

Effect of transportation and mixing with unfamiliar pig on Salmonella susceptibility in market weight pigs

*Rostagno, M.H.**

Lay Jr., D.C.

USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN, USA.

** 125 South Russell Street, West Lafayette, IN 47907, USA.*

e-mail: Marcos.Rostagno@ars.usda.gov; fax: 1-765-496-7946

Abstract

There is increasing evidence that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms. However, there is very little research conducted to determine the potential effects of specific pre-slaughter stressors on Salmonella infection and carriage in pigs. Understanding when pathogen loads are the highest or when animals are most susceptible to infection is critical to determine when intervention strategies for pathogen control may be most effective, and consequently, increase pork safety. Therefore, this study was conducted to determine the effect of two common pre-slaughter stressors, transportation and mixing with unfamiliar pigs, on the susceptibility of market-weight pigs to a low-dose Salmonella challenge. A total of 40 market-weight pigs were randomly assigned to one of the following four treatments: 1) control, 2) mixing with another pig for 6 hours, 3) transportation for 1 hour, and 4) transportation for 1 hour followed by mixing with another pig for 6 hours. Immediately after the transportation treatment, all pigs were individually inoculated with 10⁴ cfu of Salmonella Typhimurium. After 6 hours, the pigs were euthanized and subjected to necropsy for sample collection, including ileal and cecal contents, ileal tissue, and mesenteric lymph node. All samples were processed for the isolation and enumeration of the challenge strain. Even though a low challenge dose was used, infection and shedding were established in all market-weight pigs used in this study. Pigs subjected to any of the stress treatments had higher ($P < 0.05$) levels of Salmonella in the ileum, whereas only pigs subjected to both stressors combined (i.e., transportation and mixing) had higher ($P < 0.05$) Salmonella levels in their cecum, compared to control pigs. Therefore, it is concluded that pre-slaughter transportation and mixing with unfamiliar pigs increases the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

Introduction

Colonization of swine by Salmonella, and its subsequent dissemination along the pork production and processing chain is a major public health and economic issue for the pork industry worldwide. There is some evidence that stress in farm animals can have a significant deleterious effect on food safety through a variety of potential mechanisms (Rostagno, 2009). However, there is very little research conducted to determine the potential effects of specific pre-slaughter stressors on Salmonella-infected pigs. Moreover, there is almost no research to determine how stress affects the susceptibility of pigs to Salmonella infection/colonization.

The gastrointestinal microbiota may be disturbed by many factors, causing levels of pathogens and shedding from unapparent carriers (i.e., subclinically infected animals) to be affected. For instance, during the process of being transported from production farms to abattoirs, pigs are exposed to a variety of potential stressors before slaughter (Warriss, 2003; Averos et al., 2008). Consequently, many believe that the number of animals carrying and shedding Salmonella, as well as its levels in the gastrointestinal tract will be increased in response to stressors. It is also believed that the pig's susceptibility to new infections will increase. However, although these assumptions are widely accepted, definitive proof still lacks, as most of the current knowledge is based on limited scientific evidence. Therefore, this study was conducted to determine the effect of two common pre-slaughter stressors, transportation and mixing with unfamiliar pigs, on the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

Material and Methods

A total of 40 market-weight pigs were randomly assigned to one of the following four treatments: 1) control, 2) mixing with another pig for 6 hours, 3) transportation for 1 hour, and 4) transportation for 1 hour followed by mixing with another pig for 6 hours. Immediately after the transportation treatment, all pigs were individually inoculated with 10⁴ cfu

of *Salmonella* Typhimurium. After 6 hours, the pigs were euthanized and subjected to necropsy for sample collection, including ileal and cecal contents, ileal tissue, and mesenteric lymph node. All samples were processed for the isolation and enumeration of the challenge strain.

The study was conducted in 2 replicates of 20 pigs each, including 5 individually housed pigs per treatment (total of 10 individual observations per treatment). All bacteria count data were subjected to transformation, and analyzed as log₁₀ colony forming units (CFU) per gram (g) of sample. Treatments were compared by ANOVA, and statistical inferences were based on $P < 0.05$. Data analysis was performed using JMP 9.0.0 (SAS Institute Inc., Cary, NC).

Results

All pigs used in this study were *Salmonella*-negative prior to the challenge, based on the analysis of multiple individual fecal samples. Even though a low challenge dose was used, infection and shedding were established (verified through recovery of the challenge strain from individual fecal samples) in all market-weight pigs included in this study. No clinical signs of infection were observed during the experiment.

Pigs subjected to any of the stress treatments (i.e., transportation and/or mixing) had higher ($P < 0.05$) levels of *Salmonella* in the ileum, whereas only pigs subjected to both stressors combined (i.e., transportation and mixing) had higher ($P < 0.05$) *Salmonella* levels in their cecum, compared to control pigs. There was no difference between pigs subjected to any of the treatments and controls regarding the levels of *Salmonella* translocating from the intestinal tract to mesenteric lymph nodes (i.e., in ileal wall and lymph node samples).

Discussion

A variety of stressors may cause animals to generate a stress response, based on the activation of the hypothalamo-pituitary-adrenal axis and the autonomic nervous system, which results in the release of several mediators, including the catecholamines, norepinephrine and epinephrine (Elenkov and Chrousos, 2006). The gastrointestinal tract has long been known to be sensitive to the effects of stress mediators (Tache and Brunnhuber, 2008). However, although physiological and motor activity disturbances have been frequently shown to result from stress, recent studies have shown that mediators of the stress response can also affect the function of the intestinal mucosa as well as bacterial populations (Lyte et al., 2011). Because of the considerable cross-communication that occurs between the neuroendocrine and immune systems (Ziemssen and Kern, 2007), the ability of stress to influence the pathogenesis of bacterial infections, has mostly been attributed to stress-induced immunosuppression (Butts and Sternberg, 2008; Salak-Johnson and McGlone, 2007). However, the ability of enteric bacteria to directly respond to stress mediators, particularly catecholamines, has been demonstrated (Freestone et al., 2008), offering a new and more direct pathway for the potential effect of stress on bacterial populations. Recently, Toscano et al. (2007) examined the effects of *in vitro* pre-treatment of *Salmonella* Typhimurium with norepinephrine before infecting young pigs. Examination of the tissue distribution revealed that norepinephrine-treated bacteria were present in greater numbers and more widely distributed in gastrointestinal tissues than control bacteria.

While being transported from production farms to abattoirs, market pigs are exposed to a variety of stressors immediately before slaughter, including handling, feed withdrawal, transportation, mixing, etc. (Warriss, 2003; Averos et al., 2008). An early small study by Williams and Newell (1970) was the first to suggest that transportation of pigs could lead to increased shedding of *Salmonella*. Later, Isaacson et al. (1999) and Marg et al. (2001) reported that pigs experimentally infected with *Salmonella* exhibited increased shedding after transportation. However, in a study conducted with young pigs naturally infected with *Salmonella*, Rostagno et al. (2005) did not find difference in prevalence estimates, based on pre- and post-transportation fecal samplings. Scherer et al. (2008) also did not observe any effect of transport-induced stress on *Salmonella* shedding rates in feces. Morrow et al. (2002) observed no feed withdrawal effect on *Salmonella* prevalence in pigs at slaughter, whereas Martin-Pelaez et al. (2008) reported that increasing pre-slaughter feed withdrawal and lairage lead to increased numbers of *Salmonella* in market pigs.

Although inconsistent, these reports present some evidence linking stress with pathogen carriage and shedding in swine. However, all these studies focused on the effect of stress on *Salmonella*-infected pigs. To the best of our knowledge, our study is the first one to determine under controlled conditions the effect of specific pre-slaughter stressors on the susceptibility of market-weight pigs to *Salmonella*. It is important to know when pathogen levels are the highest in infected pigs. However, it is also important to know when animals are most susceptible to infection. Understanding these two critical points in the pork production chain is key to determine when intervention strategies for pathogen prevention and control may be most effective, and consequently, increase pork safety.

Conclusion

Based on the results of this study, it is concluded that pre-slaughter transportation and mixing with unfamiliar pigs increases the susceptibility of market-weight pigs to a low-dose Salmonella challenge.

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