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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 10^7CFU/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 ($p \leq 0.99$) negative correlation (-0.07 at confidence level 95%) between ambient temperature and survival of *S. typhimurium*, a non-significant ($p \leq 0.53$) positive correlation (+0.04 at confidence level 95%) between relative humidity and survival of *S. typhimurium* population and a highly significant ($p \leq 0.005$) positive correlation (+0.67 at confidence level 95%) between litter pH and survival. In the presence of organic matter, there was a non-significant ($p \leq 0.55$) negative correlation (-0.22 at confidence level 95%) between ambient temperature and survival, a highly significant ($p \leq 0.0001$) negative correlation (-0.12 at confidence level 95%) between relative humidity and survival and a significant ($p \leq 0.05$) 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 *Salmonellosis* 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 (A_w) 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 A_w, Opara *et al.* (1992). Turnbull and Snoeyenbos (1973) concluded that the salmonellacidal activity of used litter may be attributed to changing litter A_w 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 1331; 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, 10^7 and 10^8. 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 10^7 CFU/ml) and the third tray was used as control. The first tray was sprayed with 60 ml suspension (6 ml *S. typhimurium* suspension in 54 ml of phosphate buffered saline; resulted in count ~10^6). The second tray was sprayed with 60 ml suspension (0.6 ml *S. typhimurium* suspension in 99.4 ml phosphate buffered saline; resulted in count ~10^5). 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 ($p \leq 0.99$) 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 ($p \leq 0.55$) 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. tyhimurium* 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

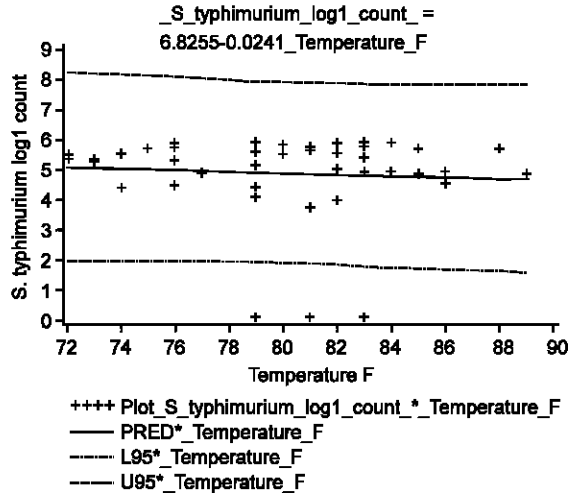

Fig. 1: Correlation between ambient temperature and *S. typhimurium* count in poultry litter in the absence of organic matter (N = 42, R^2 = 0.0051, AdjR^2 = -0.0198, RMSE = 1.4746)

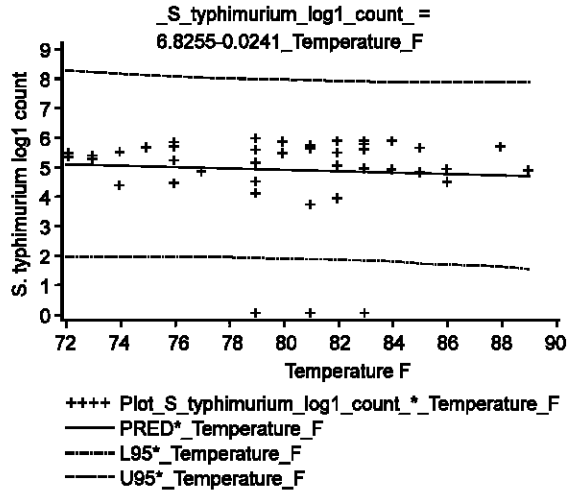

Fig. 2: Correlation between ambient temperature and survival of *S. typhimurium* in poultry litter in the presence of organic matter (N = 42, R^2 = 0.0051, AdjR^2 = -0.0198, RMSE = 1.4746)

(1,63 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 ($p \leq 0.53$) positive correlation (+0.04 at confidence level 95%) between relative humidity and survival of *S. typhimurium* in poultry litter (Fig. 3); suggesting that with t 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 ($p \leq 0.0001$) negative correlation (-0.12 at confidence level 95%) between RH

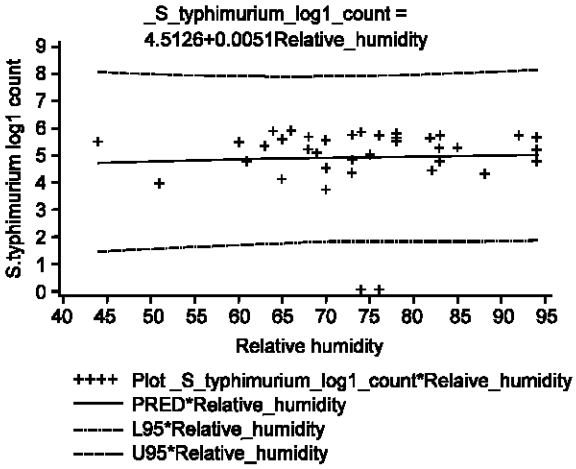

Fig. 3: Correlation between the RH and survival of *S. typhimurium* in poultry litter in absence of organic matter (N = 42, R^2 = 0.0015, AdjR^2 = -0.0234, RMSE = 1.4772)

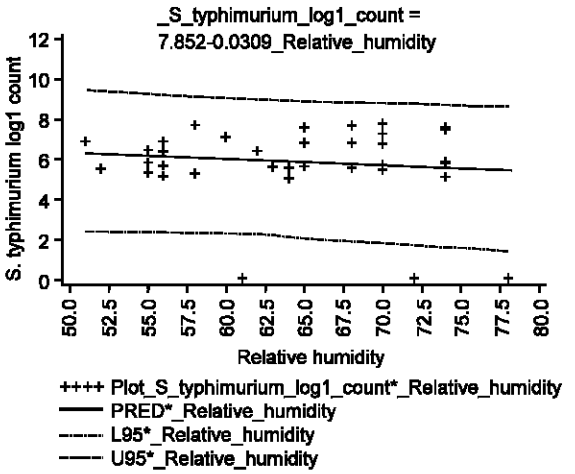

Fig. 4: Correlation between the RH and survival of *S. typhimurium* in poultry litter in the presence of organic matter presence of organic matter (N = 42, R^2 = 0.0167, AdjR^2 = 0.0079, RMSE = 1.8164)

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

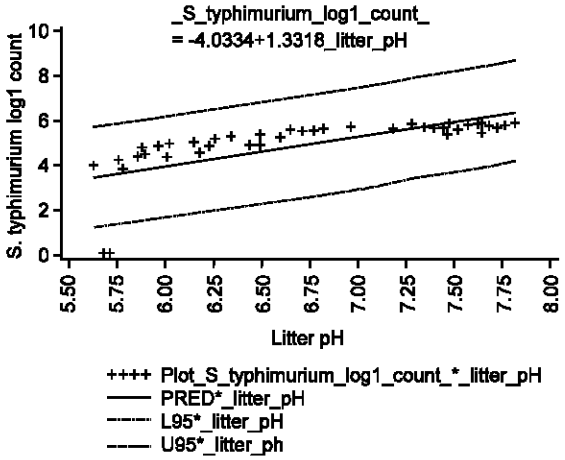

Fig. 5: Correlation between litter pH and survival of *S. typhimurium* in poultry litter in the absence of organic matter (N = 42, R^2 = 0.4535, AdjR^2 = 0.4398, RMSE = 1.0929)

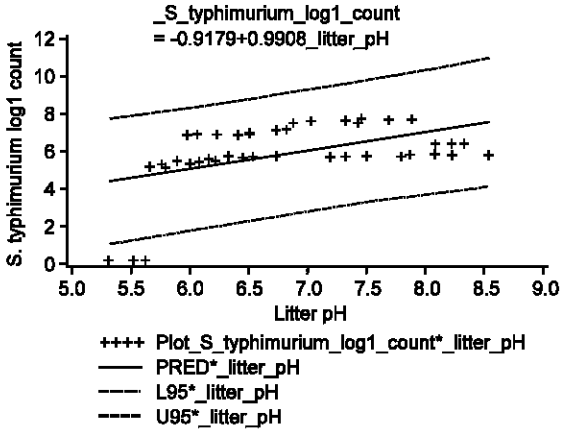

Fig. 6: Correlation between litter pH and survival of *S. typhimurium* in poultry litter in the presence of organic matter (N = 42, R^2 = 0.2396, AdjR^2 = 0.2206, RMSE = 1.5973)

3.6 and 9.5 with optimal growth at near neutral pH, D'Aoust (2001).

Studies have shown that the reduction of litter pH to more acidic levels (pH 4) resulted in a decline in microbial population, including *E. coli, Salmonella* and *Clostridium*, to below detectable limits, Hardin and Roney (1989).

Litter treatments are commonly used in poultry houses to reduce harmful ammonia emissions, but they may also be used to reduce litter pathogens by lowering litter pH. Pope and Cherry (2000) reported that significant declines in litter pH and ammonia levels along with reduced total aerobic bacteria and *E. coli* population in litter with a $NaHSO_4$ product as compared with non-treated houses. Payne *et al.* (2002) also showed that lowering litter pH to 2.68 and 3.48 using H_2SO_4 and $NaHSO_4$ 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 ($p \leq 0.005$) positive correlation (+0.67 at confidence level 95%) (Fig. 5). In the presence of organic matter there was also a significant ($p \leq 0.05$) positive correlation (+0.48 at 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 10^8 *Salmonella*/gm of fecaes for up to 2 weeks duration; Bailey (1987). Chick mortality has been observed to reach its peak at 3-7 days; Gast (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; Bahtia *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

Arakawa, A., T. Fukaton, E. Baba, L.R. McDougald, J. S. Bailey and L.C. Balnkenship, 1992. Influence of coccidiosis in broiler chickens under floor pen conditions. Poult. Sci., 71: 59-63.

Bahtia, T.R.S., G.D. McNabb, H. Wyman and G.P.S. Nayar, 1979. *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. Avian Dis., 23: 838-847.

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

Carr, L.E., E.T. Mallinson, C.R. Tate, R.G. Miller, E. Russek-Cohen, L.W. Stewart, O.O. Opera 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.

Chung, K.C. and J.M. Goepfert, 1970. Growth of Salmonella at low PH. J. Food Sci., 35: 326-328.

D'Aoust, J.Y., 1989. *Salmonella*. Pages 327-445 in Foodborne Bacterial Pathogens. M.P. Doyle, Ed. Marcel Dekker Inc., New York, NY.

D'Aoust, J.Y., 2001. *Salmonella*. Pages 163-191 in guide to Foodborne Pathogens. R.G. Labbe and S. Garcia, Ed. John Wiley and Sons Inc., New York, NY.

Durecko, R., D. Saladiova, P. Popelka and I. Simanska, 2004. Epidemiological and epizootiological aspects of Salmonellosis. Bratisl Lek Listy., 105: 414-8.

Farkas, J., 2001. Physical methods of food preservation. Pages 497-519 in Food Microbiology: Fundamentals and Frontiers. M.P. Doyle, L.R. Beuchat and T.J. Montville, Eds. ASM Press. Washington, DC.

Foster, J.W., 1993. The acid tolerance response of *Salmonella Typhimurium* involves transient synthesis of key acid shock proteins. J. Bacteriol. 175: 1981-1987.

Gast, R.K., 1997. *Salmonella* infections. Paratyphoid infections. pages 97-121 in Diseases of Poultry. W. B. Calnek, ed. Iowa State Univ. Press, Ames.

Hardin, B.E. and C.S. Roney, 1989. Effects of pH on selected bacteria. Alabama Department of Agriculture and Industry Report.

Herigstad, B., M. Hamilton and J. Heersink, 2001. How to optimize the drop plate method for enumerating bacteria. J. Microbiol. Meth., 44: 121-129.

Kumar, J., M. York, J. McDowell and B. Pomeroy, 1971. Dynamics of *Salmonella* infection in fryer roaster turkey. Avian Dis., 15: 221-232.

Liljebjelke, K.A., C.L. Hofacre, T. Liu, D.G. White, S. Ayers, S. Young and J.J. Maurer, 2005. Vertical and horizontal transmission of Salmonella within integrated broiler production system. Foodborne Pathog. Dis., 2: 90-120.

Mallinson, E.T., S.W. Joseph and L.E. Carr, 1998. *Salmonella's* Achilles' heel.broiler Ind., 61: 22-32.

Milner, K.C. and M.F. Shaffer, 1952. Bacteriological studies of experimental *Salmonella* infections in chicks. J. Infect. Dis., 90: 81-96.

Mollenhorst, H., C.J. Van Woudenbergh, E.G. Bokkers and I.J. de Boer, 2005. Risk factors for Salmonella enteritidis infections in laying hens. Poult. Sci., 84: 1308-13.

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.

Payne, J.B., E.C. Kroger and S.E. Watkins, 2002. Evaluation of litter treatments on *Salmonella* recovery from poultry litter. J. Appl. Poult. Res., 11: 239-243.

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.

Simmons, G. and R. Byrnes, 1972. The origin of Salmonellas in chickens and chicken carcasses. Aust. Vet. J., 48: 186-189.

Small, P., D. Blankenship, D. Welty, E. Zinser and J.L. Slonczewski, 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: Role of rpoS and growth pH. J. Bacteriol., 176: 1729-1737.

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.

Valentine, H., 1964. A study of the effect of different ventilation rates on the ammonia concentration in the atmosphere of broiler houses. Br. Poult. Sci., 5: 149-159.

Wales, A., M. Breslin, B. Carter, R. Sayers and R. Davies, 2007. A longitudinal study of environmental Salmonella contamination in caged and free ranged layer flocks. Avian Pathol., 36: 187-97.

William, J.E., Mallenson and G.H. Soeynebos, 1975. Isolation and identification of avian pathogens. AM. ASS. Avian Pathologist.

Zander, D.V., A.J. Bermudez and E.T. Mallinson, 1997. Principles of disease prevention: Diagnosis and control. Pages 1-45 in Diseases of Poultry. W.B. Calnek, Ed. Iowa State University Press, Ames.

Zelver, N., M. Hamilton, B. Pitts, D. Goeres, D. Walker, P. Sturman and J. Heersink, 1999. Measuring antimicrobial effects on biofilm bacteria: in R.J. Doyle, *et al*. (Eds), biofilm: methods in enzymology, Academic Press, San Diego, CA, pp: 608-628.