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Internal and External Carriage of Inoculated *Salmonella* in Broiler Chickens¹

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Abstract: External and internal persistence of inoculated *Salmonella* and spread to uninoculated broiler chicks in the same pens were studied by sampling ceca and rinses of feathered carcasses in two experiments. Half of the day-old chicks in pens were orally inoculated with a nalidixic-acid-resistant strain of *Salmonella* Typhimurium at three levels of inoculum (0.1 mL delivering approximately 4×10^2 , 10^4 , or 10^6 cfu). At 3, 6, and 8 weeks of age, equal numbers of inoculated and non-inoculated pen mates were individually electrocuted and rinsed in 400 mL of diluent, after which ceca were removed aseptically, with a total of 654 chickens sampled in the two experiments. There were no differences in *Salmonella* incidence between inoculated and non-inoculated birds at any age, so the marker *Salmonella* was well distributed within pens. Total incidence was 70%, 86%, and 83% at the 10^2 , 10^4 , and 10^6 inoculum levels, respectively. Considering both cecal and rinse samples, incidence was 81%, 84%, and 72% at 3, 6, and 8 weeks of age respectively. There were 95 positives in the cecal samples only, 149 positives in the rinses only, and 277 positives in both ceca and rinse samples, so sampling either ceca or carcass rinses alone underestimated the total incidence of the marker *Salmonella*.

Key words: *Salmonella*, colonization, external carriage, internal carriage

Introduction

Day-old chicks are highly susceptible to intestinal colonization by *Salmonella* bacteria at relatively low challenge doses (Cox *et al.*, 1990). The process of flock colonization by *Salmonella* is thought to involve a few chicks, called "seeder birds," that become colonized and then produce high numbers of *Salmonella* in their intestinal tracts. The non-colonized pen-mates are then exposed to high numbers of *Salmonella* in the feces of the seeder birds, resulting in increasing numbers of colonized chicks within the flock (Cox *et al.*, 1996, 1998). Typical flock colonization with *Salmonella* is thought to peak at three or four weeks of age, after which the rate of intestinal colonization declines, although incidence of *Salmonella* in environmental and feather samples may increase due to accumulated fecal contamination (Linton *et al.*, 1985).

Incidence of *Salmonella* is highly variable, with positive and negative flocks and many negative birds within flocks that are *Salmonella* positive. It is not surprising if any short-term survey of *Salmonella* in poultry flocks finds an incidence of 0%, 100%, or any number between the extremes. Although personnel in our laboratories have years of experience in culturing *Salmonella* from tissues or environmental samples from poultry farms, there was a recent period of approximately 6 months when an unusually low number of on-farm samples were positive even though many birds, farms, and houses were sampled in a variety of ways including rinses of whole feathered carcasses. The purpose of the experiments reported here was to challenge broiler chicks on a research farm with a marker strain of

Salmonella to see if colonization in the ceca and carriage of *Salmonella* on the exterior of birds followed patterns seen in previous work.

Materials and Methods

For each experiment, one-day-old Cobb X Cobb males from the female broiler breeder line were obtained from a commercial hatchery and transported to a research farm. Birds (40 per pen in Experiment 1 and 34 per pen in Experiment 2) were placed in floor pens. Litter in the pens had been used previously, but not with nalidixic acid-resistant strains of *Salmonella*. Each pen was set up to simulate a commercial broiler house with nipple drinkers, pan feeders, and bird density similar to that seen in industry.

In both experiments, half of the birds in each pen were wing banded and orally inoculated with 0.1ml of a suspension of nalidixic acid-resistant *Salmonella* Typhimurium on the day of placement. Calculated inoculum levels were 10^2 , 10^4 , or 10^6 cells. Actual counts of the inoculum indicated that approximately 380, 38,000, or 3,800,000 cells were delivered in Experiment 1 and 490, 49,000, or 4,900,000 in Experiment 2.

At 3, 6, and 8 weeks of age in both experiments, birds from each pen (n=10; half inoculated and half non-inoculated) were sampled for the presence of inoculated *Salmonella*. A total of 654 birds were sampled in the two experiments. Each bird was euthanized by electrocution and placed into a plastic bag. Carcasses were rinsed by shaking for 1 min in 400 ml of distilled water, after which ceca were removed aseptically from each carcass and bagged. Ten ml of the carcass rinse sample was added

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Table 1: Percentage of *Salmonella*-positive ceca and whole carcass rinse samples from broiler chicks sampled at 3, 6, and 8 weeks of age after inoculation of half of the chicks in each pen with approximately 10^2 , 10^4 , or 10^6 cfu at one day of age in Experiments 1 and 2 (n = 654)

	Approximate inoculum level	Inoculated chicks		Uninoculated chicks		
Age		Ceca	Rinse	Ceca	Rinse	Mean
		----- (% <i>Salmonella</i> positive) -----				
3 weeks	10 ²	58	55	62	58	58
	10 ⁴	60	80	75	65	70
	10 ⁶	60	68	65	78	68
6 weeks	10 ²	58	58	52	52	55
	10 ⁴	66	79	55	75	69
	10 ⁶	61	74	56	90	70
8 weeks	10 ²	40	33	40	50	41
	10 ⁴	47	80	50	50	57
	10 ⁶	53	63	57	50	56

to 90 ml of 1% buffered peptone (BP). Ceca and rinse samples were incubated overnight at 37°C after which Brilliant Green Sulfa agar (BGS) plates containing 200 ppm nalidixic acid were streaked with a loop from each incubated sample. Plates were incubated overnight at 37°C and then the presence of marker *Salmonella* was evaluated on a qualitative basis. Approximate enumeration was also performed on the original samples utilizing a 3-swab method described in Blanchfield *et al.* (1984), a method faster than counting colonies, but with a higher standard deviation. Two swabs were immersed directly into the original samples and streaked directly on BGS + nalidixic acid plates or diluted in 10 ml of BP and then streaked. A third swab was dipped in the dilution blank and streaked. Plates were incubated overnight at 37° C. Ceca were weighed, diluted in 1% BP equal to three times the weight of the sample, and crushed with a rubber mallet. Ten ml of the original sample was also placed in 90 ml of BP and incubated overnight, followed by streaking on BGS plates as described above. Counts of bacteria were converted to \log_{10} (cfu/ml). Statistical analysis was done using analysis of variance and Chi Square tests in SAS (SAS Institute, 2000).

Results and Discussion

Approximately 80% of all samples were *Salmonella* positive, so the marker strain was a better colonizer than anticipated. Cecal colonization occurred to a high degree in both experiments and uninoculated penmates of the challenged birds were also colonized, resulting in a high level of *Salmonella* contamination. No difference in incidence or numbers of the marker *Salmonella* was found between the two experiments or between inoculated birds and non-inoculated pen mates in samples taken at 3, 6, and 8 weeks of age, so the marker *Salmonella* was well distributed within pens (Table 1). Total incidence was 70%, 86%, and 83% at the 10^2 , 10^4 , and 10^6 inoculum levels, respectively, indicating that inoculum level was not a major factor as long as the challenge level was high enough to

establish colonization in the flock. Considering both cecal and feathered carcass rinse samples, incidence was 81%, 84%, and 72% at 3, 6, and 8 weeks of age respectively. The pattern usually reported for *Salmonella* colonization was seen in the trends toward a lower level of colonization at the lowest challenge dose and a lower incidence in 8-week-old birds versus 3-week-olds, but the differences were not significant.

Jarolmen *et al.* (1976) reported that incidence of inoculated, nalidixic acid-resistant *Salmonella* Infantis, S. Enteritidis, and S. Typhimurium in droppings of control chicks challenged at one or six days of age remained high until at least six weeks of age, although numbers dropped off sharply within one or two weeks after inoculation. Inoculum levels in those experiments were $6.0 \log_{10}$ cfu at one day of age and $9.0 \log_{10}$ cfu at 6 days of age. In the present experiment, chicks were inoculated with approximately 2, 4, and $6 \log_{10}$ cfu at one day of age, so one treatment was about four orders of magnitude lower than in Jarolmen *et al.* (1976), one was two orders of magnitude lower, and another was about the same. In the two experiments reported in Jarolmen *et al.* (1976), 4% and 100% of the birds were orally challenged versus 50% in the present experiment. Whole carcass rinses were not performed in those experiments, however.

Mean numbers of *Salmonella* found in the positive cecal samples across both experiments and all three sampling ages were $2.0 \log_{10}$ (cfu/ml) in the ceca of inoculated birds versus 1.8 in the ceca of non-inoculated pen mates. Whole carcass rinse means were $1.8 \log_{10}$ (cfu/ml) in whole carcass rinses of both inoculated and non-inoculated birds. Counts of marker *Salmonella* in feces of three-week-old chicks were approximately $2.0 \log_{10}$ (cfu/g) with means of about 1.0 in later weeks, so the numbers recovered from intestinal contents were in the same range as in the present experiment even though incidence remained high versus the declining incidence after three weeks of age reported in Jarolmen *et al.* (1976).

There were 95 positives in the cecal samples only, 149 positives in the external rinses only, and 277 positives in both ceca and rinse samples, so sampling ceca or rinses alone underestimated the total incidence of the marker *Salmonella*. Numerous publications have indicated higher incidence of *Salmonella* in external samples of broiler chickens than in internal samples. In studies that compared incidence of *Salmonella* in whole carcass rinses of feathered carcasses to incidence in cecal and intestinal samples, external incidence was more than twice as great as internal incidence (McBride *et al.*, 1980; Rigby *et al.*, 1980, 1982; Line, 2002).

The incidence of *Salmonella* on the exterior of carcasses is of interest because *Salmonella* bacteria on the exterior of birds may be a more important source of later contamination than *Salmonella* carried in the intestinal tract (Rigby *et al.*, 1980, 1982; Izat *et al.*, 1989, 1990; Trampel *et al.*, 2000). Inoculating bacteria on the exterior of test carcasses has been shown to cause more cross-contamination during processing than inoculating bacteria directly into the cloaca (Mulder *et al.*, 1978).

The results of the work conducted at the experimental farm indicated that marker *Salmonella* colonized both inoculated chicks and non-inoculated penmates just as easily as in previous experiments. The earlier period in which it was difficult to find *Salmonella* on commercial farms was probably caused by unknown conditions or was a statistical anomaly.

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