ZAP: The role of routine surveillance data in understanding the geography and timing of Salmonella on UK pig farms.

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Abstract

The Zoonoses Action Plan (ZAP), at its inception in 2002, sought to reduce prevalence of Salmonella infection in quality assured pigs at slaughter by 25% within three years. Salmonella levels are monitored by Meat Juice ELISA tests on samples from individual pigs and aggregated to indicate farm-level Salmonella status. By combining the ZAP scheme and quality assurance scheme datasets we generated a large geographically referenced data set which allows us to investigate aspects of the spatial and temporal epidemiology of Salmonella on GB pig farms. We seek in this study to address two questions. First, is there evidence that Salmonella in GB pigs varies seasonally? Secondly, do close farms tend to have similar levels of Salmonella? We suggest explanations for spatial and temporal effects where evidenced. Knowledge of seasons or GB regions which have atypically high Salmonella risk informs the design of control strategies.

Geostatistical and statistical modelling techniques provide evidence that (i) farm Salmonella status varies by season, with a peak in Autumn and early Spring and (ii) farm Salmonella burden is spatially structured, having allowed for seasonality and all currently available explanatory variables. This latter may indicate a true spatial effect, or may reflect as yet unmeasured explanatory variables. These latter are currently being collected.

Introduction

The Zoonoses Action Plan (ZAP) Salmonella monitoring scheme was introduced by the British Pig Executive in 2002. It sought to reduce prevalence of Salmonella infection in pigs slaughtered at British Quality Assured Pork (BQAP) abattoirs by 25% within three years. Farms which wish to sell their meat through BQAP abattoirs must participate in ZAP. This initiative supports a target set by the UK Food Standards Agency to reduce human food-borne infectious intestinal disease by 20% by 2006 and to reduce Salmonella infection in pigs by 50% by 2010.

Previous epidemiological studies have identified factors associated with increased levels of Salmonella on-farm, for example herd size (Mousing et al, 1997). We hypothesise a priori other risk factors for elevated Salmonella levels; amongst these is herd type (breeder/finisher or specialist finisher), and interest concerns whether finisher herds, experiencing a greater level of movement of pigs on-farm, might show higher Salmonella levels. Of further interest is whether, having taken account of explanatory variables, there remains any evidence of spatial (or temporal) structure – so that farms which are close together geographically (or measurements close in time) have inherently more similar Salmonella levels than those further apart. Evidence of this nature is valuable for designing control strategies which are efficiently targeted; an awareness of high-risk times of year, for example, is valuable for determining when resources should be targeted, and an enhanced knowledge of intrinsically high-risk regions is similarly informative. An understanding of spatial or temporal trends may also contribute to our general epidemiological understanding of this infection – clustering may indicate a common exposure, spread between neighbouring units, an effect of climate, or shared management practices.
Material and methods

The data consist of longitudinal records from two Government Office Regions; East of England, (Cambridgeshire, Norfolk, Suffolk, Essex, Hertfordshire and Bedfordshire) and Yorkshire and the Humber (North, South, East and West Yorkshire). These represent the two highest density pig farming regions in the UK and we are interested in any similarities (and discrepancies) between them. ZAP scores are assigned on the basis of number of samples positive according to a mix-ELISA which detects antibodies against Group B and C1 Salmonella in meat juice samples collected from every batch of pigs slaughtered in BQAP abattoirs. The MJ ELISA results are indicative of levels of circulating antibodies, not of infection and thus, peak exposure to Salmonella infection on farm will have preceded the peak ELISA result noted in the abattoir. Antibody levels are not well correlated with shedding so the peak risk of Salmonella shedding from pigs at the abattoir may precede or co-incide with the peak of antibody positives. However, knowledge of both associations between ELISA levels and risk factors and an understanding of inherent spatial and temporal variation is useful for interpreting this surveillance data and informing a targeted approach to monitoring and control in the future.

Previous studies of these data (Clough et al., submitted) aggregated the data by year, fitted a common mean prevalence of MJ-ELISA positive pigs to all farms in a given year in a given region using a generalized linear model; constructed residuals (observation minus fitted mean) quantifying how different each farm was from the common average; farm-level residuals were then investigated using the variogram (Cressie, 1993) for any evidence of spatial structure, so that farms which are close together have more similar residuals (unexplained variation) than those far apart. Spatial structure is common in such applications and may indicate shared risk factors, either spatially (e.g. weather, topography) or non-spatially structured (farms in the same region may be of similar types (for example, all outdoor herds) or may share common management practices), or of transmission. Some evidence of spatial structure in the residuals from farms in the East of England was discovered, warranting the development of a model-based approach (described fully in Sanderson (2005) and being prepared for publication).

For each sample, several variables (MJ-ELISA concentration, date of sample, holding number, holding location, breeder/finisher status of holding) are recorded. The outcome is MJ-ELISA concentration; predictors are seasonal effects (modelled using smooth terms representing 12-month and 6-month cycles) and finisher status. We take a three-tiered approach.

- First, we fit a linear regression model (model 1) to the MJ-ELISA test result data to search for preliminary evidence of either seasonality or an association with breeder/finisher status. The MJ-ELISA data were logged to assist in analysis. This simple approach ignores both the geographical element and the fact that there are repeated measurements at each holding.

- Secondly, we extend the first approach to reflect the fact that multiple observations from the same premises are present and hence that our observed sample results may not be independent. We achieve this by incorporating a holding-level random effect, which is a standard statistical approach for any kind of clustering (model 2).

- Thirdly, we extend model 2 to include a spatial component (model 3). Our approach had to take into account three aspects of our data: time, location and multiple observations at each location. We had to consider time and location together and we had to consider that farms close together might still be more similar than expected by chance. These added complexities preclude the use of many published spatial statistics approaches. Consequently, we designed a method which included a spatially-varying random effect term at the same time as the farm-level random effect. We do not describe the spatial methodology in detail; description of the statistical approaches is in preparation for publication in a statistical journal.

- In summary, our novel approach to the data allowed us to analyse spatial, temporal and farm-level effects whilst taking account of clustering and repeated measures.

All analyses are implemented in the statistical package R (http://www.r-project.org) and using the libraries geoR (Ribeiro and Diggle, 2001) and lme4 (Bates and Sarkar, 2007).
Results

There was evidence of a seasonal component to *Salmonella* levels from all three modelling approaches, in both the East of England and Yorkshire and Humber regions. In most models, a primary peak in September with a secondary peak in February were evidenced in the East of England, with similar peaks occurring marginally later (October and March) in Yorkshire and Humber (Fig. 1 shows seasonal components from model 2).

**Figure 1:** Seasonal components of models for *Salmonella* levels in East of England and Yorkshire and Humber

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There was a suggestion of an effect of being a breeder farm by comparison with finishers; breeders have lower infection levels (from model 2, in East of England $\hat{\beta}_{\text{breeder}} = -0.2389$ (SE = 0.0830); in Yorkshire and Humber $\hat{\beta}_{\text{breeder}} = -0.2716$ (SE = 0.0710)). Note that the analysis at this stage does not consider confounders; we discuss their possible effects below.

Having taken account of seasonality and breeder/finisher effect, there was some preliminary evidence of a differential spatial component to the distribution of *Salmonella* levels; in the East of England, for a given separation, farms were likely to have more similar intrinsic *Salmonella* levels than farms in Yorkshire and Humber separated by the same distance.

Having identified a component of spatial variation, we assess relatively the amount of unexplained between-farm variation which can be explained by spatial structure, by comparing the variance of spatial and non-spatial farm-level random effect terms. Spatial variation was small by comparison with farm-level non-spatial variation, indicating that unexplained spatial variation, though present, has a limited role to play in explaining total variability by comparison with other sources. The largest source of variation is between samples over time.

**Table 1:** Contributions to variance in *Salmonella* levels in the two regions, having controlled for seasonality and farm type. $\sigma^2 = $ between-animal within-farm (non-spatial); $\sigma^2 = $ between-farm (non-spatial); $\sigma^2 = $ between-farm (spatial).

<table>
<thead>
<tr>
<th>Region</th>
<th>$\sigma^2$</th>
<th>$\sigma^2$</th>
<th>$\sigma^2$</th>
<th>Ratio ($\sigma^2 / \sigma^2$)</th>
</tr>
</thead>
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<td>1.3567</td>
<td>0.6370</td>
<td>0.4084</td>
<td>2.4343</td>
</tr>
<tr>
<td>Yorkshire &amp; Humber</td>
<td>1.3884</td>
<td>0.5984</td>
<td>0.3504</td>
<td>2.9161</td>
</tr>
</tbody>
</table>

Discussion

Our analysis has provided a suggestion of a seasonal component to the behaviour of *Salmonella* levels on UK pig farms, with the most pronounced peak in Autumn and a secondary peak in late Winter/early Spring. This finding of a double peak is in support of previous findings of Hald and Anderson (2001) in Denmark, but only the Autumn peak is consistent across studies. Note that this
work has been conducted with only two years’ data so that conclusions regarding seasonality, and the secondary peak in particular, are tentative. Studies are ongoing which use a longer data series; these will clarify the seasonal profile.

It is interesting that farms which had atypically high intrinsic Salmonella levels (quantified as random effect values) were the same farms which had atypically high maximum-over-time-period ZAP scores via the ZAP categorisation scheme. Small discrepancies occurred in that two farms which were never awarded ZAP 3 scores achieved high random effects; these were farms from which small numbers of samples were taken and this disagreement therefore makes sense because a minimum number of sample requirement (15 samples in a given quarter) must be fulfilled before a ZAP score of 2 or 3 can be awarded. This demonstrates that our novel approach is useful for quantifying risk in the same broad terms as the ZAP scoring, but has the added advantage that it is taking into account factors such as seasonality, covariate effects and spatially varying factors before classifying farms as high risk.

There is evidence in both regions that breeder-finisher herds experience lower Parvovirus than specialist finishing herds, which purchase weaners from one or more breeding unit. One explanation is that breeding herds see less animal movement; since a major route of Salmonella introduction is through intake of sub-clinically infected animals (Cook, 2004), the probability of introducing infection into a previously uninfected herd will increase with the number of animals introduced. For breeding herds, the population is more self-contained and so a lower risk of infection might be expected. Further studies explicitly examining pig movement could strengthen this hypothesis. The matter is complicated further by possible confounding via associations between herd health (including PMWS), finisher status, herd size and whether any part of the production cycle was maintained outdoors. There is a theoretical (though as yet unreported) possibility that this effect is further confounded with herd size. For example, if finisher herds were larger, more samples would be taken from these herds which would hence have a greater probability of testing positive when prevalence is non-zero. These problems can be alleviated by incorporation of relevant covariates.

An important finding is that the variation between animals within farms over time contributed to the greatest extent to the farm level non-spatial variation; batches of pigs from the same farm at different times may have a different prevalence of MJ ELISA positive pigs, indicating that sampling multiple animals per batch is warranted.

Collection of more information about confounders and explanatory variables is ongoing, and these will be incorporated into the models, enhancing understanding further. Outputs will feed into risk assessment models; insight provided by our novel approaches represents an important step toward a fully integrated risk-based approach to the control of Salmonella on UK pig farms.

References

BATES, D. AND SARKAR, D., 2007. lme4: Linear mixed-effects models using S4 classes.


