Analysis of Meat Juice ELISA results and questionnaire data to investigate farm-level risk factors for Salmonella infection in UK pigs

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Abstract
The study used exposure data, collected from pig quality assurance schemes and a postal questionnaire, and serology results from ongoing abattoir Salmonella surveillance, to complete a multivariable risk factor analysis on a large number of farms with a comprehensive set of potential risk and protective factors. The results highlighted that seasonal factors, such as temporal cycles and meteorology were associated with Salmonella infection. Two feed variables, farm type, number of annual pig deliveries and dead stock collections, locality, and main cause of death on the farm were also found to be significantly associated with Salmonella infection.

Introduction
Salmonella infection in pigs can cause a range of clinical signs, from scouring to fever, and death, although infection is often sub-clinical. The prevalence of Salmonella in pig caecal samples, collected for a large abattoir study in Great Britain, was high (23.4% (19.9-27.3)), when compared to both cattle and sheep (1.4% and 1.1% respectively) (10). Salmonella is an important foodborne pathogen for the human population, with 13,213 laboratory confirmed cases of salmonellosis in the United Kingdom (UK) identified in 2007, with 13% of these human cases infected with the serovar S. Typhimurium, which is the predominant type detected in samples from UK pigs (3; 19).

Many studies have tried to ascertain the factors that influence Salmonella prevalence and identify on-farm controls, to reduce the Salmonella burden in pigs. However, some of these studies were limited to a small and potentially unrepresentative subset of the pig farm population, whilst others analysed only a small number of variables and may not have had sufficient statistical power to detect modest associations between Salmonella infection and putative risk factors.

The UK Zoonoses Action Plan (ZAP) monitoring programme was designed to run in conjunction with Quality Assurance schemes (QAS), to estimate the burden of Salmonella from a sample of slaughtered pigs. Farms that had a prevalence of more than 50% meat juice (MJ) ELISA positive pigs were required to implement an action plan or face eventual loss of their QAS status, to motivate pig farmers to develop Salmonella control plans. This paper reports how data from QAS, ZAP and a postal questionnaire were used to examine relationships between Salmonella seroprevalence results and farm characteristics on a large population of pig farms, to identify risk factors.

Method
A questionnaire was designed to collect a large number of covariates including pig stocking levels; feeding practices; housing systems; biosecurity and geographical location, to supplement those routinely collected by the QAS. The questionnaire was posted to all 2,064 farms listed under three QAS:- Approved British Pigs (ABP); Genesis Quality Assured (GQA); and Quality Meat Scotland (QMS). Monthly regional summaries of meteorological data, including actual and anomaly (difference from long-term averages) records, were downloaded (http://www.metoffice.gov.ukclimateukindex.html) and linked to the dataset by the region of farm and the month of sample collection.

The ZAP data were limited to results collected up to four years prior to the postal questionnaire completion date, to maximise the amount of holdings included in the study population and to allow a comparison of temporal trends over a number of years. For the ZAP scheme, small pieces of skeletal muscle were removed from pigs at the abattoir and placed in a MJ tube which was frozen and then (Placha, Venglovskythawed to collect the fluid. The MJ sample was tested by a mix-ELISA serological
test for a “host” response of antibodies to Salmonella (28) which produces a sample positive ratio (41) that correlates to the titre of circulating antibodies. Samples were categorised as positive in the ZAP scheme according to a defined cut-off of 0.25.

All associations were analysed by univariable mixed linear regression (STATA 10, Stata corp., college station, Tx), with the farm holding identifier selected as the random effect, to test for associations between the logged ELISA ratio (response) and explanatory variables. Explanatory variables with a p-value of 0.25 or more were omitted from the forward stepwise multivariable model. The variable with the lowest (most significant) p-value was entered first into the model, and each subsequent variable was then independently introduced into this model to select the next variable with the lowest p-value. This method continued until no further variables could be added with a p-value under 0.01. Records with missing data for the selected variables were dropped from the model. All rejected variables were added separately into the final model to ensure no significant variables had been omitted. Likelihood ratio tests were used to compare models of the same population size to determine whether the included variable significantly improved the model. The standardised residuals were plotted against the fitted values to examine signs of heterogeneity and non-normality. If explanatory variables included in the model were correlated then the least significant variable was dropped.

Results
A total of 566 questionnaires were returned, and could be linked to the ZAP database, these consisted of 305 ABP, 171 GQA and 90 QMS holdings. The holdings linked to a total of 119,906 ZAP samples (mean 224, range 1-1,671). The results of the linear regression model are presented in Table 1.

Table 1: Multivariable mixed linear regression of logged meat juice ELISA results collected from slaughtered pigs (N=109,912 samples (474 holdings))

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region: Scotland</td>
<td>-0.747</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pig farm density within 10km radius</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Conventional farm enterprise</td>
<td>-0.518</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primary cause of pig deaths in the last 12 months - respiratory or wasting</td>
<td>0.290</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean temperature anomaly (°C)</td>
<td>0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rainfall actual (mm)</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sunshine actual (hours)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Finishers fed homemix</td>
<td>-0.377</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of barley in grower feed</td>
<td>-0.007</td>
<td>0.003</td>
</tr>
<tr>
<td>Pig deliveries - 6-11 year</td>
<td>0.439</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- &gt;11 year</td>
<td>0.289</td>
<td>0.001</td>
</tr>
<tr>
<td>Collection of dead stock - &gt;6/year</td>
<td>0.245</td>
<td>0.007</td>
</tr>
<tr>
<td>Yearly cycle - cos</td>
<td>-0.100</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- sin</td>
<td>0.042</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quarterly cycle - cos</td>
<td>-0.046</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- sin</td>
<td>-0.041</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>constant</td>
<td>-2.866</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion
In total, over a quarter (27%) of the QAS population participated in the study and on average each holding was linked to over two hundred ZAP samples, providing a large dataset for analysis. In the final model both yearly and quarterly cycles were found to be significant and improved the final model, with the highest mean S.P ratio in autumn and the lowest in spring. Large differences to long term averages in the mean temperature, and high actual rainfall and hours of sunshine were identified as risk factors. These results agree with a previous study which presented increased temperature variability as associated with Salmonella prevalence (17). Air temperature has been linked to pig stress, which in turn can increase the shedding of Salmonella and can lower immunity (20). The meteorological results came from monthly...
averages from weather stations within each of the regions, whereas the temporal cycles may represent the influence of specific local or daily weather conditions.

The selected spatial factors showed that farms in Scotland have a lower prevalence of *Salmonella* possibly because the farms in Scotland are more likely to use certain management procedures (e.g. all indoor production; home-mixing) and, due to their geographical isolation, are more likely to purchase similarly low prevalence animals from Scottish farms. Location and farm density were identified by another study of UK pig *Salmonella*, which noted that the “type, number and density of pig holdings in a two kilometre radius is crucial” (34).

It has been described in other studies that health conditions, especially respiratory and wasting diseases such as PRRS and PMWS, have a synergistic relationship with *Salmonella*, either by lowering the immune system or increasing transmission by sneezing or shedding *Salmonella* in larger numbers and for a longer period of time, and this relationship was also identified in the model (39)16; 20; 2; (3). A larger number of pig deliveries was also shown to be a risk, and the introduction of pigs onto a farm was agreed to be the most likely cause of pig infection by a panel of international experts (42). A larger number of deliveries may indicate a larger number of suppliers, which has been shown to be a risk when farms recruit pigs from more than three herds in comparison to herds that breed their own replacements or recruit from a maximum of three herds (24). A higher number of dead stock collections might indicate that the farms have a greater amount of health condition problems, possibly caused by *Salmonella* or from health conditions associated with *Salmonella* infection.

Being a conventional pig enterprise was found to be protective, and this may be because the other types of enterprise (organic, freedom foods) utilise a higher degree of outdoors production and a decreased use of antibiotics, and these enterprises have been shown to have significantly higher *Salmonella* seroprevalence in pigs (18). Procedures to control *Salmonella* transmission which are used in indoor production are harder to implement outdoors and so the pigs may be at an increased risk of infection from wildlife and the environment.

Feed has been identified in numerous studies as a factor that influences *Salmonella* infection. Specific feed types can disrupt the microbial ecosystem in the gut, especially feed with a high level of acid, which can inhibit *Salmonella* and encourages gram-positive bacteria which favour acidic environments and can out-compete *Salmonella* (12) (32); 9). The use of feedmill, rather than that mixed on farm, was a significant unvariable risk factor for *Salmonella* in another study (36). The use of other feed types, such as a higher percentage of barley in the diet fed to growers, was found to be protective, which concurs with the findings from other studies (7; 8).

Collecting information from only one time point for each holding may have introduced error into the analysis as, the management of the farm may have changed in the four year period from which samples were collected. The four year period was decided upon to provide a suitable dataset to analyse the temporal variation in the data, but an improvement to this study design would be to collect data on any changes to the farm over the period. Utilising a study population drawn from the QAS may also have biased the results, as although the QAS are believed to contain around 50% of all the pig holdings and 90% of the pigs in the UK, it is unknown whether the farms are representational of the remaining farms. Anecdotal evidence suggests that non-assured farms are more likely to be smaller, non-conventional holdings. The use of serological samples from the ZAP study also provided some issues, as the results represent historical, rather than current, infection of Salmonella and this may have caused bias, particularly with the temporal results. However, studies have compared Salmonella serological results and caecal content results and although the serology results fluctuated between visits to the same farm, the serology and caecal results were significantly correlated (35).
Conclusion
The study provided a comprehensive risk factor analysis and examination of the spatial and temporal trends of Salmonella prevalence, with a study population large enough to detect even weak associations. The utilisation of data collected routinely via the QAS and ZAP schemes, as well as a one-off postal questionnaire allows a cost effective means to produce a large scale risk factor study.

References