Social Stress Increases Fecal Shedding of *Salmonella Typhimurium* by Early Weaned Piglets†

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§Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and exclusion of others that may be suitable.

Abstract

“Segregated early weaning” (SEW) of pigs reduces exposure to pathogenic bacteria, but upon arrival at grower facilities pigs may be co-mingled regardless of farm of origin. The present study was designed to examine the effect of mixing (social) stress on populations of *Salmonella enterica* Typhimurium in SEW pigs. Piglets (7 days old; \( n = 28 \) in each of 2 replicates) were separated into 2 treatments (control and mixed groups) of 2 pens per treatment (\( 7 \) piglets/pen). One \( (n = 1) \) “seeder” pig/pen was inoculated with \( 10^9 \) CFU of *S. Typhimurium*. Each seeder was placed with non-inoculated “contact” piglets \( (n = 6) \). A “contact” piglet was swapped each day between the “mixed” pens for 5 days; pigs in control pens were not exchanged. On day 5, the incidence of fecal *Salmonella* shedding was higher in the mixed contact pigs \( (P < 0.05) \). Rectal *Salmonella* and cecal coliform populations in mixed pigs were significantly \( (P < 0.05) \) greater than in control pigs but cecal *Salmonella* populations were not different. Mixed pigs were more susceptible to tissue invasiveness (i.e., *Salmonella*-positive tonsils and lymph nodes) than control pigs. These results indicate that social stress of weaned pigs may increase susceptibility to and/or fecal shedding of *Salmonella*.

Food-borne *Salmonella* infections in the United States are estimated to cost the economy $2.4 billion annually (ERS/USDA, 2001). Approximately 6–9% of human salmonellosis is associated with the consumption of pork products (Frenzen et al., 1999). *Salmonella* is relatively common on swine farms and has been isolated from all stages of the pork production chain (Davies et al., 1999; Fedorka-Cray et al., 1997b; Rostagno et al., 2003). *Salmonella* is a threat to the pork industry not only from a food-safety perspective as a public health concern, but some *Salmonella* serotypes can cause clinical illnesses in swine, negatively impacting production efficiency and profitability (Schwartz, 1991).

In today’s swine production industry, segregated early weaning (SEW) of piglets (weaning at less than 14 days of age) has become commonplace because it reduces disease transmission between sows and piglets and results in increased weight gain for healthy SEW pigs (Alexander et al., 1980; Amass, 1998; Fedorka-Cray et al., 1997a). Under the conditions of SEW, it would not be unusual for piglets to be farrowed on multiple farms and be transported together to a separate facility hundreds of kilometers away. Concerns about animal health and safety have been raised, especially when piglets are weaned, grouped with piglets from other litters, and transported to a new location on the same day. This type of environmental and social stress coupled with an immature digestive tract (Shields et al., 1980) and decreased immune system performance (Blecha et al., 1998) suggests that SEW pigs could be susceptible to colonization by pathogenic bacteria, including *Salmonella*. Little research has been conducted in SEW piglets regarding stress and adverse effects on production, behavior, or shedding of food-borne pathogenic bacteria. Therefore the present study was designed to investigate the effects of mixing (social) stress on SEW piglet colonization by and fecal shedding of *Salmonella*.

Results

Pig behaviors were different between control and mixed groups. Control pigs spent more time eating \( (P = 0.018) \) than did mixed pigs for all days combined (Fig. 1). Control pigs also spent more time rooting on the last day of sampling compared to mixed pigs, who showed little or no rooting behavior (Mixing by Day \( P = 0.006; \) data not shown).

Rectal swabs from all pigs on the day of arrival were negative for *S. Typhimurium*, but most “contact” pigs and all “seeder” pigs were positive for *S. Typhimurium* in their feces 24 hours after inoculation (Fig. 2). Over the course of the study, all “contact” pigs shed *S. Typhimurium* on at least two days. On day 5, the number of “contact” pigs positive for *S. Typhimurium* was greater \( (P < 0.02) \) in mixed than in control groups (Fig. 2).

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Upon slaughter, the rectal populations of *S. Typhimurium* were greater \( (P < 0.05) \) in contact pigs of the mixed group compared to control contacts (Fig. 3a). However, cecal *S. Typhimurium* populations were not different between treatments (Fig. 3a). Cecal coliform populations were greater \( (P < 0.05) \) in the mixed contact pigs than in controls, and rectal populations also tended \( (P < 0.10) \) to be greater in the mixed “contact” pigs (Fig. 3b).

Rectal and cecal *S. Typhimurium* and coliform populations in “seeder” pigs were higher than those in “contact” pigs, but were not different between treatments (data not shown). Overall, the number of tissues from contact pigs positive for *S. Typhimurium* was greater \( (P < 0.07) \) in the mixed groups compared with controls. This difference
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can be attributed primarily to an increase \( (P < 0.01) \) in the number of positive rectal tissues in contact pigs from the mixed group, and a tendency \( (P = 0.07) \) for an increase in the number of positive cecal tissues from these mixed "contact" pigs (Fig. 4).

**Discussion**

*Salmonella* spp. are a major problem to the swine industry and have received much research interest over recent years. *Salmonella* infections represent a challenge to swine producers both as food-borne pathogens and as production/animal health issues. It has been estimated that between 25% and 48% of the U.S. swine herd is colonized by *Salmonella* on the farm (Davies et al., 1997; Funk et al., 2001). Transmission of *Salmonella* is primarily fecal-oral, however respiratory and other environmental transmission routes have been implicated in the spread of *Salmonella* in lairage pens and during transport (Rostagno et al., 2003; Winfield and Groisman, 2003). Several intervention strategies have been implemented across the swine industry in a concerted attempt to reduce the incidence of *Salmonella* in herds and consequently in pork products.
Segregated early weaning (SEW) of piglets has been used to produce pigs free from swine pathogens, including *Salmonella*, found in the maternal herd (Alexander *et al.*, 1980; Dritz *et al.*, 1996). This beneficial effect is often thought to be conferred through colostral antibodies (Amass, 1998). The use of segregated early weaning in swine has been shown to reduce the incidence of the food-borne pathogenic bacteria *Salmonella* (Fedorka-Cray *et al.*, 1997a). Because of the health and resultant economic benefits of SEW, this process has been adopted throughout the swine industry (Amass, 1998; Schwartz, 1991). However, the logistical procedures involved with SEW, especially the mixing of pigs originating from different farms at large grower facilities, may have inadvertently introduced co-mingling (social) stress.

The definition of stress in production animals is highly controversial. Some describe stress only as pain or other negative experiences, others have defined stress as the high performance demands placed on the animals for growth and production (e.g., high-producing dairy cows), still others regard any and all handling of animals by humans as a form of stress. However, it is to the economic benefit of swine producers to minimize stress and its resultant effects on their animals; but stress still occurs in the production continuum. Generally accepted stressors in swine include: weaning, transport, handling, co-mingling, change in social groups or pen density, food deprivation or change, temperature changes, noise and environmental changes (Biondi and Zannino, 1997).

Stress alters immune system function, and this neuro-immunomodulation increases the susceptibility of animals to infections (Biondi and Zannino, 1997; Jones *et al.*, 2001; Morrow-Tesch *et al.*, 1994). It has been suggested that this immunosuppression could increase the susceptibility of pigs to colonization by *Salmonella* spp. and has been shown to stimulate fecal shedding by *Salmonella* colonized pigs (Schwartz, 1991). Animals under stress display increased levels of glucocorticoids and catecholamines that reduce immune function and smooth muscle contraction, respectively (Biondi and Zannino, 1997). Interestingly, catecholamines have been shown to stimulate growth of *E. coli* and *Salmonella* in *in vitro* studies (Burton *et al.*, 2002; Lyte *et al.*, 1996; Rahman *et al.*, 2000). However the impact of these results on pathogen survival and shedding has not been demonstrated in animals.

Efforts to understand the impact of stress on intestinal pathogenic bacterial populations in swine have met with mixed results. Transportation stress in swine has been found to increase the shedding of *Salmonella* (Berends *et al.*, 1996; Isaacson *et al.*, 1999), but other studies have shown that transportation decreases fecal *Salmonella* shedding (Marg *et al.*, 2001). 2-Deoxy-D-glucose (2DG) is a sugar analog that has been shown to produce a controlled, simulated stress response in pigs (Stabel, 1999). However, the use of 2DG has not been shown to affect fecal shedding or persistence of *Salmonella* in pigs (Stabel and Fedorka-Cray, 2004).

When pigs are mixed with novel penmates, they fight in order to establish a dominance hierarchy (Morrow-Tesch *et al.*, 1994). This establishment of a social order stresses the animal and directly impacts immunological measures in pigs, including decreasing lymphocyte and monocyte levels (Biondi and Zannino, 1997; Jones *et al.*, 2001; Morrow-Tesch *et al.*, 1994). The addition of social stress to swine reduces body weight gain and decreases resistance to bacterial infection (House *et al.*, 1988; Kelley, 1980). Mixing of novel animals together has been shown to be stressful to the animal and to increase fecal shedding of pathogenic enterotoxigenic *E. coli* (ETEC) (Jones *et al.*, 2001).

In addition to the effects of stress on the immune system or directly on pathogens, stress has also been shown to affect gastrointestinal motility (la Fleur *et al.*, 2005) and a change in feeding behavior could also alter gut motility. A change in digesta flow through the lower GI tract could impact the site of fermentation of starch and other complex carbohydrates in the gastrointestinal tract leading to a change in the luminal concentrations of short chain fatty acids (SCFA) which have been shown to be toxic to pathogenic bacteria in the gut (Bird *et al.*, 2000). Other studies have suggested that the fermentation of dietary complex carbohydrates in the lower gastrointestinal tract of pigs and chickens can help exclude pathogens (Corrier *et al.*, 1997; Durmic *et al.*, 2002). Although the present study was not designed to measure these important and complex variables and possibilities, the involvement of the site of fermentation of complex carbohydrates in our results cannot be discounted.

In the present study, the pigs altered the percentage of time devoted to eating and rooting behaviors, indicating that they were stressed by the daily introduction of novel pigs (mixing stress) into their social structures. Each day following inoculation with *Salmonella*, the number of *Salmonella*-shedding pigs was greater in the mixed group compared to controls reaching the level of statistical significance only on days 5 and 6. This
potentially indicates that stress-induced changes in the gastrointestinal populations take a period of time to occur, or that stress must reach a cumulative threshold prior to affecting intestinal populations. After 6 days of mixing stress, the number of pigs that were positive for *Salmonella* was greater among the mixed pigs compared to the controls. Although the numerical differences of pigs positive in their tonsils and ileo-cecal lymph nodes are apparent, this difference was only statistically significant within the intestinal tract. Interestingly, cecal and rectal populations of *Salmonella* and coliforms were always numerically higher in mixed pigs than in controls, but significant differences were only found in either the cecum or the rectum for coliforms or *Salmonella*, respectively.

Collectively, our results indicate that the relatively mild social stress of mixing can impact intestinal populations of *Salmonella* and coliforms in early weaned pigs. To our knowledge this is the first time that social stress has been demonstrated to have an effect on fecal shedding or populations of *Salmonella*. Further research is needed to elucidate the role that different types of stress may play on the gastrointestinal microbial population, and specifically on food-borne pathogenic bacteria living within this important consortium.

**Experimental procedures**

All animal procedures were reviewed and approved by the USDA/ARS Food and Feed Safety Research Unit’s Institutional Animal Care and Use Committee. To determine the effects of social stress on *Salmonella* populations and shedding, crossbred weanling pigs (7 days of age) were purchased from the Texas Department of Criminal Justice and transported to our facilities at the Food and Feed Safety Research Unit in College Station, TX in each of 2 replicates (*n* = 56 pigs total; 28 pigs per replicate). Immediately upon arrival, piglets were weighed, ear-tagged, rectally swabbed and randomly assigned to pens (4 pens/replicate; 7 pigs/pen) and treatment (2 control pens and 2 mixed pens). Rectal swabs were enriched and plated on brilliant green agar (BGA) containing novobiocin (25 µg/mL) to screen for the presence of *Salmonella* prior to experimental infection (described below). Pigs were individually marked (Australian system) to facilitate identification. Time spent eating, rooting and behaviors were determined by trained observers using The Observer software package, (Noldus Software, Wageningen, The Netherlands) (Mitloehner et al., 2001).

**Behavior**

Pigs were video taped in 72 hour time lapse mode for the entire 6 day study. Instantaneous scan samples were collected every 10 minutes for each 24 hour period of the study. Behaviors recorded included lying, drinking (bowl and nipple) agonistic, oral and nasal contact and rooting. Pigs were individually marked (Australian system) to facilitate identification. Time spent eating, rooting and behaviors were determined by trained observers using The Observer software package, (Noldus Software, Wageningen, The Netherlands) (Mitloehner et al., 2001).

**Quantitative enumeration of bacterial populations**

Fecal samples were serially diluted (ten-fold increments) in phosphate buffered saline (PBS; pH 7.0) for enumeration of total coliforms and inoculated *Salmonella Typhimurium*. Enumerative dilution series were plated on MacConkey's agar (Difco Laboratories, Sparks, MD) to enumerate total coliforms and Brilliant Green Agar (BGA<sub>NN</sub>; Oxoid Ltd., Basingstoke, UK) supplemented with novobiocin (25 µg/mL) and nalidixic acid (20 µg/mL) to enumerate inoculated *Salmonella Typhimurium*. Colonies displaying typical morphology of coliforms on MacConkey's plates and typical *Salmonella* morphology on BGA<sub>NN</sub> were counted on plates after 24 hours of incubation at 37°C.

**Qualitative enrichment of salmonella from rectal swabs and intestinal contents**

To determine the presence of *Salmonella* via rectal swabs, each swab was added to a tube containing 10 mL of tetrathionate broth (Difco Laboratories), mixed and incubated at 37°C for 24 hours (Difco, 1998). After this incubation, 200 µL of the Tetrathionate enrichments were added to 5 mL Rappaport-Vassiliadis R10 broth (Difco Labs) and incubated for 24 hours at 42°C before being streak-plated onto BGA<sub>NN</sub>. The BGA<sub>NN</sub> plates were incubated for 24 hours at 37°C; colonies that exhibited typical *Salmonella* morphology were individually picked for further physiological characterization. Picked putative *Salmonella* colonies were inoculated onto Triple Sugar Iron (TSI) agar (Difco Labs) slants and Lysine Iron agar (LIA) slants (Dargatz et al., 2000; Ferris et al., 1999; Wells et al., 2001). Each slant was incubated at 35°C for 24 hours. *Salmonella*-positive samples were confirmed by slide agglutination (Difco Labs).

For qualitative enrichment of *Salmonella* in intestinal contents, feces (3 g) were added to tubes containing 27 mL of tetrathionate broth (Difco) and incubated at 37°C for 24 hours (Difco, 1998). For enrichment of intestinal tissues, tonsils and ileocecal lymph nodes, the respective tissues
were added to tubes containing 27 mL of tetrathionate broth (Difco) and incubated at 37°C for 24 hours. Following this initial incubation, samples were further enriched and plated on BGA\textsubscript{NN} as described above.

**Statistics**

The present study was conducted in two replicates with 14 control (2 “seeder” and 12 “contact” pigs) and 14 mixed pigs (2 “seeder” and 12 “contact” pigs) per replicate. Statistical analyses for treatment comparisons were conducted only on the 12 “contact” pigs/treatment group in each replication. Therefore, the data presented represents a total of 24 “contact” pigs from each of the control and mixed groups. Bacterial populations in “contact” pigs were compared by the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The number of contact pigs positive for *Salmonella* was analyzed using Pearson Exact X\textsuperscript{2} analysis of SAS. All behavioral analyses were performed using the GLM procedure in SAS (2001). The experimental unit was the individual pig. All behavioral data were expressed as percentages and were subjected to a square root arc sine transformation process to achieve a normalized distribution. Transformed data were analyzed as a completely randomized design. The statistical model included effects of treatment, day, and treatment by day interactions. Treatment effects were tested using the animal within treatment error term, all other effects were tested using the residual error term.

**References**


