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Effect of 2-deoxy-D-glucose induced stress on *Salmonella choleraesuis* shedding and persistence in swine ☆

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Abstract

A glucose analog, 2-deoxy-D-glucose (2DG), previously shown in swine to induce many of the hallmark parameters of stress, was administered to *Salmonella choleraesuis* carrier-swine and the effects on *Salmonella* fecal shedding and tissue colonization were evaluated. Initially, pigs were divided into two groups, one that received 1×10^6 *S. choleraesuis* and one group that received saline. At 3 or 6 weeks post inoculation (PI), half of each group received an injection of 2DG and the other half received saline. Throughout the study, individual fecal samples were collected and quantitatively cultured for *Salmonella*, tonsil and nasal swabs were qualitatively cultured, clinical signs were monitored, temperatures were measured and whole blood collected. Pigs were necropsied 8–18 days after 2DG treatment. The experimental stress induced by 2DG was not sufficient to cause recrudescence of *Salmonella* fecal shedding even when tissues were culture positive for *Salmonella*. In addition, persistent shedding was not affected by 2DG administration. Although the complex set of parameters that constitute the stress phenomenon is still relatively unknown, it is now apparent that the stressful event(s) sufficient to trigger *Salmonella* recrudescence involves more than just increased blood glucose, increased cortisol, and inhibition of lymphocyte proliferation.
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1. Introduction

Historically, *Salmonella* species have been described as enteric pathogens, and transmission was thought to occur by fecal-oral exposure. Recent data indicate that respiratory exposure may also be an important route of infection (Fedorka-Cray et al., 1995). The role of on-farm contamination cycles with endemic *Salmonella* spp. is of such magnitude that the role of other factors is difficult to determine. It has been estimated that 5–30% of finisher pigs originally infected with *Salmonella* spp. will still be shedding at the end of the finisher period (Berends et al., 1996).

Transportation of pigs is known to cause varying levels of stress, depending on a number of parameters, such as crowding, temperature, social status, and duration of trip (Dalin et al., 1993, Hessing et al., 1994). In 1995, $\geqslant 80\%$ of all pigs in the United States traveled $\leqslant 200$ miles to slaughter, and $>50\%$ traveled $\leqslant 100$ miles (Anonymous, 1996). Peak levels of cortisol occur immediately after start of transport and remain elevated throughout transport (Bradshaw et al., 1996). Weight loss and increased circulating neutrophils occur with shipping and are correlated with elevated cortisol levels in pigs. It has been suggested that only socially submissive pigs may be negatively affected by shipping stress (Mcglone et al., 1993). Although improved methods of transport and lairage will not decrease the number of *Salmonella* contaminated pigs coming from the farm, reduction of stress will at least keep the number of *Salmonella* contaminated pigs from increasing (Berends et al., 1996).

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The number of pigs shedding pathogenic organisms, such as *Salmonella* spp., at time of slaughter may include: (1) formerly naive pigs, (2) pigs with recrudescent infections (carriers), and (3) pigs that are already shedding (persistent shedders). It is believed, but not demonstrated, that stress induces *Salmonella*-free pigs to be more susceptible to infection, carrier animals to begin shedding again, and persistent shedders to excrete *Salmonella* at higher numbers. Mechanisms for these actions include: (1) release of adrenaline and corticosteroids, which reduce the activity of macrophages and lymphocytes (Nyberg et al., 1988), and (2) release of catecholamines, which decrease gastric acid production and increase stomach pH providing a more favorable environment for *Salmonella* survival and passage (Taché, 1987). Consequently, a healthy gut flora may be one of the best measures to prevent further colonization and subsequent infection with *Salmonella* spp. in swine (Van der Waaij, 1992; Fedorka-Cray and Bailey, 1996).

Only a few groups have studied the effects of transportation stress and/or feed withdrawal on *Salmonella* shedding in swine (Williams and Newell, 1970; Isaacson et al., 1999; Kephart et al., 1995). Results were mixed. On-farm studies to determine the effects of transport stress on *Salmonella* shedding can be very problematic. Each study should include evidence that stress is actually occurring (i.e. activation of the hypothalamic–pituitary–adrenocortical axis) and to what degree. Plasma cortisol concentrations are an important measure of stress (Jensen-Waern and Nyberg, 1993). If blood samples are taken before and after shipping to measure cortisol levels, the act of bleeding uncooperative farm pigs is sufficient to raise cortisol levels and make any kind of data interpretation impossible (Mcglone et al., 1993). To avoid short-term stress by snaring, restraint of pigs must be less than 0.5, 2, and 3.5 min to keep levels of catecholamines, β-endorphin, and cortisol, respectively, at normal levels (Roozen et al., 1995). The amount of time animal handlers remain in a pen, not the time spent in the room, will affect cortisol levels too. In an experimental environment, animals kept 3 to a pen is a manageable number of animals to bleed before cortisol levels begin to increase (Mcglone et al., 1993). Natural killer (NK) cell activity can also be negatively affected by the act of bleeding, such that no difference in NK activity can be detected in pigs prior to shipment or after.

Ideally, to control the maximum number of variables, a stress model would be appropriate (Stabel, 1999; Wallgren et al., 1994). Since swine are regarded as corticosteroid resistant (Griffin, 1989), dexamethasone was not considered an optimal stress inducer. Recently, however, a porcine stress model has been developed that utilizes the glucose analog 2-deoxy-D-glucose (2DG) to stimulate physiological stress (Stabel, 1999). Administration of 2DG induced many of the hallmark signs of stress such as increased blood glucose and cortisol concentrations, and decreased lymphocyte proliferation activity. The purpose of this study was to determine the effect of 2DG induced stress on the shedding and persistence of *Salmonella choleraesuis* in swine.

2. Materials and methods

2.1. Animals

Sixty-one male and female mixed-breed pigs (H&K Enterprises, Ames, Iowa, USA) were weaned at 10–14 days of age and raised in containment facilities at the National Animal Disease Center. Bacteriological culture of feces and tonsils, nasal, and rectal swab specimens was performed weekly to confirm that pigs were free of *Salmonella* spp. at time of challenge (8 weeks of age). In two separate animal studies (Exp. #1, $n = 29$; Exp. #2, $n = 32$), a *Salmonella* infection model was combined with a porcine stress model to study the effects of stress on *Salmonella* shedding.

2.2. Experimental design

Pigs were allotted to four groups. On day 0, two challenge groups received 1×10^6 cfu/pig of streptomycin-resistant *S. choleraesuis* χ3246 (courtesy of Roy Curtiss III, Department of Biology, Washington University, St. Louis, MO, USA) and two control groups received saline intranasally. Pigs were grown an additional 6 weeks (Exp. #1) or 3 weeks (Exp. #2) to allow pigs to reach carrier-state status. At 6 or 3 weeks post inoculation (PI), 1 challenge and 1 control group received a single subcutaneous injection of 2DG and 1 challenge and 1 control group received saline. Treatment with 2DG simulates the physiological change associated with stress. Clinical signs were monitored, fecal samples cultured for *S. choleraesuis*, temperatures measured and whole blood collected on various days post *S. choleraesuis* inoculation and 2DG injection (see figures for sampling times). Pigs were necropsied 18 (Exp. #1) or 8 days (Exp. #2) after 2DG stress and tissues cultured for *S. choleraesuis*.

2.3. Administration of 2DG

The 2DG (No. D-8375, Sigma Chemical Co., St. Louis, MO, USA) was suspended in saline solution and administered to pigs at 750 mg/kg body weight. Subcutaneous injections were administered under the loose flaps of skin around the elbow joint region.

2.4. Bacteriologic examinations

Individual fecal samples (approx. 1 g) were collected using adult dog fecal loops (No. J-147, Midwest Veter-

inary Supply, Des Moines, IA, USA). Samples were quantitatively cultured for *Salmonella* in GN-Hajna broth (Difco Laboratories Inc., Detroit, MI, USA) containing 200 μg streptomycin/ml on day 1, 2, 3, 8, 15, 22 post *S. choleraesuis* inoculation and day 0 (Exp. #1), 1, 2, 4, 7 post 2DG administration using the 5-tube most probable number method (Wood and Rose, 1992). Positive cultures were selectively enriched in Rappaport-Vassiliadis medium (Difco Laboratories Inc., Detroit, MI, USA) and subsequently plated on brilliant green agar with sulfadiazine (BGS; Difco Laboratories Inc., Detroit, MI, USA) containing 200 μg streptomycin/ml. Colonies having the appearance typical of *Salmonella* were picked and inoculated into triple sugar iron (Difco Laboratories Inc., Detroit, MI, USA) and lysine agar slants (Difco Laboratories Inc., Detroit, MI, USA). Test-positive isolates were confirmed as group C_1O by agglutination with *S. choleraesuis* serotype specific antiserum (Difco Laboratories Inc., Detroit, MI, USA). In addition, tonsil and nasal swab specimens from challenge groups were qualitatively examined for *Salmonella* (Gray et al., 1996). Similarly, tonsil, nasal, and rectal swab specimens were collected from control animals on identical days as the challenge group and qualitatively cultured for *Salmonella*. At necropsy, qualitative bacteriological examination was performed on spleen (S), liver (L), tonsil (T), lung (Lu), ileocolic lymph node (ICLN), and ileocolic junction (ICJ) from all pigs.

2.5. Porcine cortisol radioimmunoassay

Plasma cortisol concentration in swine was measured according to a previously described procedure (Stabel, 1999). Exp. #1 plasma samples were collected on day −3, 0, 3, 8 post *S. choleraesuis* inoculation and on day 0, 1, 2, 4, 7 post 2DG administration. Exp. #2 plasma samples were only collected just prior to 2DG injection and 2 h after 2DG injection.

2.6. Statistical analysis

Temperature and cortisol data from each pig for each of the two experiments were used to determine the experimental mean values. Results are expressed as means ± SEM. Significant differences between groups were determined by an ANOVA (InStat, GraphPad Software, San Diego, CA, USA). A value of $P < 0.05$ was regarded as significant.

3. Results

3.1. Febrile response

In Exp. #1, the peak temperature response occurred between day 4 and day 8 post inoculation (PI) with *S. choleraesuis* (Fig. 1(a)). Temperature remained unchanged after 2DG injection except at day 2 post 2DG injection when temperature decreased in the *Salmonella* infected group (Fig. 1(b)). This response to 2DG was not consistent across groups (i.e. 2DG did not cause a decrease in temperature in the saline control group).

In Exp. #2, the febrile response was similar to the response observed in Exp. #1 with a peak response occurring between day 3 and day 4 PI (Fig. 2(a)). Injection with 2DG had no effect on temperature (Fig. 2(b)).

3.2. Antemortem bacteriological examination

Prior to inoculation all pigs were determined to be *Salmonella*-free by tonsil, nasal, and rectal swabs. Using culture methods as described, the lower level of sensitivity was approximately 10 CFU *S. choleraesuis* per gram of sample (data not shown). After inoculation with

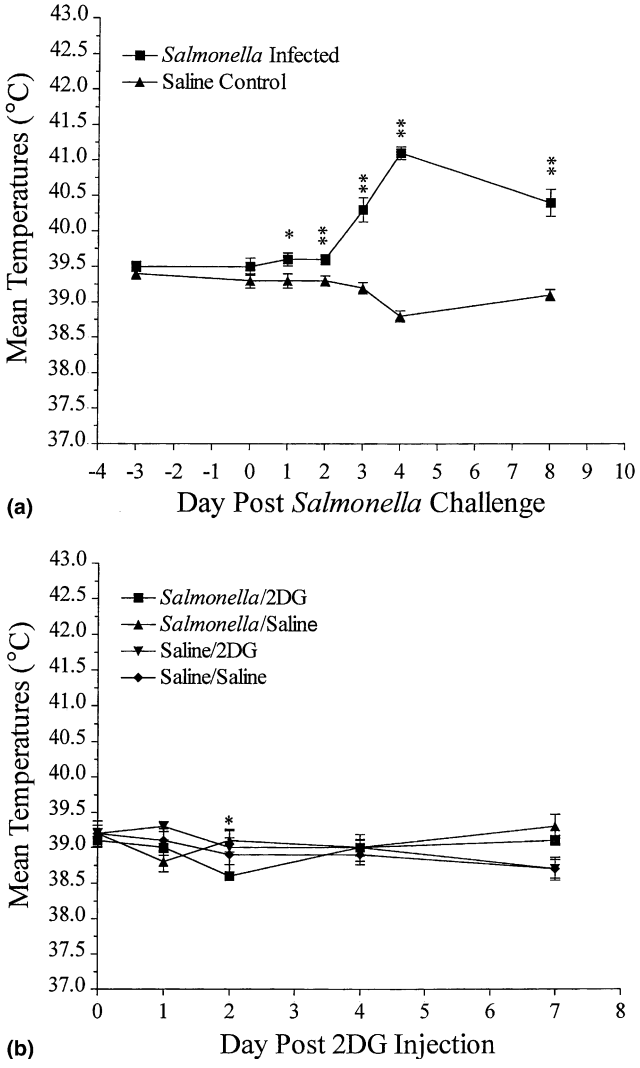

Fig. 1. Exp. #1: rectal temperatures of pigs before and after intranasal exposure to *Salmonella choleraesuis* at day 0 (a) and after subcutaneous injection of 2DG or saline (b). *$P < 0.05$ and **$P < 0.01$.

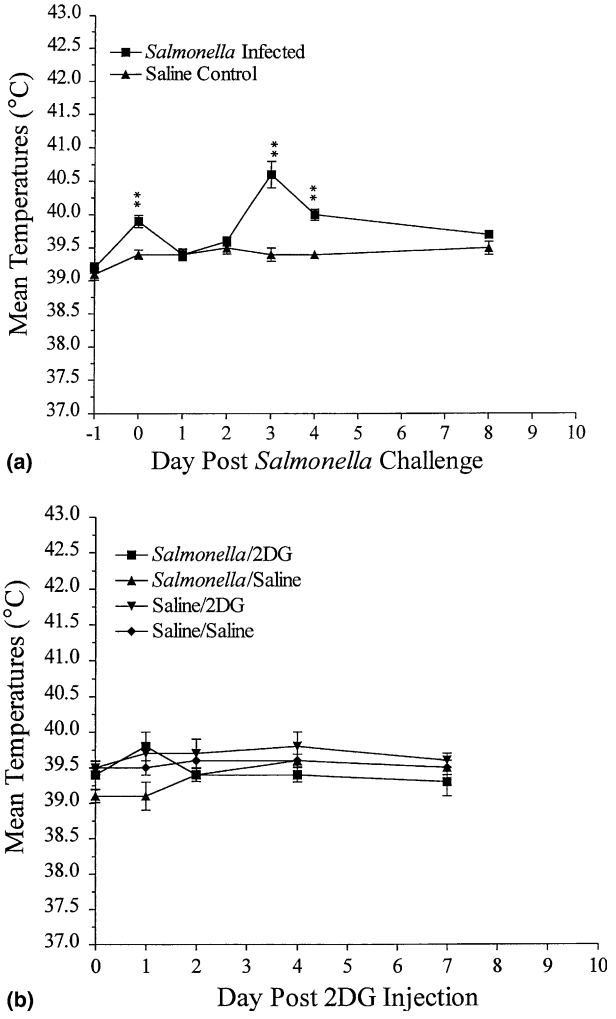

Fig. 2. Exp. #2: rectal temperatures of pigs before and after intranasal exposure to *Salmonella choleraesuis* at day 0 (a) and after subcutaneous injection of 2DG or saline (b). $^{**}P < 0.01$.

Salmonella, shedding patterns varied considerably between pigs (Tables 1 and 2).

In Exp. #1, pigs (#'s 45, 54, and 59) shed as early as day 2 PI. *Salmonella* was never recovered from two pigs (#'s 56 and 57). A third of the challenge pigs (#'s 44, 45, 47, 50, and 51) were still culture positive for *Salmonella* 3 weeks PI. Only one (#44) of 15 pigs inoculated with *Salmonella* was still shedding *Salmonella* at time of stress induction with 2DG (6 weeks PI). 2DG administration had no effect on quantity of *Salmonella* shed by this single persistent-shedder (Pig #44) nor did it cause recrudescence of *Salmonella* shedding (Table 1).

In Exp. #2, pig #8 shed as early as day 2 PI, with the maximum number of pigs shedding on day 3 and day 8 PI. *Salmonella* was never recovered from five pigs (#'s 2, 3, 6, 12, and 15). None of the pigs were shedding *Salmonella* at 3 weeks PI, the time of stress induction with 2DG. As in Exp. #1, 2DG did not cause recrudescence of *Salmonella* shedding (Table 2).

3.3. Postmortem bacteriologic examination

Other than pig #47, which had to be euthanized at 7.5 weeks PI due to a rectal prolapse, all pigs in Exp. #1 were necropsied at 9 weeks PI. At necropsy, three of 15 pigs (20%) originally inoculated with *S. choleraesuis* were determined to harbor *Salmonella* internally (Table 1). All suspect *Salmonella* were confirmed to be *S. choleraesuis* by serogroup and serotype analysis. All non-infected control pigs remained *Salmonella* negative throughout the study (data not shown).

In Exp. #2, all pigs were necropsied 4 weeks PI (1 week post 2DG injection). Seven of 16 pigs (44%) were tissue positive for *Salmonella* (Table 2).

3.4. Cortisol levels

At sampling intervals of $\geqslant$24 h (Exp. #1) plasma cortisol levels did not change significantly after IN inoculation with *Salmonella* or injection with 2DG (data not shown). Plasma samples taken 2 h post 2DG injection (Exp. #2) indicated a dramatic increase in cortisol concentration (Fig. 3).

4. Discussion

Stress is a broad based term used to identify a range of situations which alter an animal's homeostasis. Homeostasis is an integrated process involving interactions among nervous, endocrine and immune systems. The response to a stressor involves: (1) the stimulation of the hypothalamic–pituitary–adrenal (HPA) axis and eventual release of serum glucocorticoids and (2) the activation of the sympathetic nervous system (SNS) causing the release of both tissue and plasma catecholamines (norepinephrine and epinephrine). These neuroendocrine-derived factors are believed to have a direct effect on immune function. Receptors for these neurohormones are found on T and B cells, macrophages, neutrophils, and natural killer cells. These same immune cells can also produce pituitary, hypothalmic, and neural peptides (Brown and Zwilling, 1996). In addition, immunocytes secrete cytokines, such as IL-1 and TNF, which affect the neural system. Not only are receptors for cytokines found in the brain, but cytokines, such as IL-1 and IL-6, have been shown to be produced in the brain. Future research will probably show that the neuroendocrine system produces most cytokines, just like the immune system produces many neuropeptides (Blalock, 1994).

Earlier work from our laboratory has shown that 2DG causes a physiologic stress response in swine (Stabel, 1999). Specifically, an injection of 2DG causes: increased levels of blood glucose and cortisol, and decreased levels of lymphoctye activation. This 2DG stress model was used to simulate stress in both groups of pigs

Table 1
Six-week carrier pigs (Exp. #1): quantitative[a] and qualitative[b] bacteriology

	Stress (2DG)									No stress (saline)						
Pig #		44	45	46	47	48	49	50	51	52	54	55	56	57	58	59
Day post infection (PI)	0															
	1															
	2		0.2								+					+
	3	+	0.2	3.7	0.03+	0.2+		0.5		+	0.03+	+			0.1+	
	8	1.2+	0.6	+	0.02	0.07	0.03	0.2	0.4							
	15	35.1+	0.2+	+		0.3+	+	+			0.03	+				0.04
	22	53.5+	0.6+		0.02+			0.04+	+							
	28	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	35	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Inject 2DG→	42	0.5+														
	43	0.2+														
	44	0.01+														
	46	0.2+														
	49	0.05														
Necropsy→	63	T,L, ICJ			ICJ[d]						ICJ					

[a] Values represent fecal MPNs (cfu/g $\times 10^3$).
[b] A "+" found anywhere in table indicates a *S. choleraesuis* positive tonsil and/or nasal swab (qualitative only). Necropsy tissues are listed if positive for *S. choleraesuis* (qualitative only). See Section 2 for abbreviations and tissues sampled.
[c] ND, not determined.
[d] Pig #47 died 10 days after 2DG injection.

Table 2
Three-week carrier pigs (Exp. #2): quantitative[a] and qualitative[b] bacteriology

	Stress (2DG)								No stress (saline)							
Pig #		1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17
Day post infection (PI)																	
	0																
	1																
	2								5.9								
	3					1.4		7.2			2.8						21.7
	8				0.2	1.2			2.0	27.0							
	15	0.3															
Inject 2DG→	22																
	23																
	24																
	26																
	29																
Necropsy→	29	ICLN			S, L, ICLN	ICLN		S, L, LU	S, L, LU, ICLN				ICLN	ICLN		ICLN	

[a] Values represent fecal MPNs (cfu/g $\times 10^1$).

[b] Tonsil and nasal swabs were collected at each time point for qualitative bacteriology. All samples were determined to be *Salmonella* negative (data not shown). Necropsy tissues are listed if positive for *S. choleraesuis* (qualitative only). See Section 2 for abbreviations and tissues sampled.

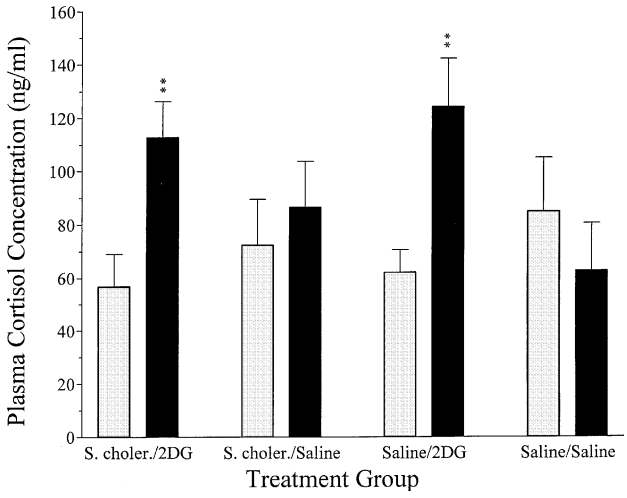

Fig. 3. Exp. #2: plasma cortisol concentration before (stippled bars) and 2 h after (solid bars) subcutaneous injection of pigs with 2DG (750 mg/kg body weight) or saline. $^{**}P < 0.01$.

described in this study. In Exp. #1, blood samples were only taken at 24 h intervals and thus the temporal (2–6 h) increase in blood cortisol concentration was missed. In Exp. #2, an early time point was added 2 h post 2DG injection to correct for this omission. Assuming, under proper conditions, marketing stress can induce fecal shedding of *Salmonella* and given that experimental stress induced with 2DG did not cause recrudescence of *S. choleraesuis* in feces, even when tissue was culture positive, there is a unique opportunity to determine which physiological factors associated with marketing stress are important for recrudescence and which factors are inconsequential. Thus, the combination of stress parameters affected by the injection of 2DG (blood glucose, cortisol, and lymphocyte blastogenesis) was insufficient to cause or predict recrudescence of *Salmonella* in feces. This important finding will allow researchers to focus on other parameters, such a neurotransmitter release and cytokine production in the gut, as possible factors that trigger release of *Salmonella* into the feces.

The lack of a cortisol response after *Salmonella* inoculation would indicate no long-term stress effects due to infection; however, short-term effects (<24 h after inoculation) were not measured.

Results using the 2DG stress model in a *Salmonella* infection study indicate that stress as induced by 2DG does not cause recrudescence of *Salmonella* fecal shedding even when tissues are culture positive for *Salmonella*. This set of animal experiments, and other unpublished data, indicates that a very small percentage of experimentally infected pigs are unable to clear the infection completely and continue to shed *Salmonella* in their feces and harbor detectable levels of bacteria in tissues such as tonsil and ileocolic junction. We have termed these animals "persistent-shedders". Future research should focus on immune and physiological mechanisms affected by stress in the persistent-shedder. This relatively small group may play a disproportionate role in the spread of *Salmonella* spp. on farm, during transport and at the abattoir.

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References

Anon., 1996. Marketing finisher pigs in the United States. Veterinary Services Info Sheet N203.696. USDA's National Animal Health Monitoring System (NAHMS), Center for Epidemiology and Animal Health, USDA:APHIS:VS, Fort Collins, CO, USA.

Berends, B.R., Urlings, H.A.P., Snijders, J.M.A., Van Knapen, F., 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. International Journal of Food Microbiology 30, 37–53.

Blalock, J.E., 1994. The syntax of immune-neuroendocrine communication. Immunology Today 15, 504–511.

Bradshaw, R.H., Parrott, R.F., Goode, J.A., Lloyd, D.M., Rodway, R.G., Broom, D.M., 1996. Behavioural and hormonal responses of pigs during transport: effect of mixing and duration of journey. Animal Science 62, 547–554.

Brown, D.H., Zwilling, B.S., 1996. Neuroimmunology of host-microbial interactions. In: Friedman, H., Klein, T.W., Friedman, A.L. (Eds.), Psychoneuroimmunology, Stress, and Infection. CRC Press, New York, pp. 153–172.

Dalin, A.M., Magnusson, U., Haggendal, J., Nyberg, L., 1993. The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count, and lymphocyte proliferation in pigs. Acta Veterinaria Scandinavica 34, 59–68.

Fedorka-Cray, P.J., Kelley, L.C., Stabel, T.J., Gray, J.T., Laufer, J.A., 1995. Alternate routes of invasion may affect pathogenesis of *Salmonella typhimurium* in swine. Infection and Immunity 63, 2658–2664.

Fedorka-Cray, P.J., Bailey, J.S., 1996. Control of *Salmonella* in swine by use of mucosal competitive exclusion culture. 77th Annual Meeting of the Conference of Research Workers in Animal Diseases, St. Louis, MO, p. A93.

Gray, J.T., Stabel, T.J., Fedorka-Cray, P.J., 1996. Effect of dose on the immune response and persistence of *Salmonella choleraesuis* infection in swine. American Journal of Veterinary Research 57, 313–319.

Griffin, J.F., 1989. Stress and immunity: a unifying concept. Veterinary Immunology Immunopathology 20, 263–312.

Hessing, M.J., Scheepens, C.J., Schouten, W.G., Tielen, M.J., Wiepkema, P.R., 1994. Social rank and disease susceptibility in pigs. Veterinary Immunology and Immunopathology 43, 373–387.

Isaacson, R.E., Firkins, L.D., Weigel, R.M., Zuckermann, F.A., Dipietro, J.A., 1999. Effect of transportation and feed withdrawal on shedding of *Salmonella Typhimurium* among experimentally infected pigs. American Journal of Veterinary Research 60, 1155–1158.

Jensen-Waern, M., Nyberg, L., 1993. Valuable indicators of physical stress in porcine plasma. Journal of Veterinary Medicine, Series A 40, 321–327.

Kephart, K., Maddox, C.M., Nesselrodt, A., Sischo, W.M., 1995. The effects of transport and feed deprivation on shedding of *Salmonella*

sp. in swine. 76th Meeting of the Conference of Research Workers in Animal Diseases, St. Louis, MO, p. A95.

Mcglone, J.J., Salak, J.L., Lumpkin, E.A., Nicholson, R.I., Gibson, M., Normon, R.L., 1993. Shipping stress and social status effects on pig performance, plasma cortisol, natural killer cell activity, and leukocyte numbers. Journal of Animal Science 71, 888–896.

Nyberg, L., Lundstrom, K., Edfors-Lilja, I., Rundgren, M., 1988. Effects of transport stress on concentration of cortisol, corticosteroid-binding globulin and glucocorticoid receptors in pigs with different halothane genotypes. Journal of Animal Science 66, 1202–1211.

Roozen, A.W., Tsuma, V.T., Magnusson, U., 1995. Effects of short-term restraint stress on plasma concentrations of catecholamines, β-endorphin, and cortisol in gilts. American Journal of Veterinary Research 56, 1225–1227.

Stabel, T.J., 1999. Evaluation of 2-deoxy-D-glucose for induction of a stress response in pigs. American Journal of Veterinary Research 60, 708–713.

Taché, Y., 1987. Central nervous system regulation of gastric acid secretion. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract, second ed. Raven Press, New York, pp. 911–930.

Van Der Waaij, D., 1992. History of recognition and measurement of colonization resistance of the digestive tract as an introduction to selective gastro-intestinal decontamination. Epidemiology and Infection 109, 315–326.

Wallgren, P., Wilen, I.L., Fossum, C., 1994. Influence of experimentally induced endogenous production of cortisol on the immune capacity in swine. Veterinary Immunology and Immunopathology 42, 301–316.

Williams, L.P., Newell, K.W., 1970. Salmonella excretion in joy-riding pigs. American Journal of Public Health 60, 926–929.

Wood, R.L., Rose, R., 1992. Populations of *Salmonella typhimurium* in internal organs of experimentally infected carrier swine. American Journal of Veterinary Research 53, 653–658.