



LACK OF A SIGNIFICANT SYSTEMIC IMMUNE RESPONSE IN PIGS EXPERIMENTALLY INFECTED WITH *SERPULINA PILOSICOLI*

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Introduction

Porcine intestinal spirochaetosis (PIS) is a diarrhoeal disease resulting from colonisation by the spirochaete *Serpulina pilosicoli* (1). Little is known about immunity in this infection (2). Individual animals may be colonised for extended periods of time, and the disease in infected piggeries appears to occur sporadically. Pig infected with PIS suffer from decreased growth rates, poor feed conversion, and weight loss (3). This experiment was conducted to determine whether infected pigs developed circulating antibodies to the spirochaete, and to evaluate the effect of vaccination.

Materials and Methods

Animals

Two groups of weaner pigs were used. Six pigs were vaccinated intramuscularly, twice at a three week interval, with a bacterin containing 10^{10} cells of *S. pilosicoli* strain 95/1000, inactivated in formalin, and emulsified in Freund's incomplete adjuvant. The other group of eight pigs were not vaccinated. All pigs were challenged orally three successive days with broth cultures of *S. pilosicoli* strain 95/1000 (10^{10} cells/day), ten days after the second vaccination. The clinical condition of the pigs was monitored daily, and colonisation was determined by faecal culture and histological analysis at post-mortem 2-3 weeks post-infection.

Serum Samples

Blood samples were taken six times during the course of the experiment, commencing prior to immunisation. The blood was left at 4°C overnight to coagulate and the serum removed and stored at -20°C. Prior to use, the sera were thawed on ice and vortexed.

Whole Cell ELISA

Whole cell sonicates of the homologous *S. pilosicoli* strain 95/1000 were used as the bound antigen for a general ELISA test. Cells (10^8) were sonicated three times for 30 seconds with 2 minutes on ice between cycles. The sonicated cells were centrifuged at 10,000g for 10 minutes and the supernatant (1.3mg/mL total protein) used for antigen. The sonicate was diluted in bicarbonate/carbonate coating buffer (pH 9.6) to a working dilution of 1.6 µg/mL. One hundred µL of the working dilution was used to coat each well of the 96 well microtitration plates. Coating was allowed to occur at 4°C overnight. The plates were washed three times with PBST before applying the serum. Sera were diluted in PBST containing 1% (w/v) BSA and incubated with gentle mixing at room temperature (RT) for 2 hours. After washing the plate three times with PBST, 100µL of diluted (1:2000) goat anti-pig IgG HRP was added to each well. The plates were incubated at RT for 1 hour with gentle mixing, before washing the plate three times with PBST. One hundred µL of OPD substrate solution (0.1% w/v OPD, 0.03% H₂O₂, phosphate-citrate buffer, pH 5.0) was added to each well and incubated for 10 minutes at RT to allow colour development. Colour development was stopped with 2M H₂SO₄ before reading the optical density (OD) at 490nm.

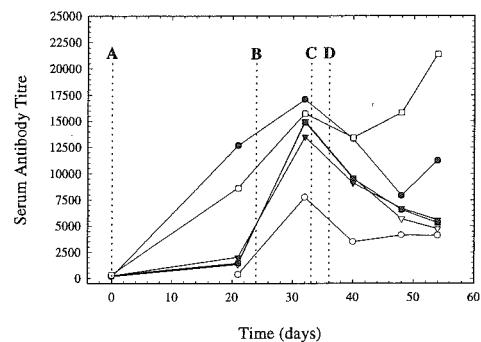
Results and Discussion

The serum titres were determined as the dilution at which the OD_{490nm} would be zero, as calculated from the linear regression of the log₁₀(time) versus OD_{490nm} plot. The serum titres of each blood sample taken from each pig were plotted against days post-vaccination (Figure 1).

Pigs in both groups became colonised by the spirochaete and developed mild colitis, indicating that the vaccine regimen was not protective. Vaccinated pigs showed a primary and secondary antibody response following the two vaccinations, indicating that the vaccine stimulated a systemic response and that the ELISA test was capable of detecting it. Titres were highest during the time of bacterial challenge, however, these levels failed to be maintained and decreased following the challenge, despite the animal being colonised throughout this period. Five of the seven unvaccinated

pigs showed little or no increase in antibody titres post-infection. Titres of the other two pigs increased marginally, but were not significantly higher than the pre-infection levels. The antibody titres of the unvaccinated pigs were significantly lower than the vaccinated pigs. These results suggest that *S. pilosicoli* can colonise the colon without inducing a significant systemic immune response and this may help explain its tendency to persist in infected pigs. Studies on local immune responses in the large intestine are now necessary.

I



II

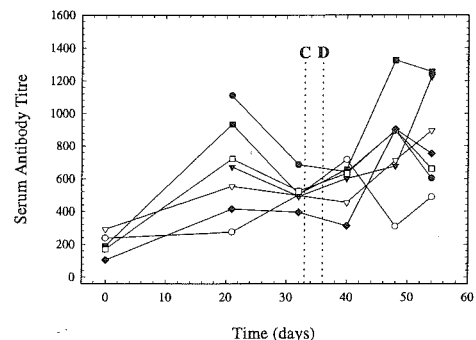


Figure 1. Serum antibody levels of vaccinated (I) and unvaccinated (II) pigs challenged with *S. pilosicoli* strain 95/1000. Vaccinated pigs were given two intramuscular injections (A,B). All pigs were challenged with *S. pilosicoli* strain 95/1000 ten days after the second vaccination (C-D).

References

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