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Cultivation of *Salmonella enterica* serovar Typhimurium in a norepinephrine-containing medium alters in vivo tissue prevalence in swine

M.J. Toscano[a,*], T.J. Stabel[b], S.M.D. Bearson[b], B.L. Bearson[c], D.C. Lay Jr.[a]

[a]*Livestock Behavior Research Unit, USDA-ARS, West Lafayette, IN 47907, USA*
[b]*Pre-harvest Food Safety and Enteric Diseases Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA 50010, USA*
[c]*Swine Odor and Manure Management Research Unit, National Soil Tilth Laboratory, USDA-ARS, Ames, IA 50011, USA*

Abstract

Transporting swine to slaughter is often linked with an increase in shedding of *Salmonella*, but little information exists to explain the role of stress. Recent research has suggested the catecholamine norepinephrine (NE) as a potential host signal during stress. The current study sought to investigate the prevalence of *Salmonella enterica* serovar Typhimurium in fecal samples and various tissues following inoculation with *S.* Typhimurium exposed to NE in vitro. The samples were collected at 3 and 24 h post-inoculation (p.i.) from pigs inoculated with *S.* Typhimurium cultured in either Luria–Bertani medium (LBC) or NE-infused, SAPI minimal medium (NEC). Bacterial quantification of tissue and fecal samples revealed a difference in the concentration of *Salmonella* between the two infections for six tissues at the two time points, five of which were greater in the NEC animals ($p < 0.05$). Upon observing an increase in the number of *Salmonella* associated with the stomach wall tissues at 3 h p.i. for the NEC culture, an experiment was conducted using an ex vivo swine contents assay to determine

Abbreviations: NE, norepeinephrine; LB, Luri–Bertani; p.i., post-inoculation; PBS, phosphate-buffered saline.

*Corresponding author. Division of Farm Animal Science, University of Bristol, Husbandry Building, Langford, Bristol, North Somerset BS40 5DU, UK. Tel.: +44 0117 928 9571; fax: +44 0117 928 9582.
E-mail address: mj.toscano@bris.ac.uk (M.J. Toscano).

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the effect of NE exposure on the ability of the organism to survive the conditions of the porcine stomach; NE treatment enhanced the survival of *S.* Typhimurium more than 2 logs ($p < 0.007$). Our results demonstrate an increase in the number of *Salmonella* associated with various swine tissues following experimental inoculation with NE-treated *S.* Typhimurium; thus, a possible scenario could be envisioned with a *Salmonella*-infected pig being stressed during transportation/mixing, resulting in the shedding of NE-stimulated *Salmonella* and exposure of naïve, stress-compromised penmates with a "primed" microorganism.

Keywords: *Salmonella*; Bacteriology; Norepinephrine; Swine

Introduction

An increase in the shedding of *Salmonella* and other infectious bacteria in swine have been found to coincide with transportation of animals to slaughter and related events (Isaacson et al., 1999; Swaneburg et al., 2001; Berends et al., 1996; Hurd et al., 2002), although the mechanisms behind the relationship remain elusive. A variety of explanations have been proposed linking transportation stress and *Salmonella* shedding, including decreased gastric acid secretion (Berends et al., 1996), inhibited immune response (Berends et al., 1996), and increased gastrointestinal motility (Williams and Newell, 1970). Despite the attention given to transportation related shedding, little work has focused on the direct relationship between the bacteria and products of the stress response. Although limited to primarily in vitro work, a large body of evidence has found the catecholamine norepinephrine (NE) to have extensive effects on the growth and production of virulence factors of Gram-negative bacteria, especially *Escherichia coli* (Belay and Sonnenfeld, 2002; Lyte and Ernst, 1992; Nietfeld et al., 1999) as well as *Salmonella* (Rahman et al., 2000). Additionally, specific mechanisms have been identified in which the presence of NE allows bacteria to overcome an iron-deficient environment, much like that of the host environment (Burton et al., 2002; Freestone et al., 2000). As a common neurotransmitter in mammalian physiology, NE is regularly released by nerve endings directly onto muscle fibers or within the adrenal medulla into the bloodstream (Genuth, 1998). Additionally, the number of neurons terminating in the gastrointestinal mucosa and enteric neural plexuses is comparable to that in the spinal cord; thus, bacteria in the gastrointestinal tract are likely exposed to high concentrations of NE (Kutchai, 1998), particularly during stressful encounters such as transportation and mixing. During stressful periods, NE release increases dramatically, inducing elevated blood pressure, glucose mobilization, and other stress-specific responses that assist the animal in overcoming the perceived stressor (Ewing et al., 1999). The pairing of a direct, NE-induced bacterial effect to enhance infection with other indirect consequences of the stress response (i.e., compromised immunity, decreased gastric pH) could play a factor in the dramatic increase of *Salmonella* shedding animals during transportation to slaughter.

The current study sought to determine whether the NE-induced effects on *Salmonella* observed in vitro would alter the prevalence of the organism in vivo.