Sources of Salmonella and Campylobacter in Poultry and Methods to Intervene

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Introduction

The presence and persistence of *salmonella* contamination in the commercial broiler hatcheries suggests that the day-of-hatch chick may be at greater risk from salmonellae in the hatchery environment than at subsequent times during growout. Contamination and penetration of the shell of fresh and incubating eggs constitute perhaps the most important link (or critical control point) in the transmission of salmonellae to young birds and should be a primary target for any intervention procedure.

Research has shown that salmonellae can rapidly penetrate the freshly laid fertile hatching egg (Williams and Dillard, 1968). The abundance of salmonellae in commercial hatcheries is evidence that the presently used chemical treatments are not killing all of the salmonellae or preventing the horizontal transmission of salmonellae through the shell and membranes of the egg. Once salmonellae organisms get past the membranes of hatching eggs, there is no way to prevent their further invasion of the egg contents or developing embryo. Therefore, for hatching egg sanitation to be effective against salmonellae, it must be rapidly applied at the breeder farm level. We are actively pursuing three lines of research to address this area. One approach is to study the penetration, proliferation, and localization of salmonellae in the fertile hatching egg (Cason et al., 1991a,b). A second approach is to determine the effectiveness of many chemicals (Cox and Bailey, 1991a; Cox and Bailey, 1991b), and the third approach is to determine the best procedure for applying a chemical that will be effective against salmonellae without adversely affecting hatchability or livability of chicks. In addition, we are exploring ways other than chemical treatment of eggs to eliminate the salmonellae influence from the breeder flocks. Also, a research project to circumvent salmonellae contamination by implanting a mature gut microflora in the unhatched embryo is presently ongoing (Cox et al., 1990). It is a consensus among poultry researchers, is that it is essential to intervene during production so that salmonella are not carried into the processing plant by birds. Also, the complexity of poultry production and the commensal intestinal colonization of chickens dictates that an integrated multi-component attack will be required to impact the problem. To summarize, breeder flocks and, hatchery environments are very important critical control points for the colonization of commercial poultry by salmonellae. *Salmonella* contaminated eggs will hatch and spread contamination throughout the hatching cabinet. Successful intervention at these critical control points is dependent on an effective chemical treatment applied to the freshly laid fertile hatching eggs. A systematic evaluation of the type and concentration of chemical, and method and time of application has been going on for the past 10 years. The results to date suggest that most eggs can be disinfected, but only with the right chemical, and only if done within minutes after exposure to *Salmonella*. The combination of eliminating salmonellae influence from breeder flock eggs followed by treatment of new hatchlings with an effective CE culture before exposure to environmental salmonellae may be a necessary approach to impact the problem.

Control of salmonellae in an integrated poultry operation is complex because there are numerous potential sources of salmonellae contamination including chicks, feed, rodents, wild birds, insects, transportation, and the processing plant environment. Effective control of salmonellae will require that intervention strategies be adopted for each of these sources. For post-hatchery salmonellae sources, competitive exclusion treatment of day-of-hatch chicks, as first described by Nurmi and Rantala (1973), has been used to help control intestinal colonization of broilers by salmonellae. Several researchers, including Mead and Impey (1985) in England and Stavric (1987) in Canada, expanded the knowledge of effectiveness and practical applications of competitive exclusion treatments.

Stern (1993) described the process for making a competitive exclusion product derived from the mucosal scrapings of pathogen-free adult broiler chickens (MCE). Large scale commercial field trials were conducted in Puerto Rico and Georgia to test the efficacy of the MCE to protect broiler chickens against natural salmonellae colonization of the intestinal tract and subsequent contamination of the processed chickens from these treated flocks. In Puerto Rico, (Blankenship et al., 1993) incidence rates of intestinal colonization with salmonellae were reduced from 11% in controls to 2% in MCE-treated flocks and from 41% in controls to 10% in treated flocks on processed carcasses (Table 1). In Georgia, incidence rates of

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intestinal colonization were reduced from 2% in controls to undetectable in treated and from 9.5% of control to 4.5% of treated processed carcasses (data not shown in tabular form). The process for making MCE was issued a U.S. Patent (95,451,400) in 1995 and was licensed to the Continental Grain Company in 1996 to develop a commercial product. This product is now called Mucosal Starter Culture MSC The current study was initiated to determine the efficacy of MSC to reduce salmonellae on processed broiler carcasses in normal commercial operations and in smaller scale swine studies.

TABLE 1. Prevalence of salmonellae among 6 to 7 weeks old chickens in Puerto Rico treated with mucosal competitive exclusion (MCE) and associated environmental contamination.

Sample	Control	Treated
Farm: ceca	17/150 (11%)[1]	3/150 (2%)
Plant:ceca	24/150 (15%)	5/150 (3%)
Processed carcasses	62/150 (41%)[a]	15/150 (10%)[b]

[a,b] Means with no common superscripts differ significantly ($P < .05$)
[1] salmonellae positive samples/total samples tested (percentage positive)

Materials and Methods

Trials were conducted in Arkansas, Alabama, and Georgia in standard 16,000 to 23,000 bird broiler houses. On the day of hatch, broiler chickens with an initial body weight of 35 to 45 grams were treated with MSC twice. Each chick was first sprayed with 0.2 ml of a 10^7 MSC organisms per ml solution after placement in the transport container. Then, each chick was supplied with approximately 8-10 ml of a MSC water solution containing 10^6 MSC organisms per ml solution as the first drinking water. Birds were placed on "built up" (used) litter. Feed was supplied to chicks via auger from feed storage bins at each house. Feed and water were available ad libitum.

One to 2 days before the end of grow-out, both ceca from 60 randomly selected chicken from each house were aseptically removed from birds which had been killed by cervical dislocation. Ceca were placed into stomacher bags and shipped overnight to the Russell Research Center. In the processing plant, sixty randomly selected broiler carcasses were pulled from the line before and after the immersion chiller. Each carcass was placed in a sterile bag and rinse sampled with 100 ml sterile water (Cox et al., 1983). The rinse solution was poured into sterile specimen containers and shipped to Russell Research Center. On the day of receipt in the laboratory, ceca were separated and a volume of universal preenrichment (UP) broth (Difco, Detroit, MI) (Bailey and Cox, 1992) equal to 3 times the weight of the ceca added to each stomacher bag. Concentrated UP (10X) was added to the carcass rinse fluid to make a single strength medium. UP tubes were incubated for 24± 2 hr at 35°C and then 0.1 ml of the-UP solution was transferred to 10 ml TT broth. After incubation for 24± 2 hr at 42°C, the VIDAS (bioMerieux/Vitek, St. Louis, MO) automated immunoassay was used according to the instructions of the manufacturer to screen each sample for salmonellae. Samples which gave a positive screen on the VIDAS assay were streaked from the TT broth onto BG sulfa and modified lysine iron agar plates for isolation of salmonellae. Typical colony forming units were serologically confirmed to be salmonellae by a latex agglutination assay. Data was recorded as positive or negative for presence of salmonellae in each sample.

The presence or absence of salmonellae was measured on the farm by analysis of the ceca from birds one day prior to processing and at the processing plant by analysis of whole carcass rinses before and after chilling. The odds ratio was calculated for each of the three test farm and associated processing plant samples. The odds ratio was then studied using the Breslow/Day test to determine homogeneity (SAS, 1996). When the odds were found to be homogenous, the data were pooled and examined together to calculate a common odds ratio, A confidence interval was then established to report the magnitude of any treatment effect observed. In addition, Chi Square analysis was done to test for a significant relationship between treatment (MSC or control) and number of positive samples.

D. *Safety of CE/MCE/MSCÔ*. The safety of all of the above has been demonstrated by veterinarian monitored challenge trials in which birds were necropsied to show effects of these treatments and no differences were found between treated and untreated flocks. Three extensive European field trial made similar observation (Huttner et al. 1991, Wierup et al. 1997; Goren et al.1988).

Results

To ensure exposure of the test chickens to salmonellae, houses with a previous history as (confirmed by drag swabs) of salmonellae were selected. Data from the carcass rinse samples pre- and post-chill were found to be homogenous by the Breslow-Day test with a likelihood ratio of .83 and 0.55 respectively. Therefore, data were pooled across locations.

The incidence of salmonellae (Table 2) was significantly less at both pre-chill (23 vs 12 positive samples; $p = .05$) and post-chill (9 vs 0 positive samples; $p < .01$). Overall these data confirm a highly significant reduction in salmonellae found on processed broiler carcasses in birds treated with MSC.

TABLE 2. Pre-chill and post-chill salmonellae data on processed carcasses from Arkansas, Alabama, and Georgia.

	Control	MSC Treated	Exact Test
Pre-chill carcasses	23+/180	12+/180	p = .05
Post-chill carcasses	9+/180	0+/180	p < .01

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