Zoonoses Action Plan for Salmonella in slaughter-age pigs: how will changes in sampling methods influence estimates of Salmonella?

O'Reilly, K. M.*, Hill, A., Miller, A., Snary, E., Cook, A.

Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency – Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom.

*corresponding author: k.o.reilly@vla.defra.gsi.gov.uk

Abstract

In June 2002 the British Pig Executive introduced the Zoonoses Action Plan (ZAP) Salmonella Monitoring Programme with the aim of reducing the prevalence of Salmonella infection in British pigs. A serological screening programme was developed where meat juice samples were collected from pigs at slaughter and tested using a mix-ELISA and herds were assigned a ZAP score from low to high on the basis of these results. We posed several questions concerning the predictive value of a ZAP score and how this may change if the frequency of sample collection were changed.

A statistical model was developed which described the different sampling strategies and the resultant ZAP score was estimated for each of the modelled farms. The model was used to assess how changing the sampling frequency would affect the ability to assign a correct ZAP score given an assumed true within-farm prevalence and how the ZAP scores may be used to predict the likely prevalence of Salmonella at a farm level.

The model predicted that reducing the number of samples tested had a large effect on correctly assigning herds with a medium (50-75%) meat juice prevalence but little effect on herds with a low (50%) or high (>75%) prevalence. A change in an individual farm’s prevalence of infection between sampling periods is unlikely to be detected. At a national and regional level the estimated prevalence had small confidence intervals and a change of approximately 2% is likely to be detected.

Currently the ZAP scheme requires that farms in the medium or high ZAP level must act to reduce the herd ZAP score to low within a specified time or face eventual loss of their quality assured status. Thus correct ZAP classification is important. As the herd ZAP level is assigned using a rolling 3 month average, incorrect classification is less likely than if monthly ZAP scores were calculated. The requirement for a farm to stay at a high level for 11 months before incurring the penalty is a further safeguard.

Introduction

The ZAP programme involves use of a meat juice mix-ELISA (MJE) system to detect antibodies to group B and C1 Salmonella in pigs (Nielsen et al. 1998), through surveillance of pigs sent to assured abattoirs in Great Britain. The optical density (OD) of the test is calculated using standard methods. A ratio between test and control samples of 0.25 is used to define a positive test result.

The ZAP scheme was developed to act as a tool to monitor the prevalence of Salmonella in pigs at slaughter in Great Britain, and to drive a reduction in prevalence (www.bpex.org), with the ultimate aim of providing safer meat to the consumer. For a holding to have a ZAP score, fifteen samples must be submitted for analysis per quarter. Currently a minimum of three pigs are sampled per batch, and the minimum number of batches per holding submitted for each quarter is five. A ZAP score is calculated using the MJE prevalence on a three-month rolling average and the score is assigned as follows; ZAP 1: ≤50% positive samples, ZAP 2; 50-75% positive samples, ZAP 3; ≥75% positive samples. Holdings assigned a ZAP score of 2 or 3 are required to adopt an action plan to reduce the level of Salmonella. If a holding is assigned to ZAP 3 for more than 11 consecutive months their assured status could be suspended. This will have a significant financial
impact on the farm and it is therefore important that holdings are not incorrectly assigned a ZAP 3 score.

The objectives of this study were to explore how varying the frequency of sampling would affect the estimate of *Salmonella* prevalence. Specifically, the following questions were phrased. If the number of samples submitted from each herd per quarter were reduced;

A. What would be the impact on the likelihood that a herd were assigned the correct ZAP score?

B. What would be the predictive value of a given ZAP score – ie. if it were stated as ZAP 1, then how likely is it that the herd truly had a prevalence of <50%?

C. What would be the impact on the likelihood of detecting a reduction in prevalence within Great Britain, nationally (England, Scotland and Northern Ireland), and at a herd level?

**Materials and methodology**

The methodology for each objective is addressed in turn;

**Objective A** A model developed by Snary *et al.* (2004) was modified. For the purposes of this paper, the estimated proportion of test positive pigs, $P_{+}$, was extrapolated from the known prevalence ($P_u$), test sensitivity and test specificity of the MJE used in the ZAP scheme, which was reported to be 0.92 and 0.93 respectively (Nielsen *et al.* (1998) and Proux *et al.* (2000)). When a batch of pigs arrives to the abattoir with a *Salmonella* prevalence ($P_i$), a sample ($S_b$) of these pigs is tested using the MJE. Multiple batches of pigs will arrive from a pig herd to an abattoir over a three-month period, where the number of pigs in each batch is denoted as $n_b$, and the total number of pigs in all batches is denoted by $N_b$ (where $N_b = \sum n_b$). The number of these pigs with a positive MJE ($n_{+b}$) is assumed to be binomially distributed with the number of samples corresponding to $n_b$, and the probability set as the proportion of test positive pigs $P_{+}$. The number of positive pigs in each batch was distributed assuming a hypergeometric distribution (Vose 2000), which accounts for sampling without replacement. From these assumptions the prevalence of MJE is assumed to be;

$$P_{MJE} = \frac{\sum_{b=1}^{N_b} \text{Hypergeometric}(S_b, n_{+b}, n_b)}{S_b N_b}$$  \hspace{1cm} (1)

Given that not all pigs are sampled in the ZAP sampling scheme, and the imperfect test sensitivity and test specificity, the true herd prevalence is not known. However, $P_{MJE}$ was simulated for a range of $P_u$ values (i.e. from $P_u = 0$ to $P_u = 1$, in steps of 0.01). For each estimate of $P_{MJE}$ a ZAP score, $\hat{Z}$, was assigned according to the definitions described in the introduction. The values of $S_b$ and $N_b$ were varied to observe the change in $P_{MJE}$ and hence $\hat{Z}$. For each incremental value of $P_u$, there is a corresponding true ZAP score, $Z$, which can be compared to the estimated ZAP score, $\hat{Z}$. The probability of assigning the correct ZAP score, $P(Z = \hat{Z})$, was estimated:

$$P(Z = \hat{Z}) = \frac{\# \text{of iterations where } Z = \hat{Z}}{\text{total} \# \text{of iterations for } P_u \text{ value}}$$  \hspace{1cm} (2)

Given a ZAP score of 1, 2 or 3, the probability that the true herd prevalence ($P_u$) lies within the range of each ZAP score is the sum of the number of correctly assigned ZAP scores for that range of prevalence over all iterations used in the model.

**Objective B** Using the same simulations described above, the ZAP scores for all iterations are stored for each value of $P_u$. For all simulations, the predictive value of each ZAP score is calculated by counting the number of correctly allocated ZAP scores divided by the number of iterations assigned that ZAP score.

**Objective C** National prevalence is defined as the proportion of positive samples submitted, irrespective of batch or holding, and gives an indication of what proportion of pigs being sent to
slaughter are MJE-positive (and hence an indication of the proportion of Salmonella-positive pigs). The observed national MJE prevalence for a quarter (3-months) was used to estimate the national Salmonella prevalence. The data used were from January-March 2006 where 37961 samples were submitted, of which 8571 (23%) were positive.

To estimate the Salmonella prevalence of all pigs sent to the abattoir, a beta distribution (with uniform priors) was used to account for the uncertainty due to the sample size (Vase 2000), and test sensitivity and specificity were also included in the estimate. This results in the estimated Salmonella prevalence being symmetrically distributed. If the number of samples submitted were reduced, the estimate of prevalence may differ. Therefore, a two-sample t-test (Petrie and Watson 1999) was used to test the null hypothesis that reducing the number of samples tested would not significantly (P<0.05) change the estimated Salmonella prevalence. The prevalence within each country (England, Scotland, and Northern Ireland) was investigated in the same way.

Similar methods as described above were used to examine the estimate of herd prevalence. However the Salmonella prevalence estimate per holding was positively skewed as there were fewer samples submitted. Consequently a t-test could not be used to compare distributions and 5th and 95th percentiles of each distribution were used to estimate Salmonella prevalence.

Results

Objective A Simulations are shown assuming that five batches were sampled per quarter. The probability of being assigned the correct ZAP score, \( P(Z = \hat{Z}) \), was reasonably high (>0.75) when the prevalence was less than 0.40 (Figure 1). As the herd prevalence of Salmonella, \( P_H \), approached the boundaries of a ZAP score (i.e. when \( P_H \) was 0.5 or 0.75) the probability of assigning a correct ZAP score reduced in value. When \( P_H \) was between 0.50 and 0.75 (which was equivalent to a ZAP 2 score) \( P(Z = \hat{Z}) \) varied from 0.4-0.75: many scores were either ZAP 1 or ZAP 3. Assuming that there was one pig sampled per batch resulted in the value of \( P(Z = \hat{Z}) \) being less than 0.50 for many values of \( P_H \). As the number of pigs sampled per batch increased, the ability to estimate the correct ZAP score increased. The main difference between sampling either three or two pigs per batch was that the estimate of \( P(Z = \hat{Z}) \) improved at low values of \( P_H \) when three pigs were sampled per batch.

Objective B When a ZAP score of 1 was assigned, the predictive value of a ZAP score was always above 0.90 irrespective of the number of batches sampled per quarter (Figure 1). The predictive value of a ZAP 2 score was comparatively low; when 2 pigs were sampled per quarter the predictive value was 0.45. The predictive value of the ZAP score had reduced to 0.40 when 1 pig was sampled per batch: more holdings were incorrectly assigned a ZAP 2 score than correctly assigned. The predictive value of a ZAP 3 score performed better and varied from 0.75-0.80 according to the number of samples submitted.

Figure 1 Effect of changing the number of pigs sampled per batch on the probability of assigning the correct ZAP score, \( P(Z = \hat{Z}) \) and the predictive value of the ZAP score. Five batches per quarter were assumed in the simulations.
**Objective C-National level** Reducing the number of pigs sampled per batch will reduce the number of submitted samples at a national and regional level by up to 33%. Reducing the number of samples submitted by 33% will not significantly ($P<0.05$) change the crude estimate of national *Salmonella* prevalence, which for January-March 2006 was 18%. A change of 2% will be detected at a national level. The *Salmonella* prevalence was estimated for each region, and 24% of samples from England were positive, 1% of samples from Scotland were positive and 5% of samples from Northern Ireland were positive. Even when considering a reduction in sample size of 33% a change in prevalence of 2% would be detected by the ZAP scheme.

**Objective C-Herd level** Assuming 15 pigs per quarter were sampled; a wide range in the estimated prevalence was estimated for each value of $P_{c,r}$. For example the 5th and 95th percentiles were 0.07 and 0.45 respectively when the true prevalence was 0.2, and 0.54 and 0.93 respectively when the true prevalence was 0.80. Therefore only a large change in prevalence, e.g. from 0.2 to 0.8, would be detected using the current minimum sampling. Using the same method, the minimum increase in MJE-prevalence that can be detected from 0.20 would be 0.70. By reducing the minimum number of pigs sampled to 10 samples per quarter, a statistically significant ($P<0.05$) change in $P_{c}$, would not be detected if a holding were to submit the minimum number of samples.

**Discussion**

Simulations have been used to show that reducing the minimum number of pigs sampled per quarter to a minimum of five pigs had a varied effect on the probability of correctly assigning a ZAP score and the predictive value of the ZAP score, as a result of testing less samples per batch. Hence, sampling two pigs per batch, rather than three, should not have a major effect on the predictive abilities of the ZAP scheme. It should be noted that the batch prevalence when two pigs are sampled will be either 0, 0.5 or 1.0. Therefore it is important that a sufficient number of batches (i.e. greater than five) are sampled per quarter in order to provide a good estimate of MJE prevalence, and hence the ability of the ZAP scheme to correctly allocate a ZAP score.

The high number of samples collected in the ZAP scheme resulted in accurate estimates of *Salmonella* prevalence at a national level; simulations suggest that a change in prevalence of 2% will be detected. At a herd level, the minimum number of samples collected to be allocated a ZAP score, currently set at 15 pigs per quarter, results in a wide estimate of true *Salmonella* prevalence. Therefore changing the sampling methods will influence the estimated holding prevalence. In practice, a majority of holdings submit more than 15 samples per quarter and therefore in these herds the estimate of *Salmonella* has small confidence intervals.

The current sampling scheme is not a sensitive method of detecting true changes in herd prevalence, and reducing the number of samples submitted would reduce this ability further. Using a rolling average of samples collected per quarter improves the sensitivity of the current sampling scheme, but herds with a prevalence close to each ZAP score cut-off are likely to change ZAP score from one month to the next without any true underlying change in *Salmonella* prevalence. However, the ZAP scheme is intended to identify those farms with persistently high levels of infection, and taking 2 rather than 3 samples per batch would not change the conclusion that herds with a ZAP 3 score for 11 consecutive months are likely to have a high MJE prevalence.

**References**


