Stress induced Salmonella Typhimurium re-excretion by pigs is associated with cortisol induced increased intracellular proliferation in porcine macrophages

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Abstract

Infections of pigs with Salmonella enterica subspecies enterica serovar Typhimurium (Salmonella Typhimurium) often result in the development of carriers that intermittently excrete Salmonella in very low numbers. During periods of stress, recrudescence of Salmonella may occur. The mechanism of stress related re-excretion of Salmonella by pigs is poorly understood and the aim of the presented study was to determine the role of the stress hormone cortisol on Salmonella re-excretion by pigs.

We showed that a 24 hour feed withdrawal increases the Salmonella Typhimurium load in pigs, which is correlated with increased cortisol blood levels. A second in vivo trial showed that the stress related re-excretion of Salmonella Typhimurium in pigs can be induced by intramuscular injection of dexamethasone. Furthermore we demonstrated that cortisol promotes intracellular proliferation of Salmonella Typhimurium in porcine alveolar macrophages, but not in intestinal epithelial cells, at a concentration (1 µM) that did not exert a notable effect on porcine cell viability and gene expression of Salmonella Typhimurium. This implies that the enhanced survival of Salmonella is probably caused by an indirect effect of cortisol on the cell.

Introduction

Pigs infected with Salmonella Typhimurium can carry this bacterium asymptomatically in their tonsils, gut and gut-associated lymphoid tissue for months resulting in so called Salmonella carriers. During periods of stress recrudescence of Salmonella may occur (Berends et al., 1996). Until now, the mechanism of stress related re-excretion of Salmonella in pigs is not well known. We hypothesized that cortisol plays a role in the stress related recrudescence of Salmonella Typhimurium in pigs.

Material and Methods

Salmonella strain: Salmonella Typhimurium strain 112910a, isolated from a pig stool sample and characterized previously by Boyen et al. (2008), was used.

In vivo trials: In a first in vivo trial, we investigated the effect of different types (feed withdrawal, isolation and overcrowding) of stress on the re-excretion of Salmonella Typhimurium by carrier pigs. In a second in vivo trial, we intramuscularly injected carrier pigs with 2 mg dexamethasone per kg body weight to test our hypothesis that cortisol plays a role in the recrudescence of Salmonella Typhimurium in pigs.

Cytotoxicity assays: The cytotoxic effect of cortisol on porcine alveolar macrophages and IPEC-J2 cells was determined using the lactate dehydrogenase cytotoxicity detection kit (Roche Applied Science, Bazel, Switzerland), in accordance to the manufacturer's instructions.

Effect of cortisol on the growth of Salmonella Typhimurium: The effect of cortisol on the growth of Salmonella Typhimurium in LB broth was examined during 24 hours.

Effect of cortisol on the gene expression of Salmonella Typhimurium: RNA was isolated from Salmonella Typhimurium using the SV Total RNA purification kit (Promega, Leiden, the Netherlands). Gene expression was measured using a Salmonella microarray constructed at the Institute of Food Research, Norwich, UK.

Invasion and intracellular survival assays: The ability of Salmonella Typhimurium to invade and proliferate in PAM and IPEC-J2 cells after exposure to cortisol was performed as described by Boyen et al., 2009.

Macrophage chemiluminiscence: The effect of cortisol was examined on the reactive oxygen species production of porcine alveolar macrophages, as described by Boyen et al., 2006.

Results

Feed withdrawal stress results in increased numbers of Salmonella Typhimurium bacteria in the gut of pigs and elevated cortisol levels

As illustrated in figure 1, carrier pigs subjected to feed withdrawal stress, 24 hours before euthanasia, showed elevated numbers of Salmonella Typhimurium in their bowel contents and organs in comparison to the control group that was not stressed. Furthermore, these pigs had significantly elevated serum cortisol levels (66.88 ± 6.72 nM) compared to the control group (48.65 ± 4.67 nM).

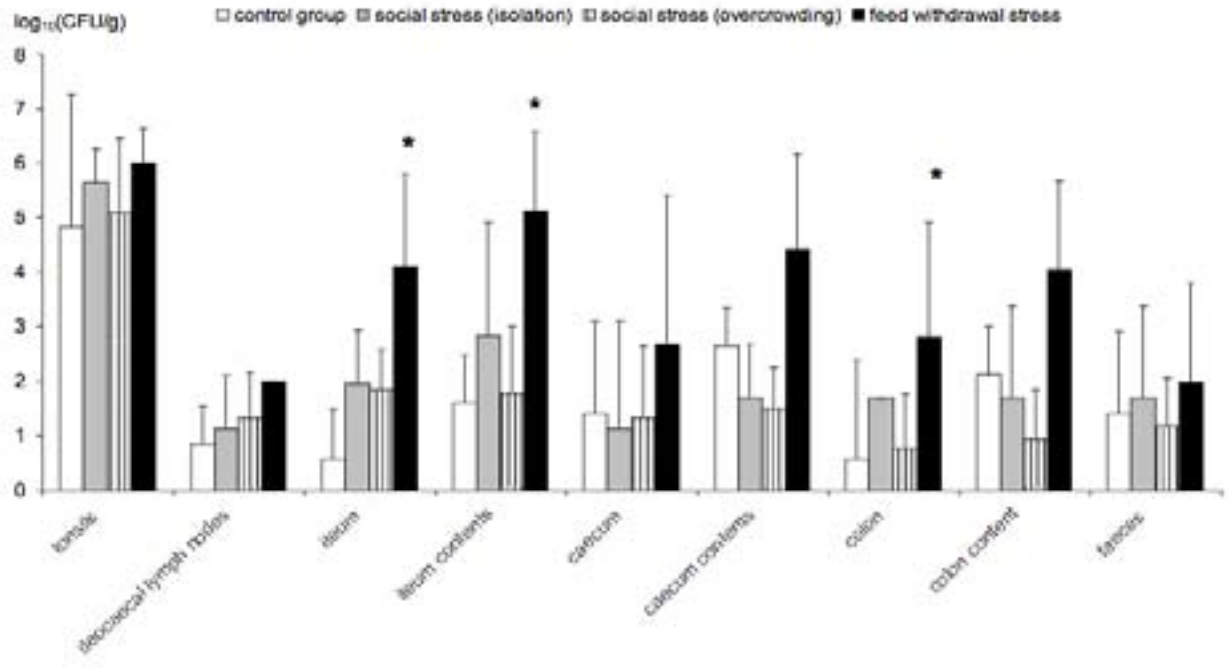

Figure 1: Recovery of Salmonella Typhimurium from pigs that were submitted to either feed withdrawal (n = 6) or social stress, isolation (n = 3) and overcrowding (n = 9), 24 hours before euthanasia. Six pigs were not stressed and served as a control group. The log10 value of the ratio of CFU per gram sample is given as the mean + standard deviation. Superscript (*) refers to a significant difference compared to the control group (p < 0.05).

Dexamethasone increases the number of Salmonella Typhimurium bacteria in the gut of pigs

As illustrated in figure 2, carrier pigs that were intramuscularly injected with 2 mg dexamethasone per kg body weight, 24 hours before euthanasia, showed elevated numbers of Salmonella Typhimurium in their gut tissues and contents in comparison to the control group.

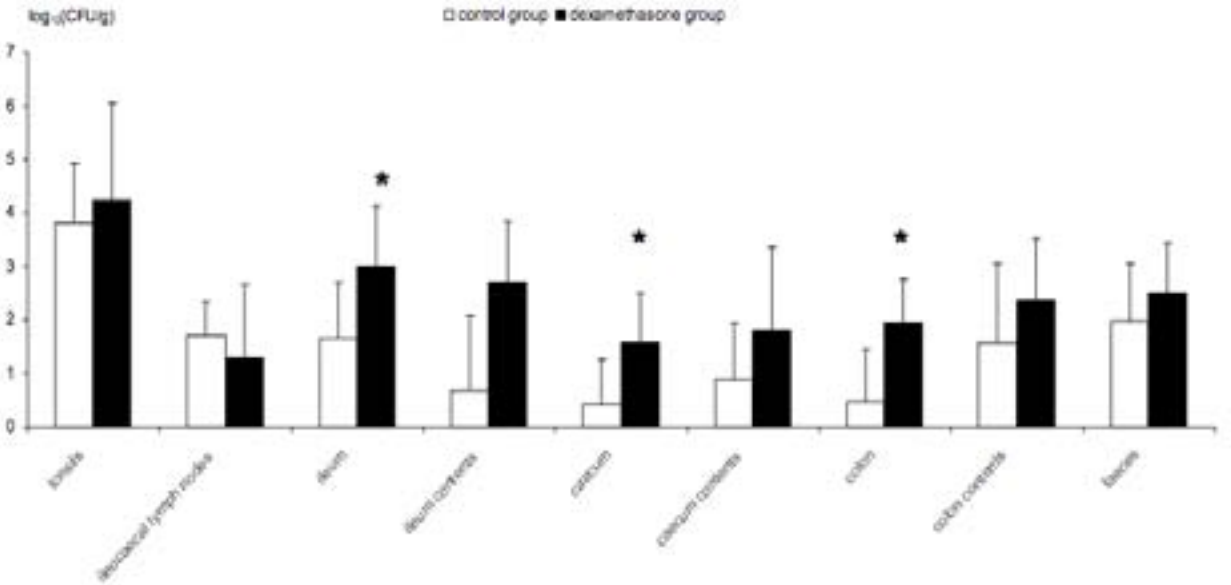

Figure 2: Recovery of Salmonella Typhimurium bacteria from pigs that were injected with either HBSS (control group, n = 9) or 2 mg dexamethasone per kg body weight (dexamethasone group, n = 9), 24 hours before euthanasia. The log10 value of the ratio of CFU per gram sample is given as the

mean + standard deviation. Superscript (*) refers to a significant difference compared to the control group ($p < 0.05$).

Cortisol does not affect Salmonella growth and gene expression, porcine intestinal epithelial cell viability and porcine macrophage viability and ROS production

Cortisol concentrations ranging from 0.001 to 100 µM did neither affect the growth of Salmonella Typhimurium, nor the viability of PAM and IPEC-J2 cells, during 24 hours. The exposure of Salmonella Typhimurium to 1 µM cortisol did not significantly affect gene expression levels. No significant differences were noticed in ROS production between Salmonella Typhimurium treated PAM in absence or presence of 1 µM cortisol.

Cortisol and dexamethasone promote the intracellular proliferation of Salmonella Typhimurium in porcine macrophages but not in porcine enterocytes

The intracellular proliferation of Salmonella Typhimurium was higher in cortisol and dexamethasone treated PAM in comparison to non-treated cells. Cortisol and dexamethasone did neither affect the intracellular proliferation of Salmonella Typhimurium in IPEC-J2 cells, nor the invasion in PAM and IPEC-J2 cells.

Discussion

Our results are in accordance with earlier studies conducted in pigs that showed that feed withdrawal is associated with increased shedding of Salmonella Typhimurium (Isaacson et al., 1999; Martín-Peláez et al., 2009; Morrow et al., 2002). Until now, the mechanism of stress related re-excretion of Salmonella in pigs remains unknown, but we showed that starvation stress results in elevated serum cortisol levels and that dexamethasone could induce recrudescence of Salmonella Typhimurium in pigs. This implies that stress induced release of cortisol in the bloodstream could alter the outcome of a Salmonella Typhimurium infection in pigs.

Earlier research in vitro has shown that norepinephrine in vitro promotes the growth and the motility of Salmonella enterica (Bearson and Bearson, 2008; Methner et al., 2008). We provided evidence that cortisol does not have similar effects on growth and does not influence gene expression of our Salmonella Typhimurium strain. Cortisol and dexamethasone nevertheless promote intracellular proliferation of Salmonella Typhimurium in porcine macrophages at concentrations that do not exert a notable effect on cell viability and ROS production by PAM.

These current results highlight the role of cortisol in the re-excretion of Salmonella Typhimurium by pigs and they provide new evidence for the role of microbial endocrinology in host-pathogen interactions.

Conclusion

In conclusion, we showed that the glucocorticoid cortisol is involved in a stress induced recrudescence of Salmonella Typhimurium in carrier pigs. In addition to this we pointed out that cortisol promotes the intracellular proliferation of Salmonella Typhimurium in pig macrophages, which is probably caused by an indirect effect through the cell.

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