Salmonella Sampling and Recovery from On-Farm Litter to Fully Processed Carcasses – Ability to Detect *Salmonella* vs. "*Salmonella*-Free"

Dianna V. Bourassa, Ph.D.
USDA-ARS, U.S. National Poultry Research Center
950 College Station Road, Athens, GA 30605
Dianna.Bourassa@ars.usda.gov (706) 546-3180

Abstract: Poultry are sampled often for *Salmonella* during growout on the farm and throughout the processing plant. While on farm sampling is not currently a regulatory requirement it can be useful in determining *Salmonella* status of each flock. On farm sampling can include varying types of both environmental and individual bird sampling techniques, and most of the composite samples exhibit a greater sensitivity in the detection of *Salmonella*. The flock status that was determined by on farm sampling has been proposed to be used for logistic slaughter in which *Salmonella*-negative flocks can be scheduled to be processed before positive flocks to minimize cross contamination within the plant. However, during feed withdrawal at the end of grow-out and prior to slaughter there can be proliferation of *Salmonella* within the bird's alimentary tract that prior to feed withdrawal was below the level of detection. At the processing plant, carcasses are sampled for the presence of *Salmonella*, which is regulated by the USDA Food Safety and Inspection Service (FSIS). Whole carcasses, parts, and comminuted products are sampled for *Salmonella* and required not to exceed a maximum number of positive samples. The standard FSIS methods as well as other isolation methods have been compared for sensitivity in *Salmonella* detection. All *Salmonella* sampling methods have a minimal level of detection so a blanket statement indicating "zero tolerance" in any raw product is an unreasonable expectation. In order to achieve Zero *Salmonella* or *Salmonella*-free status a sampling method would have to be guaranteed to detect a single *Salmonella* cell and all of the product would have to be sampled leaving no products left to eat. Cooking to 165°F/74°C or irradiation are the only known dependable methods to completely eliminate *Salmonella* from raw poultry products.

On Farm *Salmonella* Sampling

Environmental Sampling

Many different production house environmental sampling methods for the recovery of *Salmonella* on the farm have been studied. Environmental sampling methods can be divided into two categories. Direct component methods incorporate removal and analysis of samples such as feces, litter, or dust. Surface sampling methods rely on contact between the environment and the moistened sampling media such as conventional drag swabs, "socks", shoe covers, and ISODS (intermittently stepped on drag swabs).

In a study comparing direct sampling methods (feces and litter) and surface sampling methods (drag swabs = gauze squares and socks = elastic bandage), sampling with socks yielded the highest percentage of samples positive for *Salmonella* in both challenged pens with high *Salmonella* levels and the adjacent non-challenged pens with lower levels of *Salmonella* (Buhr, et al., 2007). Surface sampling methods including drag swabs, ISODS, and socks were further compared. It was demonstrated that the ISODS and sock methods that incorporated a greater contact pressure with the litter surface from using a foot to step on the sampling material were more sensitive for the recovery of low levels of *Salmonella* in comparison to conventional drag swabs, which are simply dragged across the litter surface (Buhr, et al., 2007).

When boot swabs (similar to socks), drag swabs, litter, and dust were compared in various types of turkey flocks, the boot swabs and dust had the highest percentages of *Salmonella* detection followed by litter samples. Conventional drag swabs had the lowest percentage of positive samples (Mueller-Doblies, et al., 2009). When a turkey house was sampled with boot swabs, dust, and feces, both the boot swab and dust samples were again more sensitive than individual feces samples (Arnold et al., 2009).

Overall, when taking house environmental samples for the detection of *Salmonella*, sampling methods that include stepping on the moist sampling material while in contact with the litter are more sensitive than conventional drag swab sampling of the litter surface.

Individual Bird Sampling

In addition to environmental samples, flock status can also be determined thorough individual bird sampling. These types of samples can either be non-destructive or destructive, and include cloacal swabs, individual bird feces, and intestinal ceca. In studies utilizing non-destructive methods of cloacal swabs and fecal samples, it was demonstrated that fecal samples were much more sensitive for the detection of *Salmonella* than cloacal swabs (Ayachi, et al., 2010; Garcia, et al., 2011). When using a destructive sampling approach, sampling of the ceca (vs. other intestinal segments or organs) resulted in the most positive samples (Barrow, et al., 1988). Sampling in a turkey production facility comparing ceca (destructive method) and environmental swab samples (non-destructive) demonstrated that sampling ceca was three times more sensitive than environmental swabs (Sanad et al., 2016). When sampling individual birds, ceca or fecal samples are the most sensitive in the detection of *Salmonella* because the higher resident levels of *Salmonella* than on the cloaca epithelial surface.

Sampling Time

In addition to what samples are taken, when the samples are taken can also influence the ability to detect *Salmonella*. During broiler growout (low *Salmonella* challenge) for both ISODS and ceca sampling methods, *Salmonella* can be detected at the highest frequency at about 3 weeks of age, after which detection steadily declines from 3 to 6 weeks of age (Wilson, et al., 2016). A similar trend is seen in turkeys where the greatest number of samples was positive for *Salmonella* during rearing at 14 days of age (Morris, et al., 2015). The frequency of positive samples decreased after 14 days through 20 weeks.

Feed withdrawal can also influence the ability to detect *Salmonella*. When broilers are subjected to feed withdrawal, the crop empties within 6 hours and the pH within the crop increases to near neutral leading to the growth of enteropathogens such as *Salmonella* when present (Hinton, et al., 2002). In a study where *Salmonella* negative broilers were placed on *Salmonella* positive litter during feed withdrawal, *Salmonella* was frequently detected (48%) from crops and on two occasions (5%) in the ceca after a 12 hour period (Buhr, unpublished data).

In addition to feed withdrawal prior to processing, restriction feeding of breeders may also impact *Salmonella* proliferation and persistence. In a study comparing every-day and skip-a-day feeding programs with broiler breeder pullets, pullets that were fed every day had a lower prevalence and shorter persistence of *Salmonella* than pullets that were fed with a skip-a-day program (Wilson, et al., 2013). Emptying of the crop every other day, allowing for an increase in crop pH and litter picking may contribute to a higher prevalence and persistence of *Salmonella* positive ceca.

***Salmonella* Sampling in Slaughter Plants**

During turkey and broiler processing the prevalence of *Salmonella* on carcasses changes between each step of the process. The levels of *Enterobacteriaceae*, of which *Salmonella* is a member, start high on the feathers and skin when the birds arrive at the processing plant. Levels are decreased during scalding but increase during defeathering. After defeathering, process controls including mechanical and chemical interventions continue to decrease levels of bacteria on the surface of the carcasses (Berrang and Dickens, 2000).

The Food Safety and Inspection Service samples for *Salmonella* in plant post-chill for FSIS regulated products such as whole broiler and turkey carcasses, chicken parts, and not ready-to-eat ground or comminuted products. The previous sampling set approach has been replaced with a moving window approach in which plant process control is assessed by a moving 52-week period. Poultry carcasses are sampled post-chill following all microbial interventions. Broiler carcasses are rinsed in 400 mL buffered peptone water (BPW) and 100 mL of the rinsate are collected for *Salmonella* analysis. Turkey carcasses are sampled using a sponge technique in which a sponge moistened with 10 mL BPW is wiped along the back and thigh of the carcass. For comminuted products approximately 2 pounds of product are collected for sampling of which 325 g are mixed with 1,625 mL of BPW.

Multiple sampling methods including rinse, excision (skin or muscle), swab, neck skin, and whole carcass enrichment have been evaluated for the detection of *Salmonella* on carcasses. When sampling turkey carcasses, methods that required sampling of a larger surface area of the carcasses (whole carcass swabbing and whole carcass rinse) were more sensitive in the detection of *Salmonella* than one or two site swab sampling (McEvoy et al., 2005). When neck skin and whole carcass enrichment *Salmonella* sampling methods following air chilling were compared in broilers, both methods were found to be equivalent (Bourassa, et al., 2015). However, following immersion chilling, whole carcass enrichment was the most sensitive method, neck skin less sensitive, and whole carcass rinse the least sensitive.

Although sampling methods vary in sensitivity, each method has a minimal level of detection. The most sensitive method (whole carcass enrichment) requires approximately 8-10 cells being present on the carcass to yield a positive result following 24 hours enrichment incubation (Cox and Blankenship, 1975). Therefore, any product sampled can never really be declared "Salmonella-Free". The absence of a positive result from a carcass or 325 g of comminuted product does not indicate that there is no *Salmonella* present. Sampling for *Salmonella* in processing plants is essential for evaluating process control for the minimization of contamination. Use of the phrase "Zero Tolerance" may lead consumers to believe that there is no *Salmonella* on raw product and may give a false sense of security.

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