Discussion: the proposed method seems to be effective, rapid and reproducible, the comparison with the standard method showed that neither false-positive nor false-negative results were obtained. The specificity of the reaction was confirmed by the determination of the Tm, specific for the amplicon obtained, that allows to eliminate the phase of electrophoresis, which is time-consuming and requires the use of ethidium bromide, a potent mutagenic agent, that is not suitable for routine use.

References


Development of an ELISA test for Salmonella serological monitoring in Brazil

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Summary: EMBRAPA/CNPSA developed an ELISA test based on LPS antigens from Salmonella Typhimurium. After the optimal dilution determination of the test components, four sera were chosen as controls. The interplate variation was controlled by a coefficient correlation between standard and daily curves of control sera and the coefficient variation of sample sera triplicates. The cut-off was determined by a dispersion analysis in a nursery piglet population proved to be salmonellae negative. The test performance was evaluated in experimentally and naturally S. Typhimurium infected pigs and in animals vaccinated with other Salmonella serovars. The seroconversion was observed after two weeks post inoculation in experimentally infected and vaccinated animals. In naturally infected animals, which were sampled twice during the finishing period, at the first sampling 75 % of pigs were eliminating salmonellae in feces and 25 % were positive in the ELISA. At the second sampling 76,9 % became serologically positive. These results suggest that the developed test can be used for Salmonella Typhimurium monitoring programs in swine.

Keywords: Lipopolysaccharides, S. Typhimurium, swine, serology.

Introduction: Previous studies conducted in southern Brazil indicated a wide dissemination of salmonellae infection in swine herds (Bessa et al. 2001, Kich et al. 2001). The reduction in the number of carrier pigs at slaughter is one of the most important measures for pork contamination control. Most countries started intensive programs of Salmonella control on farms based on serological monitoring (Nielsen et al., 2001). In southern Brazil serovars Typhimurium, Agona, Derby, Bredney and Panama have proved to be the most prevalent in carrier pigs sampled at slaughter (Bessa et al., 2001). As these serovars have at least two common LPS antigens with Typhimurium, an ELISA test was developed based on LPS antigens from S. Typhimurium.

Material and Methods: Phenolic extraction of LPS from Salmonella Typhimurium was done as described previously (Vidal et al. 1999). Optimal dilution was determined for serum (1:400), antigen (1:2000) and conjugate (1:25,000). For control of intraplate and interplate variation reference sera, chosen from sera
of SPF and inoculated pigs, were included on every plate. The mean OD’s for these sera in 15 assays were 0.25, 0.245, 0.4385 and 1.0995. Sample OD’s were transformed to calibrated OD, using a linear regression equation for reference OD’s on the actual plate versus mean reference OD’s. For an intraplate variation control, all sample sera were tested in triplicate and the acceptable variation coefficient among sera OD’s was below 10%. The cut-off (OD 0.159) was calculated as the mean OD of a negative population plus four standard deviation. Orally infected pigs were submitted to the ELISA test, in order to observe the seroconversion. Three 95-days-old SPF pigs were inoculated orally with $3.5 \times 10^8$ cfu of *Salmonella Typhimurium* and two animals were kept as sentinels. Blood was collected weekly from all animals during a period of 42 days. Furthermore, a field study was conducted including 56 pigs tested at the early growing phase and at slaughter. Blood and fecal samples were taken from all pigs. Finally, groups of five pigs (aged 55 days) were vaccinated with bacterins produced individually with serovars Typhimurium, Agona, Derby, Bredney and Panama. Four non – inoculated pigs were kept as negative controls.

**Results:** Seroconversion in inoculated and vaccinated pigs was observed around two weeks after the exposure. Sentinels became positive between the third and fourth week after the challenge. Higher OD values were observed in inoculated pigs than in sentinels. In the field trial, 75 % (42/56) of the growers were shedding *Salmonella Typhimurium* in feces and 25 % (14/56) were serologically positive. At slaughter the serological prevalence increased to 76.9 % in the same group of animals.

**Discussion:** Several studies described salmonellae mix ELISA tests to monitor infection on farms (Nielsen et al., 1995; Vidal et al., 1999; Proux et al., 2000). The present study showed that natural and experimental infection by *Salmonella Typhimurium* can be detected using the developed ELISA test. Furthermore, the test proved to be able to detect also the most prevalent serovars found by Bessa et al. (2001) in southern Brazil. More studies have to be conducted in order to compare the performance of the developed ELISA test with salmonellae mix ELISA tests currently in use.

**Conclusions:** The developed ELISA test detects antibodies against salmonellae serovars prevalent in southern Brazil and can be adopted for screen pigs for serological evidence of infection.

**References:**


