ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 5 (12): 1102-1109, 2006
ISSN 1682-8356

Characterization of *Salmonella* from Three Commercial North Carolina Broiler Farms

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Abstract: *Salmonella* contamination during the grow-out phase of poultry production is an area that is receiving increased attention. By assessing the relationship of on-farm *Salmonella* populations to environmental parameters and farm management practices, on-farm pathogen control strategies similar to those developed for processing HACCP programs may be identified. A small survey was conducted to determine *Salmonella* prevalence, populations, serotypes, and antibiotic resistance in fresh excreta and litter from three North Carolina broiler farms. Litter pH, temperature, ammonia levels, moisture content, and water activity were also measured. Composite litter and fecal samples were aseptically collected from three commercial farms (two houses per site) as a function of bird age (1-3 wks, 4-6 wks) and season (summer, winter) and the population of *Salmonella* species enumerated using a 3-tube Most Probable Number (MPN) method. Fifty percent of the litter (n = 24) and fecal (n = 24) samples were *Salmonella* positive. Litter and fecal mean and range of *Salmonella* populations were 1.70 and $\leq$ 1.0 to 3.6 and 1.57 and $\leq$ 1.0 to 3.1 log MPN/g, respectively. A total of four different serotypes were isolated from the six broiler houses with *Salmonella* Kentucky and *Salmonella* Heidelberg being the two most common isolates. Ninety-six percent of the isolates were resistant to $\geq$ 1 antimicrobial agent (s). The results of this limited survey indicate that while *Salmonella* spp. populations were statistically different due to a three-way interaction of farm, season and flock age, the difference could not be attributed to the main affects.

Key words: Broilers, *Salmonella*, excreta, litter

Introduction

A critical public and industry concern involving poultry production is the presence of food-borne pathogens in the production environment and in or on the bird. These food-borne pathogens are estimated to cause millions of illnesses and thousands of deaths annually in the United States (Mead *et al*., 1999). Food-borne illnesses are caused by the ingestion of a sufficient infective dose of pathogenic bacterial cells, viruses, or their toxins (Adams, 1990). Consumption of contaminated poultry products is often associated with the food-borne illness salmonellosis (Bryan and Doyle, 1995; Tauxe, 1991). Not only does *Salmonella* pose a public health threat, but it also has an economic impact on the poultry industry by threatening domestic and export consumer markets and increasing production and processing costs (Bender and Mallinson, 1991).

Contamination can arise at the pre-harvest level with sources including contaminated feed (Jones *et al*., 1991), feces, nest boxes, litter, floors, incubators (Bryan *et al*., 1979), rodents, insects, wild birds, water (Ashton, 1990; Bailey *et al*., 2001), and humans (Lahellec and Colin, 1985). Bryan and Doyle (1995) stated that commercially reared birds are in constant contact with litter and dust, both of which can be a source of contamination. *Salmonella* contamination of birds may occur before, during or after the grow-out phase of production (Barnes, 1972). Once infected, these birds may excrete concentrations of up to 10^9 *Salmonella* per gram of feces for up to a two-week duration (Bains and MacKenzie, 1974). Studies have documented that the *Salmonella* serotypes isolated from the final commercial product are also generally found in the production house litter and other areas of the production environment (Bains and MacKenzie, 1974; Lahellec and Colin, 1985). A recent study conducted by the USDA Food Safety and Inspection Service FSIS, (2003) reported that *Salmonella* prevalence in broiler carcasses collected from a wide variety of processing establishments between 1998 and 2002 ranged from 7.1% to 37.2%.

When considering the human health and economic concerns associated with *Salmonella* contaminated poultry products, the industry must constantly be searching for new and effective control measures. One area of the production continuum that has recently received considerable attention on this subject is the grow-out phase. The identification of effective critical control points or control strategies on the farm can aid in developing a food safety control program similar to that of the Hazard Analysis Critical Control Point (HACCP) program which has been implemented at the post-harvest level. In order to fully understand pre-harvest contamination problems for use in future pathogen control program development and implementation, one must properly assess the populations and prevalence of *Salmonella* present on the farm along with how varying

environmental growth parameters and management practices impact its growth, persistence and true risk for consumers. Numerous studies have been conducted to determine the presence or absence of *Salmonella* at the grow-out phase, yet few have actually determined *Salmonella* populations. Merely observing the presence or absence of *Salmonella* does little to determine if contamination levels are high or low, making it difficult or impossible to properly assess the impact of management practices, housing, and the environment on the fate of *Salmonella*. The following survey was conducted to estimate specific *Salmonella* populations in fresh excreta and litter from three commercial North Carolina broiler farms using the Most Probable Number (MPN) method for enumeration. Serotyping and antimicrobial resistance testing further characterized the *Salmonella* isolates. Litter pH, temperature, moisture content and water activity were determined along with weekly bird mortality rates. A grower survey was conducted as well in order to assess on-farm management practices.

Materials and Methods

Experimental design: Six individual houses from three commercial broiler farms (two houses per farm) were sampled in this study. Each farm consisted of tunnel ventilated, dirt-floored houses with pine shavings serving as bedding material. *Salmonella* populations were enumerated during two different seasons of the year, winter and summer. The farms were coded as F1 through F3. In addition to seasonal effects, age effects were also examined. The farms were sampled when the birds were between 1 to 3 weeks of age as well as when birds were between 4 to 6 weeks old. All three farms had similar management practices since they were integrated under the same company. According to the grower surveys, Farms 1 and 3 removed litter from the house every 2-5 years while Farm 2 cleaned out the facility once every year.

Sampling procedures: Litter samples, measuring approximately one inch (2.54 cm) in depth and weighing approximately 10 g each, were uniformly taken from 10 points in between water and feed lines following a zigzag pattern throughout each house. The 10 samples were pooled in a Ziploc® bag and stored on ice during transport to the laboratory. Fresh composite fecal droppings were also collected in between water and feed lines across the entire length of the house, placed in sterile Whirl-Pak® (Nasco, Fort Atkinson, WI) bags and stored on ice in a transport cooler.

Litter temperatures, taken at approximately one inch in depth, were measured with a calibrated thermometer (Oakton Instruments, Vernon Hills, IL) at 10 points in between the water and feed lines (same locations where litter samples were taken for microbiological analysis).

Ammonia concentration in the litter was measured at floor level (Pope and Cherry, 2000) for 3 points of the house (both ends and middle) using short-term tubes (5-70 ppm capacity) and an ammonia gas detector pump (Draeger, Pittsburgh, PA). A 1.6 L flower pot (with a small opening in its bottom) was placed up-side down on the litter where it remained for a 5-minute equilibration time. The short-term tube was subsequently inserted in the opening and ammonia readings were measured as recommended by the manufacturer. Ammonia testing tubes were used once and discarded.

Total bird mortalities were recorded during each sampling visit from house record sheets and the current weekly mortality rates were calculated.

Most Probable Number (MPN) technique: Upon arriving at the laboratory, 25 g of each composite sample were placed in separate 7x12 inch (17.78x30.48 cm) sterile filtered stomacher bags (Oxoid, Ogdensburg, NY) and 50 ml of buffered peptone water (BPW) (Oxoid) were added to each bag. The bags were then homogenized for one minute each (Wiberg and Norberg, 1996) using a stomacher (IUL Instruments, Barcelona, Spain).

A three tube MPN technique as similarly described by Voogt *et al.* (2001) was employed using BPW as a pre-enrichment broth. Ten milliliters were taken directly from each bag, placed into empty sterile test tubes (identified as 10^0 dilution), and then 1 ml of sample was transferred to 9 ml BPW dilution tubes followed by serial dilution in BPW. All tubes were then incubated at 37°C for 18-24 h before transferring 0.1 ml of the appropriate dilutions to 10 ml of Rappaport-Vassiliadis (RV) broth (Oxoid) for selective enrichment. All RV broth tubes were incubated at 42°C for 24 h. Following incubation, 10 µl from each tube was streaked for isolation onto modified lysine iron agar (MLIA) (Oxoid, Cox *et al.*, 2000) and incubated at 37°C for 24 h. Suspect colonies were picked, streaked, and stabbed onto triple sugar iron (TSI) (Difco, Sparks, MD) agar slants (Cox *et al.*, 2000) and then incubated at 37°C for 24 h. Positive *Salmonella* colonies were confirmed by agglutination using poly-O antiserum (Difco, Cox *et al.*, 2000). Populations of *Salmonella* spp. for each sample were determined using the Thomas' approximation (Blodgett, 2001).

***Salmonella* spp. Prevalence:** For determining *Salmonella* prevalence, 25 g of each sample were placed in a sterile filtered stomacher bag (Fisher Scientific, Pittsburgh, PA) containing 100 ml of lactose broth (LB) (Difco) and stomached for one minute each. An additional 125 ml of LB was added to the homogenized samples resulting in a 1:10 dilution (Andrews and Hammack, 2003). The bags were mixed for an additional 60 seconds and then incubated for 18 to 24 hours at 37°C. Similar to the MPN technique, a

selective enrichment step was performed by transferring 1 ml from each bag to 100 ml bottles of RV broth (Andrews and Hammack, 2003). Bottles were then incubated at 42°C for 24 h. Following incubation, 10 µl from each bottle was streaked for isolation onto MLIA and incubated at 37°C for 24 h. Suspect *Salmonella* colonies were confirmed as described under the MPN procedures. The prevalence procedure was employed to increase the minimum detectable level of *Salmonella* in a 25 g sample.

Serotyping: Samples that were confirmed as *Salmonella* positive were re-streaked onto MLIA plates and incubated at 37°C for 24 h. From these samples, litter (n = 11) and fecal (n = 12) isolates representative of each farm and season were selected. One of the 12 litter isolates was not recoverable after repeated culture transfers and was not further characterized. Two isolated colonies from each sample were then randomly picked, streaked onto tryptic soy agar (Oxoid) and shipped to the U.S. Department of Agriculture, Animal Plant Health Inspection Service, National Veterinary Services Laboratories, Ames, IA, for serotyping.

Antimicrobial Resistance: Partial range minimum inhibitory concentrations (MICs) were determined for the 23 serotyped isolates using a broth microdilution method (Trek Diagnostics, Westlake, OH) for the following 15 antimicrobial agents: amikacin, ampicillin, amoxicillin/clavulanic acid, ceftriaxone, chloramphenicol, ciprofloxacin, trimethoprim/sulfamethoxazole, cefoxitin, gentamicin, kanamycin, nalidixic acid, sulfisoxazole, streptomycin, tetracycline, and ceftiofur. *Salmonella* Typhimurium DT104 (ATCC 700408) was used as a control strain for susceptibility testing. Isolates were grown overnight (~ 18 hours) in brain heart infusion (BHI) broth (Oxoid) at 37°C and subsequently streaked onto BHI agar (Oxoid) plates and incubated at 37°C for 24 h. Following incubation, 2 isolated colonies of the same morphological type were transferred to 5 ml of 0.9% sterile saline solution and adjusted to a 0.5 MacFarland standard (Difco) using a nephelometer (Trek). Seventy-five microliters of saline suspension were then transferred to 10 ml of Mueller-Hinton (MH) broth (Oxoid). From the diluted MH broth 50 µl was transferred to each plate well (Trek). Plates were then incubated at 37°C for 24 h and manually read using a microtiter plate holder (Trek). The National Committee for Clinical Laboratory Standards (NCCLS, 2002) MIC breakpoint standards were used for determining antimicrobial resistance whenever possible. *Salmonella* were considered resistant whenever growth was observed at the following concentrations: $\geq$ 32 µg/ml for amikacin, ampicillin, chloramphenicol, cefoxitin, and nalidixic acid; $\geq$ 32/16 µg/ml for amoxicillin/clavulanic acid; $\geq$ 64 µg/ml for ceftriaxone; $\geq$ 4 µg/ml for ciprofloxacin; $\geq$ 8/152 µg/ml for trimethoprim / sulfamethoxazole; $\geq$ 8 µg/ml for gentamicin; $\geq$ 25 µg/ml for kanamycin; $\geq$ 350 µg/ml for sulfisoxazole; and $\geq$ 16 µg/ml for tetracycline. Resistance to ceftiofur and streptomycin was defined as a MIC of $\geq$ 8 µg/ml and $\geq$ 64 µg/ml, respectively, as previously described (Rabatsky-Ehr *et al.*, 2004).

Moisture Content, Water activity (a_w) and pH analysis: The remaining composite litter samples were used for moisture content, water activity and pH analysis. Moisture content of the litter was measured by drying 2.5 g of the composite sample overnight in a forced-air convection oven at 105°C. Water activity of litter samples was measured according to the manufacturer's instructions using a Decagon Model CX-3 Water Activity System (Decagon Devices, Pullman, WA). To determine litter pH, 1 g of sample was combined with 10 ml of deionized water, mixed by vortexing and allowed to stand for 1 minute (Pope and Cherry, 2000). Litter pH was then measured using a Corning 220 pH meter with a G-P Combo with RJ probe (Corning Inc., Corning, NY).

Statistical analysis: All data were analyzed using the general linear model (GLM) of (SAS, 1996). Houses served as the experimental units for statistical analysis and residual effects were used as the error term. All MPN data were transformed to a base-10 logarithm prior to analysis. Farm, season, age and sample were the main effects for factorial analysis. Variables having a significant F-test were compared using the least-square-means (LSMEANS) function of SAS and were considered to be significant at $P<0.05$. Besides the GLM procedure, litter MPN, temperature, moisture content, water activity, pH, ammonia concentration and weekly bird mortality data were also analyzed using the regression procedure with the stepwise selection function of SAS and the correlation procedure (1996).

Results and Discussion

The populations of *Salmonella* in commercial broiler farms are shown in Tables 1 (litter samples) and 2 (fecal dropping samples). Fifty percent of the litter (n = 24) and fecal (n = 24) samples were *Salmonella* positive. All three farms were *Salmonella* positive at least once during the sampling period. Litter and fecal mean and range of *Salmonella* populations were 1.70 and $\leq$ 1.0 to 3.6 and 1.57 and $\leq$ 1.0 to 3.1 log MPN/g, respectively (minimum detection level = 1 log MPN/g). The highest litter and fecal *Salmonella* populations (3.6 and 3.1 log MPN/g, respectively) were detected on Farm 2 during the winter months for 4-6 week old birds.

A significant 3-way interaction of season, farm and age did influence the litter *Salmonella* populations ($P = 0.02$); however, *Salmonella* populations in fecal samples were not influenced by season, age, farm or their two-way

Table 1: Log MPN/g1 and prevalence of *Salmonella* species in broiler litter samples exhibiting season and age effects within individual farms

Season	Farm 1		Farm 2		Farm 3	
	1 to 3 wk	4 to 6 wk	1 to 3 wk	4 to 6 wk	1 to 3 wk	4 to 6 wk
Winter[2]	2.4	<1.0 (+)	<1.0[b]	3.6[a]	<1.0 (+)	<1.0
Summer[3]	1.0	2.5	1.5[b]	<1.0[b] (+)	3.0	1.5
p-value						
SEM (4)[4]	0.53		0.01		0.19	
(season by age)	0.92		0.34		0.60	

[a-b]Means with different subscripts within a farm differ significantly ($P \leq 0.05$), [1]Base-10 logarithm of the most probable number of *Salmonella* detected in litter samples (average of 2 broiler houses per farm), [2]Samples taken between November and March, [3]Samples taken between April and October, [4]SEM(4): Standard error of the mean with 4 degrees of freedom, (+) Positive result for prevalence, yet below the detection limit of the MPN procedure, <Negative result from both methods: reported as below the detection limit of MPN procedure: 10 cells/gram of sample (1 log MPN/g)

Table 2: Log MPN/g1 and prevalence of *Salmonella* species in broiler fecal samples

Season	Farm 1		Farm 2		Farm 3	
	1 to 3 wk	4 to 6 wk	1 to 3 wk	4 to 6 wk	1 to 3 wk	4 to 6 wk
Winter[2]	1.4	<1.0	<1.0	3.1	2.0	1.0
Summer[3]	1.1	2.5	<1.0	2.3	1.5	<1.0
p-value						
SEM (4)[4]	0.55		0.22		0.39	
(season by age)	0.78		0.67		0.42	

[1]Base-10 logarithm of the most probable number of *Salmonella* detected in fecal samples (average of 2 broiler houses per farm). [2]Samples taken between November and March, [3]Samples taken between April and October, [4]SEM (4): Standard error of the mean with 4 degrees of freedom, < Negative result from both methods: reported as below the detection limit of MPN procedure: 10, cells/gram of sample (1 log MPN/g)

interactions. A two-way analysis of season and age effects on litter *Salmonella* populations for each individual farm was then conducted and a season and age interaction was observed for Farm 2 (Table 1).

As previously stated in the materials and methods, there was variation between farms in litter cleanout cycles with Farms 1 and 3 replacing litter less frequently than Farm 2. It has been shown that older and more built-up litter, such as that found in Farms 1 and 3, can exhibit an inhibitory effect on *Salmonella* when compared to newer litter, similar to the litter present on Farm 2 (Botts *et al.*, 1952; Corrier *et al.*, 1992; Fanelli *et al.*, 1970; Olesiuk *et al.*, 1971; Reiber *et al.*, 1990; Snoeyenbos *et al.*, 1967; Tucker, 1967; Williams and Benson, 1978). According to (Turnbull and Snoeyenbos, 1973), the salmonellacidal effects of older litter may be explained by the rise in ammonia producing bacteria, which will result in increased ammonia production, thus increasing litter pH to a more alkaline level. However, at our sample times, no correlation was observed between ammonia levels and litter *Salmonella* populations. Another argument is that used litter can aid in competitive exclusion by developing organisms to compete against *Salmonella* colonization in the chicks gut (Fanelli *et al.*, 1970; Gustafson and Kobland, 1984). Other management practices such as rodent and insect control, water quality and biosecurity practices could also influence *Salmonella* populations.

The microbiological, physical and chemical properties of litter samples are summarized in Table 3. The environmental parameters monitored in this study did not significantly influence the litter *Salmonella* populations. As anticipated, litter moisture content and A_W were positively correlated ($r = 0.70$; $P = 0.0001$) in addition to fecal and litter *Salmonella* populations ($r = 0.55$; $P = 0.005$). Weekly mortality rate, litter temperature, moisture content, and A_W varied across all farms, while little variation was observed for litter pH. The mean and range for weekly mortality rate, litter temperature, moisture content, A_w, pH and ammonia levels were 0.59% and 0.24 to 1.55%; 31.5°C and 28.6 to 34.3°C; 29.6% and 18.7 to 41.8%; 0.91 and 0.79 to 0.94; 8.09 and 6.79 to 8.88; 38.2 ppm and 15 to 65.9 ppm. As expected, litter ammonia levels were generally higher during the winter months and in older birds. This can be explained due to decreased ventilation for heat conservation during the winter months and an increased amount of fecal shedding occurring in older birds. With the exception of one *Salmonella* positive composite litter sample with a A_W value of 0.79, A_W values for *Salmonella* positive composite litter samples ranged from 0.88-0.94. These results agree with Carr *et al.*, (1995) and Opara *et al.* (1992) who found that higher litter A_W values (0.90-0.95) were associated with *Salmonella* positive flocks. According to (D'Aoust, 2001), *Salmonella* grow at A_W values $\geq$ 0.93; however, *Salmonella* positive litter samples $\leq$ 0.84 A_W have been previously reported (Hayes *et al.*, 2000). These researchers suggested the possibility of a more resistant organism present in the samples or that the measured A_W values may not have

Table 3: Microbiological, physical and chemical properties of litter samples of 1 to 3 week and 4-6 week old broilers

Winter	1 to 3 wk			4 to 6 wk			
	F1	F2	F3	F1	F2	F3	p-value (SEM)
Log MPN/g[1]	2.4	<1.0 (+)	<1.0	<1.0	3.6	<1.0	
MORT[2] (%)	0.32^{bc}	0.24^{c}	0.65^{a}	0.59^{a}	0.67^{a}	0.41^{b}	0.0002 (0.029)
TEMP[3] (°C)	34.3^{a}	30.1^{bc}	29.5^{c}	31.5^{b}	30.5^{bc}	31.0^{bc}	0.0068 (0.514)
M.C.[4] (%)	41.8^{a}	26.4^{c}	31.9^{bc}	36.3^{ab}	24.9^{c}	25.2^{c}	0.0120 (2.44)
a_W[5]	0.94^{a}	0.93^{a}	0.91^{ab}	0.94^{a}	0.89^{b}	0.92^{a}	0.0250 (0.008)
pH[6]	7.83	8.30	6.79	7.95	8.28	8.67	
NH_3[7] (ppm)	40.9^{bc}	50.0^{ab}	21.7^{c}	41.7^{bc}	64.2^{a}	65.9^{a}	0.0210 (6.47)
Summer							
Log MPN/g[1]	1.0	1.5 (+)	3.0	2.5	<1.0	1.5	
MORT[2] (%)	0.46^{z}	1.55^{y}	0.59^{z}	0.65^{z}	0.36^{z}	0.56^{z}	0.0210 (0.170)
TEMP[3] (°C)	32.7^{wx}	33.5^{wx}	30.3^{y}	28.6^{z}	32.2^{x}	34.2^{w}	0.0009 (0.456)
M.C.[4] (%)	22.6^{yz}	18.7^{z}	35.4^{x}	31.6^{xy}	22.5^{yz}	37.9^{x}	0.0250 (3.25)
a^{W}[5]	0.88^{y}	0.79^{z}	0.94^{v}	0.91^{x}	0.92^{wx}	0.93^{vw}	0.0001 (0.006)
pH[6]	8.24	8.10	7.55	8.88	7.97	8.57	
NH_3[7] (ppm)	30.9^{xy}	15.0^{z}	16.7^{z}	25.8^{yz}	42.5^{x}	43.4^{x}	0.0110 (4.24)

$^{a-c}$Winter means with different subscripts within a row differ significantly (P< 0.05), $^{x-z}$Summer means with different subscripts within a row differ significantly (P < 0.05), [1]Base-10 logarithm of the most probable number of *Salmonella* detected in litter samples (average of 2 broiler houses per farm), [2]Weekly mortality rate within seasons, [3]Average of 20 measurements per farm (10 measurements/ house), [4]Moisture content of the composite litter sample, [5]Water activity of the composite litter sample (a_W scale: 0-1, where 1 is pure water), [6] pH of the composite litter sample, [7]Average of 6 measurements of ammonia level per farm (3 measurements/house), $^{(+)}$Positive result for prevalence, yet below the detection limit of the MPN procedure, < Negative result from both methods: reported as below the detection limit of MPN procedure: 10 cells/gram of sample (1 log MPN/g)

Table 4: *Salmonella enterica* serotypes identified in broiler litter and fecal dropping samples

Litter			Fecal droppings		
Serotype	n	(%)[1]	Serotype	n	(%)[1]
Kentucky	7	63.6	Kentucky	8	66.7
Heidelberg	2	18.2	Heidelberg	2	16.6
Mbandaka	2	18.2	Mbandaka	1	0.08
			8, (20):-:z6	1	0.08
Total No. of isolates serotyped	11			12	

[1]Percentage of total isolates serotyped

Table 5: *Salmonella enterica* serotypes identified by farm during the winter and summer months

	Farm		
Season	1	2	3
Winter[1]	Kentucky (4)[3]	Heidelberg (4)	Kentucky (4)
Summer[2]	Kentucky (3)	Kentucky (3)	Mbandaka (3)
	8, (20):-:z6 (1)		Kentucky (1)

[1]Samples taken between November and March, [2]Samples taken between April and October, [3]Frequency of isolation

represented the immediate environment in which the organism was present. Based on the findings from previous studies (Carr *et al.*, 1995; Hayes *et al.*, 2000), it does appear that by lowering litter A_W values to $\leq$ 0.84, *Salmonella* populations can be reduced.

As documented in Table 4, *Salmonella* Kentucky was the most commonly isolated serotype from both litter and fecal dropping samples, followed by *Salmonella* Heidelberg, *Salmonella* Mbandaka, and a classified serotype expressing the antigenic formula 8, (20):-:z6. Table 5 shows that *S.* Kentucky was well distributed across the three farms, while *S.* Heidelberg, *S.* Mbandaka, and *S.* 8, (20):-:z6 were not as widely distributed. *S.* Heidelberg was associated with the higher populations detected on Farm 2.

The *Salmonella* serotypes identified in the present study have also been identified as common poultry serotypes (Byrd *et al.*, 1997; Byrd *et al.*, 1999; Jones *et al.*, 1991; Roy *et al.*, 2002; Schlosser *et al.*, 2000). Previous studies have indicated that *S.* Kentucky and *S.* Heidelberg accounted for the highest percentage of total serotypes isolated from commercial broiler production facilities (Byrd *et al.*, 1997; Byrd *et al.*, 1999). Roy *et al.*, (2002) found that 47.4% of the total serotyped *Salmonella* isolates from poultry, poultry products, and the poultry production environment were typed as *S.* Heidelberg and *S.* Kentucky. In a second study conducted by the USDA FSIS, *S.* Kentucky followed by *S.* Heidelberg were the two most common *Salmonella* serotypes isolated from broiler processing establishments (USDA FSIS, 1999; USDA FSIS, 2001).

The antimicrobial agents to which the *Salmonella* isolates (n = 23) exhibited the most resistance were sulfisoxazole (61%), tetracycline (52%), streptomycin (52%), ceftiofur (48%), cefoxitin (43%), amoxicillin-clavulanic acid (43%), ampicillin (39%) and kanamycin (4%). No *Salmonella* isolates demonstrated resistance to amikacin, chloramphenicol, ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid, or trimethoprim-sulfamethoxazole. Tessi *et al.* (1997) observed similar results when out of 93 *Salmonella* broiler carcass isolates tested, the largest numbers were resistant to tetracycline (52.7%), sulfisoxazole (45.2%), and streptomycin (37.6%). Other researchers have observed

Table 6: Antimicrobial resistance characterization of *Salmonella enterica* serotypes identified in broiler litter and fecal dropping samples

Serotype	Serotype Code[1]	FOX[2]	AMI[2]	CHL[2]	TET[2]	AXO[2]	AUG[2]	CIP[2]	GEN[2]	NAL[2]	TIO[2]	FIS[2]	SXT[2]	KAN[2]	AMP[2]	STR[2]	Total
8,(20):-:z6	1-6-S-F	R[3]			R		R				R	R				R	6
Mbandaka	3-3-S-L																0
Mbandaka	3-3-S-F											R					1
Mbandaka	3-6-S-L											R					1
Heidelberg	2-6-W-L	R					R				R						3
Heidelberg	2-6-W-F	R					R				R				R		4
Heidelberg	2-6-W-L	R					R				R				R		4
Heidelberg	2-6-W-F	R					R				R				R		4
Kentucky	3-6-W-F				R							R				R	3
Kentucky	3-6-W-F				R						R					R	3
Kentucky	3-3-W-L	R					R				R	R			R		5
Kentucky	3-3-W-L	R					R				R	R			R		5
Kentucky	1-3-W-L				R							R			R		3
Kentucky	1-3-W-F				R							R				R	3
Kentucky	1-3-W-L				R							R				R	3
Kentucky	1-3-W-F											R					1
Kentucky	1-6-S-L	R			R		R				R	R			R	R	7
Kentucky	1-6-S-L	R			R		R				R				R	R	6
Kentucky	3-3-S-F	R			R		R				R				R	R	6
Kentucky	2-6-S-L											R		R		R	3
Kentucky	2-6-S-F				R											R	2
Kentucky	2-6-S-F				R							R				R	3
Kentucky	1-3-S-F				R							R				R	3
Total		10			12		10				11	14		1	9	12	

[1]Farm (1-3), age (3-1 to 3 wk; 6-4 to 6 wk), season (S-summer; W-winter), source (F-fecal; L - litter), [2]FOX-Cefoxitin (R ≥32 µg/ml); AMI-amikacin (R ≥32 µg/ml); CHL-chloramphenicol (R ≥32 µg/ml); TET - tetracycline (R ≥16 µg/ml); AXO-ceftriaxone (R ≥64 µg/ml); AUG-Aaoxicillin/clavulanic acid (R ≥32/16 µg/ml); CIP-ciprofloxacin (R ≥4 µg/ml); GEN-gentamicin (R ≥8 µg/ml); NAL-nalidixic acid (R ≥32 µg/ml); TIO-ceftiofur (R ≥8 µg/ml); FIS-sulfisoxazole (R ≥350 µg/ml); SXT-trimethoprim/sulfamethoxazole (R ≥8/152 µg/ml); KAN-kanamycin (R ≥25 µg/ml); AMP-ampicillin (R ≥32 µg/ml); STR-streptomycin (R ≥64 µg/ml), [3]R: resistance

a 57% resistance to tetracycline, streptomycin, sulfisoxazole gentamicin, or a combination of trimethoprim and sulfamethoxazole from 1,824 *Salmonella* serotypes isolated from broiler carcasses (Lee *et al*., 1993). Of the 23 isolates, 22 (96%) were resistant to ≥ 1 antimicrobial agent tested, 19 (83%) were resistant to ≥ 2 antimicrobial agents, 18 (78%) were resistant to ≥ 3 antimicrobial agents, 9 (39%) were resistant to ≥ 4 antimicrobial agents, 6 (26%) were resistant to ≥ 5 antimicrobial agents and 4 (17%) were resistant to ≥ 6 microbial agents (Table 6). Isolates resistant to ≥ 6 microbial agents were all recovered during the summer months.

There is current concern over an increasing resistance of pathogens to fluoroquinolones, which are powerful antibiotics used for the treatment of human infections involving gram (-) bacteria. Enrofloxacin is the veterinary equivalent to ciprofloxacin, which is an important fluoroquinolone becoming increasingly used for human treatment (Asperilla *et al*., 1990). No resistance was found to either ciprofloxacin or nalidixic acid (a quinolone) in this study.

Resistance was found to amoxicillin-clavulanic acid, ampicillin, cefoxitin, kanamycin, streptomycin, sulfisoxazole, and tetracycline, all antibiotics used for the treatment of human infections. Resistance was also observed to ceftiofur, a third generation cephalosporin developed strictly for veterinary use and available for use in poultry. Interestingly, isolates were resistant to antibiotics not used in poultry production, which include kanamycin, amoxicillin-clavulanic acid, ampicillin, and cefoxitin. This suggests that resistant isolates may have been introduced into poultry from different sources or vectors such as rodents, insects, and humans or an already contaminated farm environment and could be unrelated to direct antibiotic use in poultry production (Rajashekara *et al*., 2000). Novick, (1981) stated that approximately 2% of "wild type" bacteria are resistant to any given antibiotic.

Tetracyclines, virginiamycin, lincomycin, tylosin, bambermycin, penicillin, sulfonamides and bacitracin are approved for use as feed additives for poultry and other animals in order to enhance growth rate, feed efficiency and prevent disease (Muirhead, 1999; Rajashekara *et al*., 2000). This may help to explain the resistance found to ampicillin, sulfisoxazole and tetracycline. Whenever multiple antibiotic resistant strains are found in the animal production system, a public health concern arises due to the difficulty in treating infections caused by these bacteria. Treating infections caused by multiple antibiotic resistant bacteria can involve a prolonged recovery and more expensive antibiotics resulting in increased treatment costs (Kelley *et al*., 1998).

The findings of this survey indicate that while *Salmonella* spp. populations and their prevalence on three commercial North Carolina broiler farms were statistically different due to a three-way interaction of farm, season, and flock age, the difference could not be attributed to any of the main effects. While the present

study was unable to relate observed litter properties to organism populations, perhaps more in depth studies involving a larger number of broiler farms over several years would show increased relationships between *Salmonella* populations, environmental parameters and management practices. From the 23 isolates serotyped, *S.* Kentucky and *S.* Heidelberg were the two most common isolates. Ninety-six percent of the isolates were resistant to = 1 antimicrobial agent (s). Efforts to determine pre-harvest *Salmonella* populations, such as described in this study, can aid in developing new and effective control strategies for reducing food-borne pathogen prevalence and populations entering processing plants from contaminated flocks and their subsequent transmission risk to humans.

References

Adams, M.H., 1990. Production and processing alternatives for the elimination of *Salmonella* in broilers: Effects of formic acid and calcium formate on colonization and the efficacy of ozone as a disinfectant in chill water. Master's thesis. University of Arkansas, Fayetteville, AR 72701.

Andrews, W.H. and T.S. Hammack, 2003. *Salmonella*. Bacteriological Analytical Manual Online, Chapter 5. Available at: http: //vm. cfsan. fda. gov/~ebam/bam-5.html. Hypertext source: Bacteriological Analytical Manual, 8th Edition, Revision A, Chapter 5, 1998. Accessed 18 April 2003.

Ashton, W.L.G., 1990. Enterobacteriaceae, pp: 11-41. In F.T.W. Jordon, (ed.), Poultry Diseases. Bailliere Tindall, Philadelphia, PA.

Asperilla, M.O., R.A. Smergo and L.K. Scott, 1990. Quinolone antibiotics in the treatment of *Salmonella* infections. Rev. Infect. Dis., 12: 873-889.

Bailey, J.S., 1987. Factors affecting microbial competitive exclusion in poultry. Food Tec., 41: 88-92.

Bailey, J.S., N.J. Stern, P. Fedorka-Cray, S.E. Craven, N.A. Cox, D.E. Cosby, S. Ladely and M.T. Musgrove, 2001. Sources and movement of *Salmonella* through integrated poultry operations: A multi-state epidemiological investigation. J. Food Prot., 64: 1690-1697.

Bains, B.S. and M.A. MacKenzie, 1974. Transmission of *Salmonella* through an integrated poultry operation. Poult. Sci., 53: 1114-1118.

Barnes, E.M., 1972. Food poisoning and spoilage bacteria in poultry processing. Vet. Rec., 90: 720-722.

Bender, F.E. and E.T. Mallinson, 1991. Healthy birds are lower cost birds. Broiler Industry, pp: 62-64.

Blodgett, R., 2001. Most probable number from serial dilutions. Bacteriological Analytical Manual Online, Appendix 2. Available at: http://cfsan.fda. gov/~ebam? bam-a2.html. Hypertext source: Bacteriological Analytical Manual, 8th Edition, modified from Revision A, CD-ROM version, 1998 on June 21st, 2000. Accessed 15 February, 2005.

Botts, C.W., L.C. Ferguson, J.M. Birkeland and A.R. Winter, 1952. The influence of litter on the control of *Salmonella* infections in chicks. Am. J. Vet. Res., 13: 562-565.

Bryan, F.L. and M.P. Doyle, 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Prot., 58: 326-344.

Bryan, F.L., M.J. Fanelli and H. Riemann, 1979. *Salmonella* infections, pp: 74-130. In H. Riemann and F.L. Bryan, (ed.). Food-borne Infections and Intoxications. Academic Press Inc., London.

Byrd, J.A., D.E. Corrier, J.R. DeLoach and D.J. Nisbet, 1997. Comparison of drag-swab environmental protocols for the isolation of *Salmonella* in poultry houses. Avian Dis., 41: 709-713.

Byrd, J.A., J.R. DeLoach, D.E. Corrier, D.J. Nisbet and L.H. Stanker, 1999. Evaluation of *Salmonella* serotype distributions from commercial broiler hatcheries and grower houses. Avian Dis., 43: 39-47.

Carr, L.E., E.T. Mallinson, C.R. Tate, R.G. Miller, E. Russek-Cohen, L.E. Stewart, O.O. Opara and S.W. Joseph, 1995. Prevalence of *Salmonella* in broiler flocks: Effect of litter water activity, house construction, and watering devices. Avian Dis., 39: 39-44.

Corrier, D.E., A. Hinton, Jr., B. Hargis and J.R. DeLoach, 1992. Effect of used litter from floor pens of adult broilers on *Salmonella* colonization of broiler chicks. Avian Dis., 36: 897-902.

Cox, N.A., N.J. Stern, S.E. Craven, M.E. Berrang and M.T. Musgrove, 2000. Prevalence of *Campylobacter* and *Salmonella* in the cecal droppings of turkeys during production. J. Appl. Poult. Res., 9: 542-545.

D'Aoust, J.Y., 2001. *Salmonella*, pp: 163-191. In R.G. Labbe and S. Garcia, (ed.), Guide to Food-borne Pathogens. John Wiley and Sons, Inc., New York.

Fanelli, M.J., W.W. Sadler and J.R. Brownell, 1970. Preliminary studies on persistence of *Salmonella* in poultry litter. Avian Dis., 14: 131-141.

Gustafson, R.H. and J.D. Kobland, 1984. Factors influencing *Salmonella* shedding in broiler chickens. J. Hyg., 92: 385-394.

Hayes, J.R., L.E. Carr, E.T. Mallinson, L.W. Douglass and S.W. Joseph, 2000. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. Poult. Sci., 79: 1557-1561.

Jones, F.T., R.C. Axtell, D.V. Rives, S.E. Scheideler, F.R. Tarver, R.L. Walker and M.J. Wineland, 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot.., 54: 502-507.

Kelley, T.R., O.C. Pancorbo, W.C. Merka and H.M. Barnhart, 1998. Antibiotic resistance of bacterial litter isolates. Poult. Sci., 77: 243-247.

Lahellec, C. and P. Colin, 1985. Relationship between serotypes of *Salmonella* from hatcheries and rearing farms and those from processed poultry carcasses. Br. Poult. Sci., 26: 179-186.

Lee, L.A., V.L. Threatt, N.D. Puhr, P. Levine, K. Ferris and R.V. Tauxe, 1993. Antimicrobial-resistant *Salmonella* isolated from healthy broiler chickens during slaughter. J. Am. Vet. Med. Assoc., 202: 752-755.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCraig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food Related Illness and Death in the United States. Emerg. Infect. Dis., 5: 607-625.

Muirhead, S. (ed.), 1999. Feed additive compendium. The Miller Publishing Co., Minnetonka, MN.

National Committee for Clinical Laboratory Standards, 2002. Performance standards for antimicrobial susceptibility testing; Twelfth informational supplement M100-S12. NCCLS, Wayne, PA.

Novick, R.P., 1981. The development and spread of antibiotic-resistant bacteria as a consequence of feeding antibiotics to livestock. Ann. NY Acad. Sci., 368: 23-59.

Olesiuk, O.M., G.H. Snoeyenbos and C.F. Smyser, 1971. Inhibitory effect of used litter on *Salmonella* Typhimurium transmission in the chicken. Avian Dis., 15: 118-124.

Opara, O.O., L.E. Carr, E. Russek-Cohen, C.R. Tate, E.T. Mallinson, R.G. Miller, L.E. Stewart, R.W. Johnston and S.W. Joseph, 1992. Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. Avian Dis., 36: 664-671.

Pope, M.J. and T.E. Cherry, 2000. An evaluation of the presence of pathogens on broilers raised on Poultry Litter Treatment®-treated litter. Poult. Sci., 79: 1351-1355.

Rabatsky-Ehr, T., J. Whichard, S. Rossiter, B. Holland, K. Stamey, M.L. Headrick, T.J. Barrett, F.J. Angulo and the NARMS Working Group, 2004. Multidrug-resistant strains of *Salmonella enterica* Typhimurium, United States, 1997-1998. Emerg. Infect. Dis., 10: 795-801.

Rajashekara, G., E. Haverly, D.A. Halvorson, K.E. Ferris, D.C. Lauer and K.V. Nagaraja, 2000. Multidrug-resistant *Salmonella* Typhimurium DT104 in poultry. J. Food Prot., 63: 155-161.

Reiber, M.A., R.E. Hierholzer, M.H. Adams, M. Colberg, and A.L. Izat, 1990. Effect of litter condition on microbiological quality of freshly killed and processed broilers. Poul. Sci., 69: 2128-2133.

Roy, P., A.S. Dhillon, L.H. Lauerman, D.M. Schaberg, D. Bandli and S. Johnson, 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis., 46: 17-24.

SAS Institute Inc, 1996. SAS/STAT User's guide, Version 6, Fourth Edition, Vol. 2. SAS Proprietary Software Release 8.2. SAS Institute, Inc., Cary, NC.

Schlosser, W., A. Hogue, E. Ebel, B. Rose, R. Umholtz, K. Ferris and W. James, 2000. Analysis of *Salmonella* serotypes from selected carcasses and raw ground products sampled prior to implementation of the Pathogen Reduction; Hazard Analysis and Critical Control Point Final Rule in the U.S. Int. J. Food Microbiol., 58: 107-111.

Snoeyenbos, G.H., V.L. Carlson, B.A. McKie and C.F. Smyser, 1967. An epidemiological study of salmonellosis of chickens. Avian Dis., 11: 653-667.

Tauxe, R.V., 1991. *Salmonella*: A postmodern pathogen. J. Food Prot., 54: 563-568.

Tessi, M.A., M.S. Salsi, M.I. Caffer and M.A. Moguilevsky, 1997. Drug resistance of Enterobacteriaceae isolated from chicken carcasses. J. Food Prot., 60: 1001-1005.

Tucker, J.F., 1967. Survival of *Salmonella* in built-up litter for housing of rearing and laying fowls. Br. Vet. J., 123: 92-103.

Turnbull, P.C.B. and G.H. Snoeyenbos, 1973. The roles of ammonia, water activity, and pH in the salmonellacidal effect of long-used poultry litter. Avian Dis., 17: 72-86.

USDA, FSIS., 2003. Progress report on *Salmonella* testing of raw meat and poultry products, 1998-2002. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C. Available at: http: //www. fsis. usda.gov/OPHS/haccp /salm5year.htm. Accessed 15 March 2005.

USDA, FSIS, 1999. *Salmonella* serotypes isolated from raw meat and poultry, 1998-1999. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C. Available at:http://www. fsis. usda. gov/ ophs/ haccp/ sero1yr. htm. Accessed 15 April 2005.

USDA., FSIS, 2001. Serotypes profile of *Salmonella* isolates from meat and poultry products, 1998-2001. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C. Available at: http: //www. fsis. usda.gov/ ophs / haccp /sero2001.htm.

Voogt, N., M. Raes, W.J.B. Wannet, A.M. Henken and A.W. van de Giessen, 2001. Comparison of selective enrichment media for the detection of *Salmonella* in poultry faeces. Lett. Appl. Microbiol., 32: 89-92.

Wiberg, C. and P. Norberg, 1996. Comparison between a cultural procedure using Rappaport-Vassiliadis broth and motility enrichments on modified semi-solid Rappaport-Vassiliadis medium for *Salmonella* detection from food and feed. Int. J. Food Microbiol., 29: 353-360.

Williams, J.E. and S.T. Benson, 1978. Survival of *Salmonella* Typhimurium in poultry feed and litter at three temperatures. Avian Dis., 22: 742-747.