methods for the detection of *Salmonella*. In experiment 1, chicks were challenged orally with a suspension of naladixic acid-resistant *Salmonella* and wing banded, and additional nonchallenged chicks were placed into each of 2 challenge pens. Nonchallenged chicks were placed into each nonchallenge pen located adjacent to the challenge pens. At 7, 8, 10, and 11 wk of age the litter was sampled using 4 methods: fecal droppings, litter grab, drag swab, and sock. For the challenge pens, *Salmonella*-positive samples were detected in 3 of 16 fecal samples, 6 of 16 litter grab samples, 7 of 16 drag swabs samples, and 7 of 16 sock samples. Samples from the nonchallenge pens were *Salmonella* positive in 2 of 16 litter grab samples, 9 of 16 drag swab samples, and 9 of 16 sock samples. In experiment 2, chicks were challenged with *Salmonella*, and the litter in the challenge and adjacent nonchallenge pens were sampled at 4, 6, and 8 wk of age with broilers remaining in all pens. For the challenge pens, *Salmonella* was detected in 10 of 36 fecal samples, 20 of 36 litter grab samples, 14 of 36 drag swab samples, and 26 of 36 sock samples. Samples from the adjacent nonchallenge pens were positive for *Salmonella* in 6 of 36 fecal droppings samples, 4 of 36 litter grab samples, 7 of 36 drag swab samples, and 19 of 36 sock samples. Sock samples had the highest rates of *Salmonella* detection. In experiment 3, the litter from a *Salmonella*-challenged flock was sampled at 7, 8, and 9 wk by socks and drag swabs. In addition, comparisons with drag swabs that were stepped on during sampling were made. Both socks (24 of 36, 67%) and drag swabs that were stepped on (25 of 36, 69%) showed significantly more *Salmonella*-positive samples than the traditional drag swab method (16 of 36, 44%). Drag swabs that were stepped on had comparable *Salmonella* detection level to that for socks. Litter sampling methods that incorporate stepping on the sample material while in contact with the litter appear to detect *Salmonella* in greater incidence than traditional sampling methods of dragging swabs over the litter surface.

Key words: *Salmonella* detection, litter sampling, broiler, drag swab, cecum

INTRODUCTION

The sampling of poultry-house litter has been used to indicate the *Salmonella* status of broiler flocks for the past 25 yr (Kingston, 1981). Once flock status has been determined, measures can be taken to minimize further cross-contamination between flocks during processing. Corrier

et al. (1995) demonstrated that *Salmonella* entering the poultry house with chicks on the day of placement could be found in the litter at 3 wk of age. Recycled poultry litter has previously been identified as a possible source for external *Salmonella* contamination on preprocessed broiler carcasses (Reiber et al., 1990). In a recent study by Cason (unpublished data), only 10% more broilers sampled directly from floor pens were determined to be *Salmonella* positive by external feathered-carcass rinse (81.7%) than by ceca samples (71.3%) from the same individuals. Other investigators have reported a greater incidence of *Salmonella* recovery from the outside of the feathered carcass compared with ceca samples. Rigby et al. (1980, 1982) found a greater percentage of exterior samples positive (42 and 50%) than intestinal-cecal or cecal samples from pretransport carcasses (18% intestinal-cecal and 7% cecal). Line (2002) recorded twice as many *Salmonella*-positive samples from 6-wk-old feathered whole-carcass rinses (44%) than from ceca samples (20%) from the same carcasses. These studies indicate that broilers

do not have to maintain detectable colonization levels within the intestines to be a potential source of *Salmonella* contamination when entering the processing plant. When ceca, feathered skin, and litter were sampled from a 6-wk-old flock of broilers, 19% of ceca samples were positive, 21% breast feathered-skin samples were positive, and 65% of litter samples were positive (Corrier et al., 1995). Sampling of the environment (a composite sample) may be more representative of a flock's *Salmonella* status than sampling ceca from individual broiler carcasses. However, other literature has shown that a litter sample may not be an accurate way to determine a flock's *Salmonella* status. Kingston (1981) was able to detect 9 *Salmonella*-positive flocks using drag swabs, but only 3 of these same flocks were *Salmonella* positive by culturing the litter directly. Turnbull and Snoeyenbos (1973) showed that *Salmonella* levels are decreased in litter as the flock ages due to low water activity and high pH from ammonia in the litter. In this unsuitable environment, *Salmonella* may be present but difficult to culture from the litter. In this case, direct litter sampling was not sufficient to predict external carcass contamination.

Evidence is still unclear as to how to sample the environment in an efficient and sensitive manner to enable accurate prediction of flock status upon arrival at the processing plant. Typical sampling methods for litter of occupied and vacant broiler houses have included litter collection, drag swab sampling, fecal dropping sampling, disposable shoe covers, and sampling with socks (Rigby and Pettit, 1980; Kingston, 1981; Caldwell et al., 1998; Skov et al., 1999; Pope and Cherry, 2000; McCrea et al., 2005). Some of these methods are occasionally more sensitive than others. Byrd et al. (1997) reported a greater incidence of *Salmonella* recovery for wet drag swabs (47.5%) compared with dry drag swabs (23.3%). Disposable shoe covers have been typically used dry, whereas socks are wet prior to sampling (Skov et al., 1999; McCrea et al., 2005).

If a flock is found to be positive, the litter can be treated or replaced to minimize possible contamination to the subsequent flocks reared in the same house (Payne et al., 2002). Lahellec et al. (1986) demonstrated that the majority of the *Salmonella* serotypes isolated from chicks and the environment on the day of placement (69.3%) are recovered from the house on the last day. This finding indicated that placement of chicks into an environment already containing *Salmonella* is more important for colonization than if *Salmonella* is introduced later during grow out. Flocks determined to be positive can be processed at the end of a processing plant shift, therefore minimizing cross-contamination from the positive flocks to negative flocks yet to be processed. The objective of this study was to evaluate several environmental sampling methods for *Salmonella* in occupied and vacant pens to determine the best type of sampling method to accurately predict flock *Salmonella* status.

MATERIALS AND METHODS

In experiment 1, 2 sets of 25 broiler chicks were wing banded and challenged by oral gavage with 0.1 mL of a 10^5 suspension of naladixic acid-resistant *Salmonella* Typhimurium. Two additional sets of 25 nonchallenged chicks were placed in the challenge pens (39.6 m^2). We placed 50 nonchallenged chicks (nonchallenge pens) in pens adjacent to those with the challenged chicks. All chicks were placed on clean pine shavings and raised in an environmental-type house. At 6 wk of age, 12 challenged and 12 nonchallenged broilers from the challenge pens, and 12 nonchallenged broilers from each of the nonchallenge pens were euthanized by electrocution, the abdominal cavity opened aseptically, and the ceca collected for determination of *Salmonella* status. Broilers remained in the challenge pens throughout the litter-sampling period but were removed from the adjacent nonchallenge pens at 6 wk of age. At 7, 8, 10, and 11 wk of age the litter was sampled from challenge and nonchallenge pens using 4 methods (duplicate samples per pen for each sample time): feces, litter grab, drag swabs (7.62 × 7.62 cm; Kingston, 1981), and socks (7.5-cm by ~10-cm section of elasticized tubular bandage worn over disposable plastic boots; Skov et al., 1999). Feces samples were a collection of 2 fresh fecal droppings for each of the duplicate samples. Litter grabs were approximately 25 g of litter collected from 2 areas within the pen (near the feeder and the drinking line) on each sample day. Drag swabs (DS-001, Solar Biologicals Inc., Ogdensburg, NY) presoaked in skim milk were unwound and dragged in a figure 8 around the pen perimeter, 2 times per sample. At the same time, socks (Tubigrip #1451, SSL International Plc., Oldham, England) soaked in saline (0.85%) were worn over new disposable plastic boots that were donned upon entering each pen. After one figure 8 around the pen perimeter, the socks were turned aseptically so that the top section could come in contact with the litter during the second pass. All samples were transported back to the lab aseptically in individual plastic bags on ice for *Salmonella* analysis.

For each fecal sample, 1% buffered peptone (**BP**) was added to a 50-mL conical vial to reach a total volume of 45 mL. Litter grab samples had 150 mL of BP added per sample, and 100 mL of BP was added to each of the drag swab and sock samples. All samples were shaken and then incubated at 35°C for 24 h before a loopful (3-mm loop) of liquid was plated onto brilliant-green sulfa agar with 250 mg of naladixic acid/L. Plates were incubated in an inverted position at 35°C for 24 h, and *Salmonella*-positive plates were recorded.

Experiment 2 had a total of 12 challenge and 12 adjacent nonchallenge pens containing reused litter that had been determined to be *Salmonella* negative (naladixic acid resistant). Chicks, by pen, were challenged orally with 0.1 mL of 10^2, 10^4, or 10^6 suspension of naladixic acid-resistant *Salmonella* in an attempt to provide a variable level of *Salmonella* in the challenge pens. For each challenge level, duplicate pens were located on each side of the room. In each set of pens, 30 chicks were challenged orally, wing banded, and placed in the challenge pen. Thirty nonchallenged chicks were also placed in the challenge pens. In each of the adjacent nonchallenge pens, we placed 60

Table 1. *Salmonella* detection by feces, litter grab, drag swabs, and socks sampling methods, experiments 1 and 2

	Feces	Litter grab	Drag swabs	Socks	Total
Experiment 1					
Challenge pens					
7 wk	0/4	1/4	2/4	0/4	3/16
8 wk	1/4	1/4	4/4	4/4	10/16
10 wk	1/4	2/4	1/4	2/4	6/16
11 wk	1/4	2/4	0/4	1/4	4/16
Total					
n	3/16	6/16	7/16	7/16	23/64
Percentage	19	38	44	44	36
Nonchallenge pens					
7 wk[1]	NS[2]	1/4	2/4	2/4	5/12
8 wk[1]	NS	0/4	4/4	3/4	7/12
10 wk[1]	NS	1/4	3/4	0/4	4/12
11 wk[1]	NS	0/4	0/4	4/4	4/12
Total					
n	NS	2/16	9/16	9/16	20/48
Percentage	NS	13[b]	56[a]	56[a]	42
Experiment 2					
Challenge pens					
4 wk	7/12	10/12	6/12	10/12	33/48[m]
6 wk	1/12	5/12	2/12	8/12	16/48[n]
8 wk	2/12	5/12	6/12	8/12	21/48[mn]
Total					
n	10/36	20/36	14/36	26/36	70/144
Percentage	28[b]	56[a]	39[ab]	72[a]	49[y]
Nonchallenge pens					
4 wk	3/12	3/12	2/12	8/12	16/48
6 wk	3/12	0/12	1/12	4/12	8/48
8 wk	0/12	1/12	4/12	7/12	12/48
Total					
n	6/36	4/36	7/36	19/36	36/144
Percentage	17[b]	11[b]	19[b]	53[a]	25[z]

[a,b]Total percentage within a row with no common superscripts differ significantly ($P < 0.05$).

[m,n]Total ratios within a column with no common superscripts differ significantly ($P < 0.05$).

[y,z]Total percentage within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Pens vacant after 6 wk of age.

[2]Feces were not sampled (NS) in these pens because pens were vacant.

chicks. Chicks were placed in the same room of the environmental-type house used in experiment 1 but in pens that had not been previously exposed to a *Salmonella* challenge.

At 4, 6, and 8 wk of age, the litter was sampled from challenge and adjacent nonchallenge pens for each challenge level using the same 4 sampling methods described above: feces, litter grab, drag swab, and sock (duplicate samples per pen for each sample time). At 6 and 8 wk of age, 10 challenged and 10 nonchallenged broilers from the challenge pens were euthanized, and the ceca were collected for determination of *Salmonella* status. Broilers remained in the challenge and the adjacent nonchallenge pens throughout the litter-sampling period. All litter samples were analyzed for the presence of naladixic acid-resistant *Salmonella* as described above.

In experiment 3, at 7, 8, and 9 wk of age, litter was sampled from a separate flock of broilers that was reared on used one-flock litter in a separate room of the same environmental-type house. This flock had been challenged on the day of placement as described in experiment 2, except that only a single challenge level was used (0.1 mL of a 10^4 suspension of naladixic acid-resistant *Salmonella*), and there were initially a total of 40 broilers

per pen (20 challenged and 20 nonchallenged) with a total of 12 challenge pens. In addition to drag swabs and sock sampling, a third method consisting of stepping on a drag swab 4 times during sampling (with disposable foot covering that was put on while entering the pen) of the pen was also evaluated. At 6 wk of age, 5 challenged and 5 nonchallenged broilers from each of the 12 challenge pens were euthanized as described above, and ceca were aseptically collected for the determination of *Salmonella* status. At 7 and 8 wk of age broilers were present in the pens during sampling and at 9 wk of age the pens had been vacant for 1 wk. A single sample (drag swab, sock, and stepped on drag swab) was collected from each of the 12 challenge pens on each sampling time. *Salmonella* status was determined on all samples as previously described.

Statistical Analysis

Challenge status of pens, broiler age, litter sampling method, and challenge level results (within each experiment) were analyzed by experiment using the GLM procedure of SAS software (SAS Institute, 1998). *Salmonella* incidence results by sampling method were further ana-

Table 2. *Salmonella* detection by drag swabs, stepped on drag swabs, and socks sampling methods, experiment 3

Sampling time	Drag swabs	Stepped on drag swabs	Socks	Total
7 wk	11/12 (92%)	11/12 (92%)	12/12 (100%)	34/36 (94%)[y]
8 wk	4/12 (33%)	8/12 (67%)	7/12 (58%)	19/36 (53%)[z]
9 wk[1]	1/12 (8%)	6/12 (50%)	5/12 (42%)	11/36 (30%)[z]
Total	16/36 (44%)[b]	25/36 (69%)[a]	24/36 (67%)[a]	64/108 (59%)

[a,b]Total percentage values within a row with no common superscripts differ significantly ($P < 0.05$).

[y,z]Total percentage within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Pens vacant for 1 wk prior to sampling.

lyzed by challenge vs. nonchallenge pens as well as challenge level by using the χ^2 test procedure. For all analyses, significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

At 6 wk of age, cecal samples in experiment 1 were *Salmonella* positive from 4 of 12 challenged broilers and from 5 of 12 nonchallenged broilers raised commingled in the challenge pens and from 6 of 24 nonchallenged broilers raised in the nonchallenge adjacent pens. In the present study, there were no overall significant differences in the incidence of positive ceca between challenged and nonchallenged broilers within the same pen or between adjacent pens. The challenged broilers were able to horizontally transmit *Salmonella* to pen mates and to the chicks in the nonchallenge adjacent pens. *Salmonella*–positive air samples during brooding have been demonstrated as a mode for the horizontal transmission between chicks and turkeys in adjacent pens (Hoover et al., 1997; Gast et al., 1998) and among caged laying hens (Holt et al., 1998).

At the 4 sampling times (from 7 to 11 wk) in experiment 1 for the challenge pens, *Salmonella* was detected in 3 of 16 fecal samples, 6 of 16 litter grab samples, 7 of 16 drag swabs, and 7 of 16 sock samples (Table 1). Samples from the adjacent nonchallenge pens were *Salmonella* positive in 2 of 16 litter grab samples, 9 of 16 drag swab samples, and 9 of 16 sock samples. No significant differences in the incidence of *Salmonella* were found between challenge and nonchallenge pens ($P = 0.6644$), broiler age ($P = 0.2346$), or sampling methods ($P = 0.2126$). Within the nonchallenge pens, drag swabs and socks detected significantly more *Salmonella*-positive samples (56%) than litter grab samples (13%).

In experiment 2, at 6 wk of age, cecal samples were *Salmonella* positive from 32/56 (57%) challenged broilers and from 43/60 (72%) nonchallenged broilers raised commingled in the challenge pens. Broiler cecal samples in challenge pens were 75% positive for 10^2 challenge, 62.5% positive for 10^4 challenge, and 83.3% positive for the 10^6 challenge pens. In experiment 2, the challenge vs. nonchallenge pen ($P = 0.0025$), broiler sample age ($P = 0.0237$), and sampling method ($P = 0.0011$) all significantly differed in the recovery of *Salmonella*.

Pens with chicks that were challenged had a *Salmonella* recovery incidence from the litter of 49%, whereas the adjacent pens that were not challenged had only 25% of samples that were positive. For the challenge pens, *Salmonella*-positive samples were detected in 10 of 36 (28%) fecal samples, 20 of 36 (56%) litter grab samples, 14 of 36 (39%) drag swab samples, and 26 of 36 (72%) sock samples (Table 1). In the challenge pens, socks and litter grab had significantly greater incidence of *Salmonella* recovery than feces sampling. Drag swabs were not significantly different from the other sampling methods. Samples from the nonchallenge pens were *Salmonella* positive in 6 of 36 (17%) fecal samples, 4 of 36 (11%) litter grab samples, 7 of 36 (19%) drag swab samples, and 19 of 36 (53%) sock samples. In the nonchallenge pens, socks had a significantly greater incidence of *Salmonella* than all other sampling methods. For the challenge and nonchallenge pens, socks had significantly greater incidence of *Salmonella* recovery than feces samples. The incidence of *Salmonella* recovery in the challenge pens was greatest at 4 wk of age 69% (33 of 48 samples), declined to 33% (16 of 48 samples) at 6 wk of age, and was intermediate 44% (21 of 48 samples) at 8 wk of age. The incidence of *Salmonella* recovery from the litter in the adjacent nonchallenge pens did not differ with sample age and was about half (25%) the incidence for the challenge pens (49%).

Overall, sampling litter by socks was the most sensitive method of sampling and fecal sampling was the least sensitive. Both litter grab and drag swab sampling were intermediate methods. Hayes et al. (2000) also demonstrated a greater sensitivity of drag swab sampling (92%) in comparison to litter grab sampling (46%) for detecting the *Salmonella*-positive commercial houses (48 of 86 houses). Surgical shoe cover sampling (6 of 48 or 12.5% positive) has been reported to result in a greater incidence of *Salmonella* recovery than drag swab sampling (1 of 48 or 2.1% positive; McCrea et al., 2005). Perhaps the increased detection of *Salmonella* in these lower incidence broiler houses was due to pressing of the sock or shoe cover into the litter, resulting in greater exposure to litter than the surface contact made by drag swabs.

In experiment 3 at 6 wk of age, cecal samples were *Salmonella* positive from 47 of 60 challenged broilers and from 40 of 60 nonchallenged broilers raised commingled in the challenge pens for an average of 72.5% positive. At 7 wk of age, 94% of the litter sampling methods were *Salmonella* positive with only 1 negative sample of the 12 samples for the drag swabs (Table 2). At 8 wk of age, *Salmonella* was detected in only 4 of 12 drag swabs but

at a greater incidence from stepped on drag swabs (8 of 12) and from sock samples (7 of 12). At 9 wk of age, socks (5 of 12) and stepped on drag swabs (6 of 12) continued to detect greater levels than drag swabs that were not stepped on (1 of 12). At 8 and 9 wk of age, when drag swabs were stepped on during sampling, the incidence of *Salmonella* detection was greater in comparison to drag swabs that were not stepped on. Overall, *Salmonella* recovery incidence was significantly greater for sock (67%) and drag swabs that were stepped on (69%) during sampling than for drag swabs (44%) that were not stepped on. These results indicate that when the sampling material comes in greater contact with the litter by stepping on the sample material (socks or drag swabs), the samples are more likely to detect *Salmonella* when *Salmonella* is present. Stepping on drag swabs can apparently improve the incidence of *Salmonella* detection without an increase in cost or sample time, because shoes are typically covered with disposal plastic boots upon entering each house.

These experiments were designed specifically to evaluate environmental sampling methods for *Salmonella* in pens with diverse levels of *Salmonella* to evaluate the sampling methods to accurately predict flock *Salmonella* status. The 3 experiments were replicated within each experiment, but each experiment contained different initial parameters, and, therefore, comparisons between experiments for *Salmonella* recovery should not be made regarding new vs. reused litter, the age of the flock at the time of sampling, or if broilers were present or vacant in the pens.

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