EXPERIMENTAL STUDY OF POSSIBLE INTERACTION BETWEEN SALMONELLA ENTERICA AND ASCARIS SUUM IN PIGS

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Abstract An experimental inoculation study was performed to study possible interactions between Salmonella enterica and Ascaris suum in pigs. Pigs were inoculated with 10^9 cfu Salmonella Typhimurium, 10,000 infective A. suum eggs, or both, or left as uninfected controls. There was no significant difference in number of Salmonella-excreting animals or Salmonella excretion level in feces between pigs inoculated with Salmonella + Ascaris and pigs inoculated with Salmonella only. Occurrence of Salmonella in liver, lungs, intestines or intestinal lymph nodes was not different between these two groups either. Some effect was seen of Salmonella on number of white spots and liver pathology, compared to pigs inoculated with Ascaris only. Serological results supported the bacteriological findings. No evidence was found that Ascaris infection exacerbates concomitant Salmonella infection in pigs.

Introduction Concomitant infections with different pathogens are very common in animals. Domesticated animals such as pigs may harbor significantly fewer infections than their wild living counterparts, but concurrent infections with bacteria, viruses and/or parasites are still quite frequent. Concomitant infections have the potential to exacerbate each other, with possible detrimental effects for the infected animal. Of special interest are infections with zoonotic potential, as their amplification by secondary infections may pose a food safety hazard.

In recent years, research has focused on possible interactions between bacteria and parasites in pigs, in particular the capacity of parasitic infections to possibly exacerbate infections with zoonotic bacteria such as Salmonella, Campylobacter and Escherichia coli. For example, Steenhard et al. (2002) found that infection with the nodular worm Oesophagostomum spp. increased and prolonged excretion of Salmonella Typhimurium in pigs.

In Denmark, the most common parasitic worm in pigs is the roundworm Ascaris suum. In contrast to the nodular worm, A. suum is more pathogenic for pigs, especially because this parasite has a migratory phase through the intestines, liver and lungs of the pig. The lesions in these organs caused by the larval migration may create increased opportunities for pathogenic bacteria to enter the circulatory system of the host. Therefore, A. suum has the potential to exacerbate Salmonella infection in pigs, which merited further study.

Firstly, a database investigation was performed, comparing the occurrence of Salmonella and A. suum in finisher pigs at herd level. A significant association was found between the presence of Salmonella (measured as positive meat juice samples) and the presence of A. suum (measured as liver white spots at slaughter) at herd level (Boes and Enoe, 2003). However, there was no quantitative association between the number of positive meat juice samples and the number of livers with white spots within herds, nor was it possible to conclude if A. suum influenced Salmonella or vice versa. It was therefore decided to carry out an experimental study of concurrent infection with Salmonella Typhimurium and A. suum in growing pigs. The aim of this study was to investigate, if infection with A. suum would cause an increase of Salmonella in concomitantly infected pigs.

Materials and Methods The study was performed as an experimental inoculation study. Growing pigs weighing approx. 30 kg were purchased from a specific pathogen free farm in which no Ascaris or Salmonella had been detected previously. The pigs were randomly assigned to one of four experimental groups:

1) Inoculation with both Salmonella and Ascaris (n=21)
2) Inoculation with Salmonella only (n=21)
3) Inoculation with Ascaris only (n=8)
4) Non-inoculated pigs serving as controls (n=8)
On day 0, pigs in groups 1 and 2 were inoculated with 109 cfu *Salmonella Typhimurium* DT12 and pigs in groups 1 and 3 were inoculated with 10,000 infective *A. suum* eggs. Inoculation was performed by oral gavage.

Excretion of *Salmonella* following inoculation was monitored semi-quantitatively by examining fecal samples on day 1, 4, 7, 14, 21 and 28. Presence of *Salmonella* in organs was recorded qualitatively by necropsy of 2-3 pigs in each group on day 1, 4 and 7, respectively. The remaining pigs were necropsied on day 28 and also examined for *Salmonella*. The organs examined on each occasion were: liver, lungs, small intestinal wall, large intestinal wall, and cecal lymph nodes. On day 4 and day 7, respectively, livers and lungs were also examined for migrating *Ascaris* larvae. Liver white spots caused by migrating *Ascaris* larvae were recorded for all pigs at necropsy.

On day 0, 7, 14, 21 and 28, blood samples were collected from all pigs. Serum was examined for antibodies against *Salmonella enterica* and *A. suum* using ELISA. Finally, pigs were weighed at the start of the study and at necropsy.

**Results**

The results of *Salmonella*-positive fecal samples in pigs inoculated with *Salmonella + Ascaris* and pigs inoculated with *Salmonella* only is shown in Figure 1. All but one pig excreted *Salmonella* bacteria in their feces 24 hours after inoculation. From day 4 the number of positive samples declined gradually, reaching a prevalence of 50% in pigs infected with *Salmonella* and 33% in pigs with concomitant infection four weeks post inoculation. However, the number of *Salmonella*-positive samples was at no point significantly different between the two groups.

There was no significant difference in *Salmonella* excretion level between groups 1 and 2 at any time following inoculation. On day 1, mean excretion levels were between $10^2$ and $10^4$ cfu/g feces, while from day 4 to day 28 excretion declined to $10^2$ cfu/g feces on average in pigs that were still positive.

*Salmonella* was isolated from livers and lungs of a number of pigs from groups 1 (2 pigs) and 2 (8 pigs) during the first week following inoculation, but not on day 28. However, the limited number of pigs necropsied on each occasion did not allow any statistical comparison.

All pigs necropsied in groups 1 and 2 within the first week after inoculation were positive for *Salmonella* in the intestinal lymph nodes as well as the small and large intestine. On day 28, *Salmonella* was isolated from 83% of lymph nodes, 50% of small intestines and 58% of large intestines, but no significant differences were found between the two groups.

Due to technical problems, *Ascaris* larvae from livers could not be enumerated on day 4. On day 7, a mean number of 1,635 larvae was found in the lungs of pigs with concomitant infection, compared to 1,628 larvae in pigs inoculated with *A. suum* only (group 3). This difference was not significant. On day 28, *A. suum* worms were recovered from the intestinal contents of 83% of pigs with concomitant infections, compared to 50% of pigs infected with *A. suum* only. However, due to small group sizes this difference could not be tested statistically.

The number of white spots in livers due to migration of *Ascaris* larvae is shown in Figure 2. The prevalence peaked on day 4, to decline gradually from day 7 to 28. There was no significant difference in white spot prevalence between pigs with concomitant *Salmonella + Ascaris* infection and pigs infected with *Ascaris* only.

The ELISA results showed no significant differences in *Salmonella* antibody response between pigs inoculated with *Salmonella + Ascaris* and pigs inoculated with *Salmonella* only. All pigs in these groups had seroconverted on day 14 after inoculation and remained seropositive during the rest of the study period. In contrast, the results of the anti-Ascaris ELISA showed higher antibody levels in pigs with...
Salmonella + Ascaris infection, compared to pigs infected with Ascaris only. However, due to the small number of pigs in group 3 this difference could not be tested statistically.

There was no difference in average daily gain between the experimental groups at any point during the 4-week study period.

Discussion A previous study (Boes and Enøe, 2003) has shown a possible association between Salmonella and Ascaris in pigs at herd level. Therefore, we carried out a study to clarify if such an association could be reproduced experimentally. Our study did not yield conclusive evidence of interaction between Salmonella and Ascaris in pigs. Neither the number of Salmonella-excreting animals nor the excretion level in the feces was increased in pigs concomitantly infected with Ascaris, despite the relatively high inoculation doses used. This suggests that, under normal circumstances, infection with A. suum in pigs is not likely to exacerbate concomitant Salmonella infections. This is supported by the serological results, which showed no differences in antibody response against Salmonella.

On the other hand, some of our results suggest that Salmonella may have an effect on A. suum. Four weeks after inoculation, liver pathology was more severe and white spot numbers slightly higher (though not significantly) in pigs with concomitant infections, compared to pigs infected with Ascaris only. In addition, antibody levels directed against A. suum were higher in concomitantly infected pigs. This observation and its possible implications deserve further attention.

Conclusions Concomitant infection with Salmonella and Ascaris:
- did not increase the number of Salmonella-excreting pigs
- did not increase the quantitative level of excretion nor the duration of Salmonella in the feces of pigs
- did not increase the number of pigs with Salmonella in their internal organs
- did not evoke a stronger antibody response against Salmonella in infected pigs

REFERENCES


Figure 2. The prevalence of liver white spots due to migration of Ascaris larvae in pigs inoculated with Salmonella + Ascaris (black bars) and pigs inoculated with Ascaris only (white bars).