The Influence of Ambient Environmental Conditions on the Survival of Salmonella enteric Serovar typhimurium in Poultry Litter

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Abstract: Combined effects of temperature, relative humidity and litter pH in the presence or absence of organic matter on the survival of S. typhimurium over time was studied. The litter (L: 30 cm x W: 25 cm x D: 6 cm aluminum trays filled with wood shavings) was inoculated with S. typhimurium at initial concentration of 4.8 x 10^7 CFU/ml, then litter trays were placed in a room with microclimate similar to that of a naturally ventilated poultry house. The periodical measurement of S. typhimurium population in poultry litter in relation to the ambient environmental conditions revealed that: in the absence of organic matter, there was a non-significant (p<0.05) negative correlation (-0.07 at confidence level 95%) between ambient temperature and survival of S. typhimurium, a non-significant (p>0.53) positive correlation (+0.04 at confidence level 95%) between relative humidity and survival of S. typhimurium population and a highly significant (p<0.005) positive correlation (+0.67 at confidence level 95%) between litter pH and survival. In the presence of organic matter, there was a non-significant (p>0.55) negative correlation (-0.22 at confidence level 95%) between ambient temperature and survival, a highly significant (p<0.0001) negative correlation (-0.12 at confidence level 95%) between relative humidity and survival and a significant (p<0.05) positive correlation (+0.48 at confidence level 95%) between litter pH and survival. The study suggested that increased litter pH and relative humidity rather than temperature presented a great influence on the increased survival of S. typhimurium. New management practice that will reduce litter pH and relative humidity should be considered in the control plans of Salmonelliosis in poultry farms.

Key words: Salmonella typhimurium, survival, poultry, organic matter, temperature, relative humidity, litter pH

INTRODUCTION
Salmonella remains one of the main causes of food borne illness all over the world and many key questions regarding the introduction and persistence in animal production system still remain (Liljebjelke et al. 2005). An everyday requirement for decreasing the incidence of Salmonellosis is based on strict hygienic conditions “from stable to table” Durecko et al. (2004). Salmonella enteric serovar typhimurium and enteritidis are known as the persistent serotypes among single age flocks, with a correlation between qualitative environmental samples and semi quantitative fecal samples and there were significant temperature and seasonal effects upon contamination that was increased significantly over time Wales et al. (2007).

Litter can be considered one of the most favorable media for the growth and transmission of Salmonella, depending on water activity (Aw) and Moisture Content (MC). High Aw values (0.90-0.95) were associated with flocks positive for Salmonella; while low Aw values (0.79-0.84) were associated with flocks negative for Salmonella and transition Aw values (0.85-0.89) were associated with flocks having increased risk for the presence of Salmonella; Carr et al. (1995). Contaminated poultry litter, serving as a reservoir for Salmonella, can be linked to both food safety concerns when contaminated birds enter processing plants and environmental concerns when used as fertilizer.

The survival of Salmonella in poultry house environment is dependent on both physical and chemical factors such as temperature, water activity (Aw) or equilibrium RH (ERH), moisture content, and pH. Whenever extrinsic factors fall outside the optimum range for microbial growth and survival, these factors can cause cellular damage. Depending on the severity of the stress factors, growth can be inhibited or cell death can occur, Farkas, (2001). The findings from previous studies indicated that extrinsic parameters can influence the presence or absence of Salmonella in broiler litter, with the most significant factor being Aw, Opara et al. (1992). Turnbull and Snoeyenbos (1973) concluded that the salmonellacidal activity of used litter may be attributed to changing litter Aw, and pH.

The aim of this study was to evaluate the survival of S. typhimurium under normal environmental conditions in artificially contaminated litter (wood shavings) in
absence or presence of organic matter (poultry dropping).

**MATERIALS AND METHODS**

**Propagation of salmonella typhimurium:** S. typhimurium ATCC 13311; genomic DNA strain NCTC74 was propagated and counted using Drop plate Technique. Zelver et al. (1999) and Herigstad et al. (2001). The procedures were carried out by pipetting 1 ml of bacterial suspension into a dilution tube containing 9 ml of tetrathionate broth; making dilution 10^{-1}. Tenfold serial dilutions were made to obtain dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-9}. Bacterial count in each dilution was obtained by inoculating on chromagar Salmonella plated (Becton-Dickinson, VMR Int.) The plates were incubated overnight for 17-20 h at 35-37°C. Viable cell counts were expressed as CFU/surface area.

The calculation was carried out using the following formula: Log (average CFU/drop vol.) (dilution factor) (Vol. scrapped into/surface area).

**Inoculation of the litter with salmonella typhimurium Trial (A):** Three aluminum foil trays (L: 30 cm x W: 25 cm x D: 6 cm) were filled with litter (wood shavings). The trays were sterilized by autoclaving at 121°C for 1 h. Sterilization was confirmed by placing 25 gm of autoclaved litter into 225 ml of Buffered Peptone Water (BPW; Oxoid, Fisher Scientific Int.) and incubated in a rotatory incubator for 3 h, followed by culturing on Chromagar Salmonella plates (BD, VMR Int.). Two of the three trays were inoculated with S. typhimurium suspension (4.8 x 10^5 CFU/ml) and the third tray was used as control. The first tray was sprayed with 80 ml suspension (6 ml S. typhimurium suspension in 54 ml of phosphate buffered saline; resulted in count ~10^3). The second tray was sprayed with 60 ml suspension (0.5 ml S. typhimurium suspension in 99.4 ml phosphate buffered saline; resulted in count ~10^3). The control tray was sprayed using 60 ml of phosphate buffered saline. The litter was mixed thoroughly with the added suspension and placed in a facility with open environmental conditions.

**Trial (B):** The same procedures were carried out as in trial (A), except that fresh poultry droppings collected from Poultry Farm and autoclaved at 121°C for 30 min was added as a source of organic matter (250 gm/tray) at the beginning of the experiment.

**Collection of litter samples:** In both Trials (A and B), three samples of 3.0 gm were collected from each aluminum tray through the whole depth of the litter. Samples were collected twice weekly. Each sample was added to 27 ml Phosphate Buffered Saline (PBS). vortexed for 20-25 min, then the mixture was filtered using filter paper 7 cm in diameter, William et al. (1975); The filtrate was used for S. typhimurium count. The ambient temperature, relative humidity were recorded daily. In addition pH of the litter was measured daily.

**Salmonella typhimurium count:** The filtrate was used for obtaining bacterial count using the drop plate technique with chromagar Salmonella plates as described previously. Viable cell counts were expressed as CFU/surface area.

The calculation was carried out using the following formula: Log (average CFU/drop vol.) (dilution factor) (Vol. scrapped into/surface area).

**Statistical analysis:** The statistical analysis was carried out by performing analysis of variance (ANOVA) and regression correlations using SAS 9.2.0 software.

**RESULTS AND DISCUSSION**

The management practices at the breeder level may have a profound effect on the transmission and persistence of Salmonellae within an integrated production system, as well as on the potential contamination of poultry derived products, Mollenhorst et al. (2005).

**Effect of ambient temperature on S. Typhimurium in poultry litter in the presence or absence of organic matter:** In absence of organic matter (autoclaved fresh poultry dropping), there was a non-significant (p=0.99) negative correlation (-0.07 at confidence level 95%) between the ambient temperature and S. typhimurium survival (Fig. 1). In the presence of organic matter, there was also non-significant (p=0.55) negative correlation (-0.22 at confidence level 95%) between the ambient temperature and S. typhimurium survival (Fig. 2). These data suggest that irrespective of the absence or presence of organic matter in face of the increase in ambient temperature there was a decline in the survival of S. typhimurium in poultry litter.

**Effect of relative humidity on the survival of S. Typhimurium in poultry litter in the presence or absence of organic matter:** Controlling RH inside the house and in the litter is an important control strategy for reducing pathogens, ammonia fumes and parasites such as coccidia in the bird’s environment, Zander et al. (1997). Proper ventilation practices are not only critical to cooling birds but are also a key management tool used to remove excess moisture from the broiler house and to maintain a certain degree of dryness in the litter. Valentine (1964) found that both ammonia and RH levels were reduced as the rates of air exchange inside well-insulated test pens (8 x 14 feet) increased. Mallinson et al. (1998) reported that low broiler litter surface airflow rates (less than 15.6 m/min or 51 ft/min) were related to increase in litter Salmonella population.
Fig. 1: Correlation between ambient temperature and S. typhimurium count in poultry litter in the absence of organic matter (N = 42, R² = 0.0051, AdjR² = -0.0198, RMSE = 1.4746)

Fig. 2: Correlation between ambient temperature and survival of S. typhimurium in poultry litter in the presence of organic matter (N = 42, R² = 0.0051, AdjR² = -0.0198, RMSE = 1.4746)

(1.83 CFU/10 gm) compared with higher airflow rates (greater than 15.6 m/min) and decreased Salmonella population (less than 1.33 CFU/10 gm).

In the absence of organic matter there was a non-significant (p<0.53) positive correlation (+0.04 at confidence level 95%) between relative humidity and survival of S. typhimurium in poultry litter (Fig. 3); suggesting that with increasing relative humidity; survival of S. typhimurium in poultry litter was increasing.

In the presence of organic matter in litter there was a highly significant (p<0.0001) negative correlation (-0.12 at confidence level 95%) between RH and survival of S. typhimurium in the poultry litter (Fig. 4); This suggests that in face of increased relative humidity; survival of S. typhimurium in poultry litter was decreasing in the presence of organic matter.

Effect of litter pH on survival of S. Typhimurium in poultry litter in the presence or absence of organic matter: Studies have shown that S. Typhimurium and E. coli grow optimally in pH environment from 5-9, Foster, (1993); Small et al. (1994), although Salmonella growth rates generally thrive from pH 6.5-7.5, Chung and Goepfert (1970); D'Aoust (1989). Others have reported that the pH growth range for Salmonella falls between
lowering litter pH to 2.68 and 3.48 using \( \text{H}_2\text{SO}_4 \) and \( \text{NaHSO}_3 \). Litter treatment products significantly reduced *Salmonella* population by 1.04 and 1.30 log CFU/ml, respectively.

In the absence of organic matter there was a highly significant \((p<0.005)\) positive correlation \((+0.67 \text{ at } \text{confidence level } 95\%)\) (Fig. 5). In the presence of organic matter there was also a significant \((p<0.05)\) positive correlation \((+0.48 \text{ at } \text{confidence level } 95\%)\) (Fig. 6), suggesting that decreasing litter pH was resulting in a decline in the survival of *S. typhimurium* irrespective of the presence or absence of organic matter.

As few as 5 cells of *Salmonella* infection have been shown to infect chicks, Milner and Shaffer (1952) and this number may even be lower if the birds are stressed; Arakawa et al. (1992). Once infected, these birds may excrete fecal concentration of up to \(10^7\) *Salmonella* per g of faeces for up to 2 weeks duration; Bailey (1987). Chick mortality has been observed to reach its peak at 3-7 days; Gost (1997). In some instances, new litter has been shown to be contaminated with *Salmonella* before bird placement; (Kumar et al., 1971; Simmons and Byrnes, 1972; Bahlia et al., 1979).

**Conclusion:** The findings from the present study indicated that some extrinsic parameters and environmental conditions can influence the survival of *Salmonella typhimurium* in broiler litter over time, with the most significant factors being litter pH and relative humidity.

**REFERENCES**


