Monitoring *Salmonella* infection on pig farms in GB by meat juice ELISA and culture of pen faecal samples

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Summary
Many pig herds in Great Britain are infected with *Salmonella*, which is seldom associated with clinical disease but may be a potential source of some human infections. In the absence of clinical disease, infected herds are unlikely to be detected through passive surveillance. An industry initiative, the Zoonoses Action Plan Salmonella Monitoring Scheme (ZAP) monitors finishing pigs at slaughter through submission of samples that are tested with a meat juice enzyme-linked immunosorbent assay (MJ-ELISA). There is a poor correlation between the MJ ELISA result of an individual pig and the presence of *Salmonella* in the caecal contents at slaughter. On farms, pooled pen floor faecal samples for culture of *Salmonella* can be collected relatively cheaply and easily. In this paper, we report that there is a significant correlation at the farm level between the prevalence of infected pen samples and MJ ELISA results.

Introduction
Salmonella infection, principally with *S.* Typhimurium, was identified in 23% of slaughtered finishing pigs at a random sample of British abattoirs (Davies et al 2001). The UK pig industry introduced ZAP with the aim of reducing the prevalence of infection in pig herds to safeguard public health. Each herd will be given a ZAP score, based on the prevalence of MJ ELISA positive pigs. Those herds with the greatest prevalence of MJ-ELISA positive pigs are required to develop an action plan to control *Salmonella*. We collected pooled pen floor faecal samples and meat samples for the MJ ELISA from 148 farms between 2001 - 2003. Pooled pen samples provide an indication of the level of active infection within a herd at a particular moment in time, whereas MJ ELISA results reflect the past exposure of individual animals to *Salmonella.* Both measures are interpreted at the herd level. The data from these studies have been used to investigate the association between MJ ELISA results and *Salmonella* infection on the farm.

Objectives
We present the prevalence of MJ ELISA positive pigs and *Salmonella* positive pen samples that were observed from a series of cross-sectional studies in British finisher pig herds. Demonstration of the correlation between these values can be used to aid interpretation of these two distinct measures of herd infection.

Materials and methods
Data from five different cross-sectional studies were combined. Four of these studies were of randomly selected farms that finished pigs under contract to larger companies. The fifth study was of a random sample of farms across GB. In all of these studies, a

similar protocol was followed. Up to 30 pens containing pigs aged between approximately 10 and 22 weeks of age were selected at random. A pooled pen floor faecal sample (25g) was collected from each pen and up to 40 samples for the MJ ELISA test were collected from a random sample of pigs presented for slaughter.

Pen faecal samples were pre-enriched in buffered peptone water and selectively enriched in Diassalm agar plates. Samples from this were inoculated onto a Rambach agar plate and suspect Salmonella colonies were subjected to a slide agglutination test using a range of typing sera and to the minimum phenotypic criteria for identification to *Salmonella* species. A subculture of each confirmed *Salmonella* isolate was submitted for full serotyping and phage typing, where applicable.

The meat juice is tested using a mix-ELISA for antibodies to group B and C1 *Salmonella* serotype lipopolysaccharide. Samples were tested in duplicate by the Guildhay VetSign Salmonella ELISA Kit, according to the manufacturer's instructions. During testing, field samples of strong and weak positive sera and meat juice were run alongside the tests, as well as the kit controls. Plate results were accepted when all controls met the expected results. A positive result was defined as a sample:positive ratio of >0.25.

All data were entered onto an ACCESS database and analysed using Stata® release 8 (Stata Corporation, Texas, USA). The prevalence of MJ ELISA positive pigs from each farm was used as the outcome variable and linear regression was used to model the correlation with the prevalence of infected pens, adjusted for study. A robust 95% confidence interval (ci) was calculated using Stata.

Results

The mean prevalence of *Salmonella* infected pens was 24% (ci 20% - 28%) and the mean prevalence of MJ-ELISA positive pigs was 29% (ci 25% - 34%). These varied significantly between studies (anova $p<0.001$), as shown in table 1. There were 26 farms that had 0% of pens detected as infected and 0% of pigs with a positive MJ ELISA. Six farms had at least one positive pen sample but no positive MJ ELISA results and 16 farms had at least one positive MJ ELISA result but no positive pen samples. There was strong evidence of an association between MJ-ELISA results and the prevalence of infected pens (P=0.009). This explained approximately 28% of the between farm variation ($R^2_{adjusted}=0.280$). The equation {MJprev=0.156+0.572Penprev} expresses the relationship, which may be crudely summarised as suggesting that the prevalence of MJ-ELISA positive pigs is about 16% if the pen prevalence is zero and increases by about 6% for every 10% increase in the prevalence of infected pens. These results are shown in figure 1, with a 95% confidence interval for the regression line.

Table 1. The prevalence of meat juice ELISA positive pigs and the prevalence of *Salmonella* infected pooled pen faecal samples from 4 studies of British pig farms.

Study	No. Farms	MJ ELISA		Pen samples	
		Prevalence	95% ci*	Prevalence	95% ci*
1	22	49%	37%-61%	40%	30%-50%
2	20	2%	0%- 4%	1%	0%- 2%
3	28	48%	39%-56%	27%	18%-35%
4	78	24%	18%-30%	24%	18%-30%

* ci = confidence interval

Figure 1. The association between the prevalence of MJ ELISA positive pigs and *Salmonella* positive pooled pen faecal samples on 148 farms in Great Britain.

Discussion

The intention of ZAP is to identify farms with the greatest prevalence of MJ ELISA positive pigs. This study shows that in general, these farms also had the greatest proportion of infected pens. However, there were some farms with anomalous results – the MJ ELISA prevalence was low yet the pen prevalence was high, or vice versa. Thus, an individual farm that was shown to have a high ZAP score should be advised to undertake microbiological testing to establish the status of the herd with respect to infection. A herd that has a low prevalence of MJ ELISA positive pigs may nevertheless harbour a significant level of infection at pen level. Further investigation of herds with these apparently paradoxical results would be valuable.

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References

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