Evaluation of Pooled Serum and ‘Meat-Juice’ in a Salmonella ELISA for Pig Herds

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Summary: Samples of ‘meat-juice’, serum, caecal contents and carcase swabs from 420 pigs from 20 finishing farms were tested for Salmonella bacteriologically and serologically by ELISA on individual samples or on pools of serum or meat juice. In addition pooled floor faeces were taken from the finishing pens on the farm of origin.

Salmonella was isolated in samples from 19 of the 20 farms. 32.8 % of pooled pen faeces and 24.3 % of caecal samples were positive but Salmonella was only found in 1.7 % of carcase swabs. 43.2 % of individual ‘meat-juice’ samples and 25.3 % of serum samples gave positive ELISA results. Neither the individual or pooled ELISA tests showed a statistically significant correlation with caecal carriage of Salmonella or contamination of carcasses, although the percentage of positive pen faecal samples did correlate significantly with caecal positives. Only serum mean optimal density from pools of 5, 10 or 20 sera correlated significantly with Salmonella prevalence in pen faecal samples but all pooled serum and meat-juice optimal density or sample/positive ratios correlated significantly with the percentage individual ELISA positives. This suggests that pooled serum or meat-juice could be used as an alternative to individual samples for ranking herds.

Keywords: serology, monitoring, comparison, swine, contamination

Introduction: Monitoring for Salmonella in slaughter pigs is important to enable targeted control measures to be applied on significantly infected farms and at the abattoir. Serological testing using a LPS based mix ELISA has been shown to be suitable for ranking herds according to likely weight of infection (Nielsen et al., 1998), but testing sufficient numbers of samples to obtain an accurate herd ranking is expensive for an industry in severe financial difficulties. Pooled samples are routinely used to maximise bacteriological detection of Salmonella with limited resources. This paper describes a study designed to assess the suitability of pooling of serum or meat juice samples for ranking the Salmonella status of pig herds.

Materials and Methods: Approximately 420 serum, meat juice, carcase swab, and caecal contents samples were obtained from groups of slaughter pigs from 20 farms. Carcase swabs were taken according to a US/Danish protocol. In addition, the farms were visited on the day before slaughter and pooled faeces collected from pens occupied by the pigs to be slaughtered. Serum and meat juice samples were tested by ELISA (Vetsign Salmonella ELISA Kit; Guildhay) as individual samples and as pools comprising 5, 10 and 20 individual subsamples. Bacteriological culture carried out by 18 hours pre-enrichment at 37 °C Buffered Peptone Water (BPW; Merck), 48 hours selective enrichment at 41.5 °C in DIASALM medium (Merck), with subculturing on to Rambach agar (Merck) plates after 24 and 48 hours culture. The plates
were incubated for 24 hours at 37 °C and suspect colonies confirmed by standard biochemical and serological tests. Serotyping and phage typing was carried out by VLA Weybridge. Statistical analyses were made on the basis of herd results and correlations and descriptive statistics calculated with Statistica (StatSoft, Inc. 2001).

**Results:** 109 (25.3 %) of the 430 serum samples taken were positive (sample/positive ratio (SP) > 0.25), placing 7 herds in the equivalent of Danish Category 1, 11 herds in Category 2, and 2 herds in Category 3, ie. the worst *Salmonella* status category, requiring further action. The mean SPs from the various poolings were 0.33 (0.08-0.83), 0.33 (0.05-1.19) and 0.37 (0.07-1.39) for pools of 5, 10 and 20 respectively. 182 (43.2 %) of 421 meat-juice samples were positive, which would place 8 of the 20 farms in Category 3. The mean SP results from the various poolings were slightly higher than the mean of the individual samples; 0.41 for individuals, 0.48 for pools of 5, 0.51 for pools of 10 and 0.48 for pools of 20.

7 (1.7 %, herd range 0-9.1 %) of 422 carcase swabs contained *Salmonella*. The positive carcase swabs originated largely, but not exclusively, from herds with high numbers of caecal positives. 102 (24.3 %) of 420 caecal samples contained *Salmonella*, predominately *S*.Typhimurium (17.4 % samples) which were mostly DT104 or related strains such as U302. 121 (32.8 %) of 369 pooled pen faeces samples contained *Salmonella*. The serotypes and phage types corresponded well with those found in caecal contents. No *Salmonella* or ELISA positives were found in one of the 20 farms.

None of the ELISA tests, either % positive individual ELISA tests or optimal density (OD) or SPs for pooled sera correlated with caecal *Salmonella*. Optimal density for pools of 5, 10 and 20 sera correlated significantly with the % positive farm pens, but there was no such correlation for ‘meat-juice’. For all pools the ODs and SPs correlated significantly with % positive individual samples and there was a significant correlation between the % positive farm pen samples and % positive caeca. Although there was a significant correlation between serum and meat-juice results there were substantially high numbers of positive samples with meat-juice.

**Discussion:** This study demonstrates that pooled serum or meat-juice could be used for ranking pig herds as part of a *Salmonella* surveillance and control programme. More work is required to refine the application of the test, and in particular more long-term studies to establish herd serological and bacteriological profiles and appropriate weighting factors for recent versus earlier herd results. The application of the test would probably be more effective if a percentage of ‘worst’ herds for further action was applied rather than using fixed banding categories. It is clear also that the correlation between test results from serum and meat-juice could be improved by attention to dilution factors.

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**References**
