

# ScsA as a major driver for increased survival of *Salmonella* during a stress response

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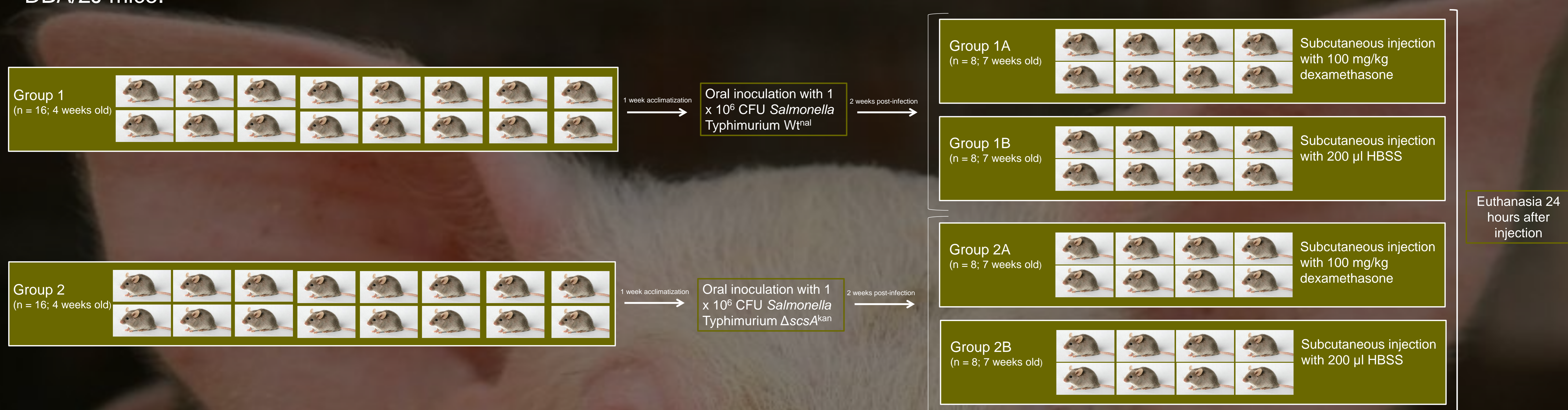
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## Introduction

Generally, pigs infected with *Salmonella* Typhimurium carry the bacterium asymptotically resulting in so called *Salmonella* carriers. Recently, we showed that a 24 hour feed withdrawal increased the intestinal *Salmonella* loads in carrier pigs, which was correlated with increased serum cortisol levels (1). This stress related recrudescence of a latent infection could be reproduced by a single injection of dexamethasone. We also showed that cortisol promotes intracellular proliferation of *Salmonella* bacteria in macrophages. The aim of the present study was to identify *Salmonella* genes that play a role during stress-induced recrudescence of a *Salmonella* infection.

## Materials and Methods

*In vivo* expression technology (IVET) was used to identify *Salmonella* genes that are intracellularly expressed in macrophages after exposure to cortisol. Following IVET, a *scsA* knock-out mutant ( $\Delta scsA$ ) and a complemented knock-out mutant ( $\Delta scsA^c$ ) were constructed. These strains were used in invasion and proliferation assays. Finally, we optimized a stress-mouse-model, mimicking the observations we have seen in pigs using DBA/2J mice.

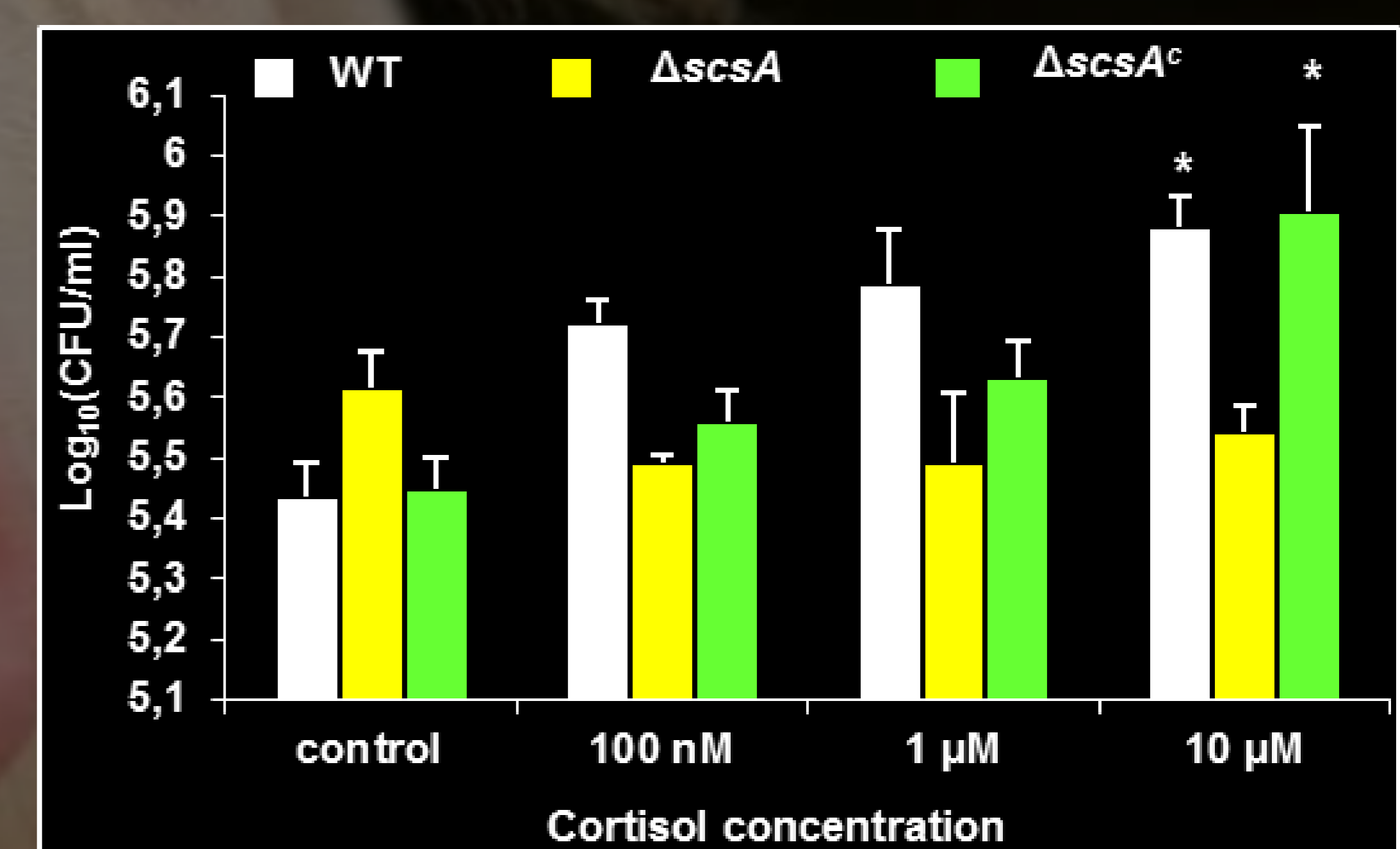


**Figure 1:** Sixteen mice were inoculated with a total of  $1 \times 10^6$  CFU of *Salmonella* Typhimurium or  $\Delta scsA$ . At day 14 p.i., eight animals of each group were subcutaneously injected with 100 mg/kg dexamethasone and eight mice were injected with 200  $\mu$ l HBSS and served as a control group. Twenty-four hours later, all mice were euthanized.

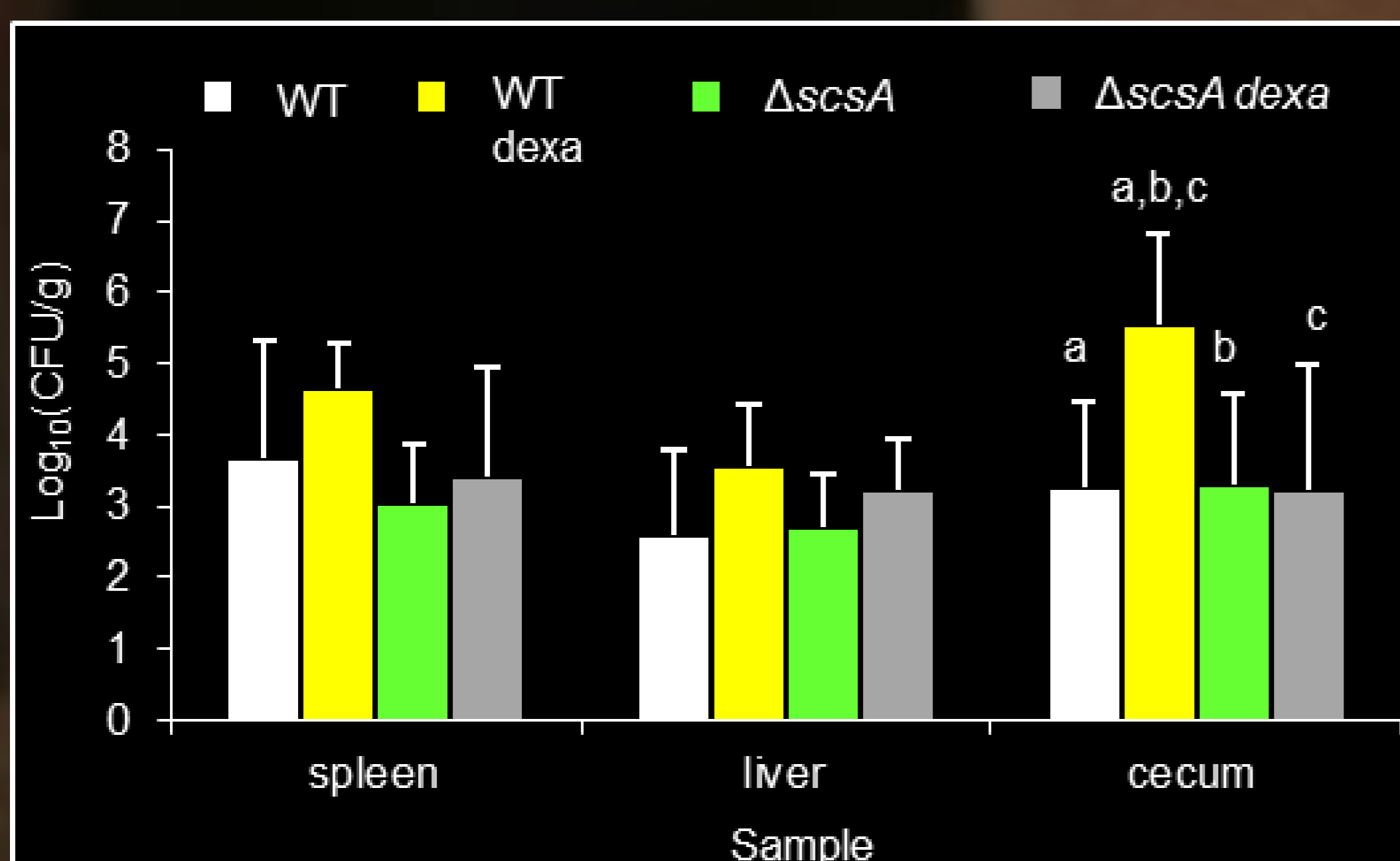
## Results and Discussion

Using IVET and intracellular proliferation tests, we identified *scsA* as a key driver of cortisol induced intracellular replication of *Salmonella* in porcine macrophages. Deletion of *scsA* abolished the increase in proliferation, an effect that was restored by the complementation of *scsA* (Fig. 2).

We then demonstrated the determining role of the *scsA* gene in glucocorticoid-induced *Salmonella* proliferation *in vivo* using an optimized DBA/2J mouse model (1). Using this model, we demonstrated that *scsA* is required for the glucocorticoid-induced increase in *Salmonella* infection load in the murine cecum *in vivo* (Fig. 3).



**Figure 2:** *ScsA* mediates cortisol-induced increase in proliferation of *Salmonella*, *in vitro*. Shown is the effect of cortisol on the log<sub>10</sub> values + standard deviation of intracellular *Salmonella* Typhimurium WT,  $\Delta scsA$  and  $\Delta scsA^c$  bacteria in porcine macrophages. Superscript (\*) refers to a significant difference compared to the condition without cortisol ( $P \leq 0.05$ ).



**Figure 3:** *ScsA* mediates cortisol-induced increase in proliferation of *Salmonella*, *in vivo*. Shown is the effect of dexamethasone exposure on the recovery of *Salmonella* WT and  $\Delta scsA$  from organs of DBA/2J mice. The log<sub>10</sub> value of the ratio of CFU/gram sample is given as the mean + standard deviation. Significant differences are signed with a, b, c ( $P \leq 0.05$ ).

## Conclusion

In conclusion, we showed that *Salmonella* senses stress conditions both *in vitro* and *in vivo* by responding to cortisol. We identified *scsA* as a major regulator during this process. The bacterium responds to cortisol in a *scsA* dependent way, with increased intestinal *Salmonella* loads as a result, which eventually can lead to increased pathogen dispersal.

## References

(1) Verbrugge E, Boyen F, Van Parys A, Van Deun K, Croubels S, Thompson A, Shearer N, Leyman B, Haesebrouck F, Pasmans F. 2011. Stress induced *Salmonella* Typhimurium recrudescence in pigs coincides with cortisol induced increased intracellular proliferation in macrophages. *Vet. Res.* 42:118