Studies to investigate factors that affect pig *Salmonella* prevalence in the United Kingdom

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By

Richard Piers Smith

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Abstract

Salmonella is the second most common human zoonosis in the UK, and infection is usually through foodborne routes or contact with contaminated faeces. The high prevalence (~20%) in pigs highlights their importance as reservoirs of *Salmonella*, especially when compared to cattle and sheep (~1%). Although studies have identified factors that would influence *Salmonella* control, further evidence was needed to understand pig *Salmonella* and provide advice specific for control in the UK. This thesis describes the use of two large datasets from an abattoir-based serological surveillance scheme and Quality Assurance schemes to answer a number of research questions.

The datasets were first described and analysed by a number of methods, to evaluate their usefulness for analysis and to indicate potential analytical approaches. Covariates, identified by a literature review, were missing from the Quality Assurance data but initial results showed farm location and the flooring used for finisher pigs, were associated with higher seroprevalence. Significant spatial clustering of high prevalence farms was also detected, as was regional differences in farm management.

A questionnaire was used to collect important missing data. Subsequent epidemiological modelling, using this comprehensive list of farm characteristics, highlighted that temporal factors (quarterly and yearly cycles), farm location, pig farm density, meteorological variables, health conditions, specific vehicle deliveries, feed types and farm enterprise type were associated with *Salmonella* presence ($P<0.01$).

A further study utilised a number of spatial techniques to examine and describe spatial heterogeneity in Britain and Northern Ireland and define the temporal trends in *Salmonella* seroprevalence. The adaptation of a geostatistical approach showed that the addition of the covariates identified in the epidemiological model accounted for the localised clustering of farm seroprevalence results.

Pig movement connections between farms were assessed to determine the interconnectivity of the pig farm network. This was the first description of the British pig movement network and data were also collected on the use of abattoirs and livestock hauliers. The network displayed high clustering and short network distances between farms, indicating that *Salmonella* might transmit quickly amongst farms but within clusters of farms. Differences in the connections between regions were detected. Farms belonging to large companies were shown to have mostly movements within that company, although movements to small companies connected large companies. This structure and the occurrence of multiple indirect routes between many pairs of farms indicates that targeting surveillance and control within companies or on farms with high network centrality characteristics would not prove effective.

The findings of the analyses are discussed in light of recommendations for control and surveillance procedures, as well as giving evidence on the effectiveness of the types of analysis and the use of the large datasets, as well as providing recommendations for further work.

Table of contents

Tables

Figures

Appendices

Chapter 2
Appendix A: Review of QA data

Chapter 3
Appendix A: Pig farm biosecurity survey questionnaire
Appendix B: Pig farm biosecurity questionnaire assessment form
Appendix C: Enrolment letter to private veterinary surgeons
Appendix D: Enrolment supporting letter from ABP
Appendix E: Enrolment supporting letter from GQA
Appendix F: Farmer enrolment letter
Appendix G: Pig farm company enrolment letter
Appendix H: Univariable model results

Chapter 4
Appendix A: Regional K-function plots

Glossary

A number of specific terms have been used throughout this thesis and the definitions of the terms are explained below:

ABP	Assured British Pigs
BPEx	British Pig Executive
CERA	Centre of Epidemiology and Risk Analysis
CPH	County Parish Holding farm identifier
Defra	Department for Environment, Food and Rural Affairs
EFSA	European Food Safety Authority
EU	The European Union
Fattener	Fattener – see finisher
Finisher	Market age pigs, approximately 15+ weeks old or 50-110kg.
Farrow-to-finish farm	Pig farming system that breeds pigs on-farm and keeps them until they are sent to slaughter. Other farrowing types of farm are farrow-to-grower and farrow-to-weaner.
Finisher farm	A farm that has no breeding stock and brings grower pigs onto the farm to finish and send to slaughter. Finisher farms are at the bottom of the breeding pyramid.
FSA	Food Standards Agency
Gilt	An immature female pig which has farrowed fewer than two litters
GB	Great Britain
GQA	Genesis Quality Assurance
Grower pig	Approximately 11-14 weeks old or 30-50kg.
Herdmark	A unique alphanumeric code that specifies a farm site and is the official individual reference for a holding (Defra, PRIMO rules).
Multiplier farm	Breeding farms receiving grandparent breeding stock from nucleus farms and producing progeny which are sent as parent breeding stock to commercial breeding farms.
Nucleus farm	Pig breeding farms at the top of the breeding pyramid with great grandparent pedigree stock that produce pigs (grandparent stock) of a particular beneficial genetic type.

NUTS	Nomenclature of Units for Territorial Statistics
QAS	Quality Assurance Scheme
QMS	Quality Meat Scotland
Slapmark	A tattoo of the herdmark (see above) on each shoulder of a pig, used to identify a pig holding at the abattoir.
UK	United Kingdom
VLA	Veterinary Laboratories Agency, now called Animal Health and Veterinary Laboratories Agency (AHVLA)
Weaner pig	Approximately 3-10 weeks old or 8-30 kg.
ZAP	Zoonoses Action Plan
ZNCP	Zoonoses National Control Plan

Introduction and overall aim

Salmonella, the 2nd most prevalent zoonotic infection of people in the United Kingdom (UK), has caused on average 12,865 reported human cases each year over the last decade (2000-2010) in England and Wales alone (HPA, 2010). However, the true number of human cases is estimated to be much larger, with five cases of *Salmonella* occurring for every one reported to national surveillance (FSA, 2011). The coincidence of temporal outbreaks in people and pigs, and similarities of the genetic consistency of the *Salmonella* types detected, have provided evidence that eating pig meat, as well as coming into contact with pig faeces, can cause human infection.

Salmonella control in pigs has been a particularly problematic task as infection in pigs is often subclinical and farmers have had little motivation to carry out interventions, especially in light of the prominence of other infectious agents that are of more direct relevance to their business (e.g. enzootic pneumonia, swine dysentery). Another factor contributing to the apparent lack of motivation for on-farm control is the need for strong, consistent scientific evidence of factors that may reduce *Salmonella* prevalence on UK pig farms and how these could be enacted (PVS, 2008). Furthermore, there was a belief within the industry that there was little that could be completed on farms that would impact upon *Salmonella* prevalence and that the responsibly for applying controls was shared with slaughterhouses, processing plants and the consumer (VLA, 2011). However, as part of the European Union Zoonoses Regulation (EC) No. 2160/2003, Defra will be required to organise a national control plan for the control of *Salmonella* in pigs, which is expected to start in 2015, in line with other European member states. The British pig industry and Defra are keen to make an impact in reducing the prevalence of *Salmonella* in pigs before European Union limits become compulsory.

The pressing need to provide strong scientific evidence on how to control *Salmonella* on pig farms has coincided with the advent of Quality Assurance schemes for pig farms that collect a range of data on farm structure and management. The schemes cover a large proportion of the professional pig farms in the UK and each member farm that sends pigs to slaughter also participates in a scheme to monitor *Salmonella* prevalence by taking serological samples at the abattoir. These datasets had not previously been used for a detailed epidemiological risk factor analysis and they represented an opportunity to design a set of novel and rigorous analyses of pig *Salmonella* on a dataset that was both representative of the professional UK pig farm population and provided high statistical power to the analysis (i.e. less likely to falsely fail to reject a null hypothesis).

This study was designed to gather the current knowledge on on-farm controls for *Salmonella* on pig farms and provide further evidence for control by testing hypotheses

against a large study population. The study also examined *Salmonella* with regards to the structure of the UK pig industry, to analyse whether any particular areas were at particular risk or where specific control or surveillance could be targeted.

The chapters in this thesis will provide:

1) A review of the symptoms and prevalence of *Salmonella* and transmission routes between pigs and people, as well as a description of the current *Salmonella* pig surveillance system and an evaluation of the test that it utilises;
2) A review of the current knowledge on the potential risk factors for *Salmonella*, from countries with similar farming methods, to highlight the areas of interest that would need to be analysed in this study;
3) The evaluation of the suitability of the Quality Assurance scheme and *Salmonella* surveillance system data for epidemiological analysis;
4) A thorough multivariable risk factor analysis, using a large study population and a comprehensive list of potentially associated variables, to identify the key explanatory variables associated with *Salmonella* prevalence;
5) An analysis to examine the spatial and temporal trends of *Salmonella* prevalence, to examine if these could provide further advice to control strategies;
6) A network analysis to test whether specific farms, or types of farms, may be of particular importance to spreading infection via direct and indirect methods, and to examine the whole network structure of pig farms to highlight which control strategies would be beneficial to this structure.

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Chapter 1: Literature Review

1.1 Introduction

A review of literature was completed to explore the current understanding of the epidemiology of *Salmonella* infection in pigs. The first report (Chapter 1.2) explains the importance of pig *Salmonella* to farming and to human infection, and describes the evidence towards the attribution of human disease from pigs. The report also reviews the current pig *Salmonella* surveillance system in the UK. The type of sample test used for surveillance is described in comparison with other surveillance options, and an understanding of the implications of the test results are described, to evaluate the usefulness of utilising these surveillance data as the *Salmonella* outcome in this study. The report highlights that the serology results from the UK abattoir surveillance scheme would provide an adequate estimate of farm prevalence that would cover a large population of pig farms, although this would not cover small holdings or breeding farms that did not send pigs to slaughter.

A structured literature review was completed to gather the current knowledge on the farm- and animal-level factors that have been identified as associated with *Salmonella* in pigs and were applicable to the UK pig industry (Chapter 1.3). The review evaluated the evidence from a large number of studies and listed eight main topic areas that were associated with *Salmonella* in pigs that would need to be accounted for in any modelling analysis covered in this thesis.

1.2 *Salmonella* and *Salmonella* surveillance

1.2.1 *Salmonella* serovars and human and animal prevalence

Salmonella are rod-shaped, flagellated, aerobic, Gram-negative bacteria belonging to two major species: *S. enterica* and *S. bongori*. Strains can be classified into more than 2,600 different serovars recognised in the Kauffmann-White-Le Minor scheme based on diversity of lipopolysaccharide O antigens and flagellar protein H antigens (Grimont and Weill, 2007Murray et al., 1995). *Salmonella* are classified into serogroups by their O antigen (from A to Z).

Salmonella is an important human pathogen in the UK. At the initiation of this research in 2007, the number of laboratory confirmed human cases of salmonellosis in the UK was 13,213 and salmonellosis was the second most common type of human foodborne illness after *Campylobacter* (57,590) (Defra, 2007). However, by 2011 the number of human cases had dropped to 9,455, whereas campylobacteriosis had risen to 72,150 human cases (Defra, 2012). Actual human case numbers were estimated to be five times higher, due to the number of unreported infections (FSA, 2011). A subset of *Salmonella* serovars has been linked to human illness, which comprise around 1,400 members of *S. enterica* sub-species *enterica*. The majority of these cases are attributed to *Salmonella enterica* subspecies *enterica* serovar Enteritidis (52.5%) and *Salmonella enterica* subspecies *enterica* serovar Typhimurium (13.8%) (HPA, 2010a). According to convention these names are shortened to *S.* Enteritis and *S.* Typhimurium respectively (Popoff, 2001). These zoonotic *Salmonella* are carried by a large variety of farmed livestock, avian species and mammalian wildlife, as well as a number of common and exotic pet animals, such as dogs and reptiles (Geue and Loschner, 2002; Simpson, 2002; VLA, 2007). Human infection is believed to be acquired predominantly from ingesting uncooked/ under-cooked food containing *Salmonella,* but also from cross-contamination, contamination after cooking and from contact with contaminated faeces from an infected person or animals (HPA, 2010b). Human cases of salmonellosis have diarrhoea, fever, abdominal cramps and vomiting. Typhoidal *Salmonella* is a much rarer form and is transmitted from human-to-human via faecal contact, causing typhoid fever. The importance of salmonellosis was evidenced by the calculation for Disability Adjusted Life-Years, which provides a score for the burden of disease, accounting for mortality, disability and the effects of ill-health, In a Dutch study, salmonellosis scored 1,600/100,000 disability adjusted life-years, whereas tuberculosis has been scored as low as 5 (EFSA, 2010; Haagsma et al. 2010).

British surveillance data shows that *S.* Enteritis was associated with infection in poultry, with the highest percentage (18%) of poultry infections being *S.* Enteritis, whereas *S.*

Typhimurium is associated (69.7%) with infection in pigs (VLA, 2007). *S.* Typhimurium has been a human pathogen of significant interest in the UK, particularly since the epidemic of multi-resistant *S.* Typhimurium DT104 in the mid to late 1990's (Threlfall, 2000). Additionally, monophasic *S.* Typhimurium (defined as lacking the second phase H antigen) has rapidly increased in prevalence in humans and in pigs, cattle and poultry since its emergence two decades ago (EFSA, 2012). There has been little conclusive evidence of the attribution of human *S.* Typhimurium infection. However, a number of *S.* Typhimurium 'phagetypes (U288, U308a, U310, DT193 and DT208) have been shown to be particularly associated with infection in pigs and subtyping analysis of isolates from people and pigs showed that both had similar VNTR (Variable Number of Tandem Repeats analysis) alleles, especially for U288 isolates, which would indicate that *Salmonella* infection in humans could be attributed to pig reservoirs (Kirchner et al., 2007; Kirchner et al., 2011). An EFSA source attribution study, using microbial subtyping data, estimated that EU human salmonellosis was mostly attributed to the laying hen reservoir (43.8%) followed by pigs (26.9%) (EFSA, 2011a). However, it is believed that the importance of pigs has increased recently due to the control of *Salmonella* in poultry. Other evidence of the possible importance of pig *Salmonella* infection for human illness was shown by the seasonal variation of the prevalence in pork and human incidence, which was shown to be similar in studies conducted in Denmark and Scotland, and a significant risk factor for human illness was the prevalence in pork sampled 4 to 5 weeks before human case registration (Hald and Andersen, 2001; Smith-Palmer et al., 2003).

Prior to the start of these studies, the prevalence of *Salmonella* in British pigs had been shown to be 23.4% (19.9-27.3) from 2,060 slaughter pig caecal samples collected in a randomised, structured abattoir study completed in 2003 (Milnes et al., 2008). This was significantly higher than the prevalence of *Salmonella* in either cattle (1.4%) or sheep (1.1%) and was consistent with a similar British study completed in 1999 (Davies et al., 2004). Of the positive samples collected in 2003, 55% were infected with S. Typhimurium (Teale, Milnes and Stewart, 2005). A risk assessment model of *Salmonella* dynamics in slaughter age pigs predicted that 17% of pigs on a farm would be infected, but with wide variation (from 0-50%), and approximately 4% would be excreting (Hill et al., 2008). In other areas of Europe, a risk assessment model found that "about two thirds of all Dutch pig farms are more or less permanently infected" (Berends et al., 1996), whereas the prevalence of *S.* Typhimurium on 96 randomly selected Danish pig farms was 14.6%, and 8.3% for other *Salmonella* (Stege et al., 2000). A small longitudinal study in the United States (US) found that the presence of multiple serotypes of *Salmonella* may be common in finishing units, possibly due to multiple sources of infection (Davies, Funk and Morrow, 1999). *Salmonella* infection in pigs can cause fever, scouring, unsteady gait and vomiting. However, infection is usually subclinical, making the detection of infected pigs on the farm difficult.

Since the start of the studies described in this thesis, a Europe-wide baseline survey for *Salmonella* in slaughtered pigs has confirmed the high prevalence of *Salmonella* (21.2%, 5th highest out of 25 Member States) in the lymph nodes of UK slaughter pigs compared with other Member States (average of 10.3%) (EFSA, 2008). Carcass contamination was also high (13.5%) when compared to the average of 13 Member States (8.3%). A similar EU-wide baseline survey of *Salmonella* in breeding pigs, using freshly voided faeces collected from pens, found that 52.2% of farms were positive in the UK (4th highest out of 26 countries) (EFSA, 2011b). A significant positive correction was found between the prevalence of breeding farms and finisher farms from each member state, indicating the importance of breeding farms in disseminating *Salmonella* through the pig production pyramid. The correlation was significant for *S.* Typhimurium, *S.* Derby and a grouping of the other serovars. This finding may highlight the importance breeding farms have in introducing novel strains to naïve farms lower down the pyramid, where low herd immunity to the incoming *Salmonella* strain can result in a large increase in prevalence.

1.2.2 Pig *Salmonella* surveillance

Salmonella is a reportable organism in Great Britain when isolated from a statutory species (including pigs), their environment and animal feed, as covered by the 1989 Zoonoses Order. The Government is required "to take effective measures to detect and control salmonellas of public health significance in specified animal species at all stages of production" (Defra, 2010). The Government works towards *Salmonella* surveillance and control alongside a number of agencies. The role of the British Pig Executive (BPEx) is to promote the pig industry and pig meat products, and to represent pig levy payers in England. BPEx introduced the Zoonoses Action Plan (ZAP) *Salmonella* Monitoring Programme in June 2002 for pigs supplied to British Quality Assured Pork (BQAP) and Scottish Guild abattoirs. The scheme was a component of the Food Standards Agency's target of reducing pig *Salmonella* in the UK by 50% by 2010, to protect the public's health by ensuring that consumers have a safe supply of food (FSA, 2005). The scheme was terminated in July 2012 as a result of failure to stimulate improvements in *Salmonella* control.

ZAP was based on the Danish surveillance system that utilised serology results from pigs sent to slaughter to categorise pig farms by *Salmonella* prevalence and encourage farms with a relatively high prevalence to carry out interventions. The Danish scheme was designed in 1995 (Mousing et al., 1997), and had contributed to a reduced prevalence of *Salmonella* in pigs. This reduction, along with controls introduced for the reduction of *Salmonella* in poultry, had contributed towards a saving of $25.5 million in the estimated cost of human infection (cost of lost labour and of laboratory tests and hospitalisation) as determined by cost-benefit analysis (Nielsen et al., 2001; Alban, Stege and Dahl, 2002; Wegener et al., 2003).

The meat juice ELISA

The ZAP scheme utilised an Enzyme Linked Immuno-Sorbent Assay (ELISA) to test meat juice (MJ) samples, collected from pig carcasses at the abattoir, for the presence of antibodies against *Salmonella*. The MJ ELISA was developed in Denmark for its *Salmonella* control programme and it has become a *de facto* standard, and is being used in schemes in Ireland, the Netherlands and Germany, as well as in some parts of the US, although different test methodologies lead to incomparable result between countries.

Small pieces (~2cm square) of muscle from the diaphragm or neck were removed from a carcass and placed in a MJ tube. Collecting samples from the abattoir ensured that the random selection of pigs could be independent from the supplying farms. The ZAP scheme samples were then frozen and the MJ fluid from the thawed sample tested by a

mix-ELISA serological test (Guildhay VETSIGN™Kit) at a single British private laboratory. The test measured a "host" response of antibodies to *Salmonella* infection.

During the ELISA, any *Salmonella* antibodies present in the MJ bind to antigens in the 'tube wall', whereas the remaining MJ contents are washed away. The next step in the ELISA is for the addition of an antiglobulin, which is chemically linked to an enzyme, which binds to the antibodies. When a substrate is added to the mixture, the bound enzyme reacts to create a colour change so that the intensity of the colour (optical density (OD)) is equivalent to the density of antibodies present in the MJ sample (Tizard, 2004). The OD of the sample and that from a negative and positive control are recorded, and the following formula is used to provide a sample:positive (S:P) ratio result for each sample:- (OD sample-OD negative sample)/(OD positive control – OD negative control). In the ZAP scheme, a S:P ratio cut-off point of 0.25 was set, so that samples found to have a ratio more than or equal to 0.25 were considered to be positive. The 0.25 cut-off is similar to a 40% optical density result (Hill et al., 2008). The intended purpose of the test is important in setting a cut-off and weighing up the impact on sensitivity (ability to detect true positives) and specificity (ability to detect true negatives). The Danish control programme originally used the 40% cut-off so that pig herds with a high prevalence of sero-reactors could be identified with minimal problems of false positive reactions, however, this was reduced in 2001 to 20% to improve sensitivity (Davies et al., 2001).

ZAP scheme ranking of farms

One muscle sample was collected from every batch of pigs sent to slaughter and additional samples were collected from that batch at a rate of one in fifty pigs thereafter (Armstrong, 2003). In May 2003, the sampling frame was defined as three samples to be randomly collected from each batch (BPEx, personal communication 2010). Each holding was required to submit 15 or more samples over a three month period, every year, to gain a ZAP score. All holdings that failed to comply with this were assigned a ZAP status of 0. The percentage of positive MJ samples from eligible holdings were categorised as follows: ZAP 1 <65%; ZAP 2 65% – 84%; ZAP 3 >84%. Producers given a ZAP 2 or 3 score were expected to develop an action plan to control the prevalence of *Salmonella* in their pigs, and holdings that persistently had ZAP scores of 2 or 3 were no longer eligible for Quality Assurance Scheme membership.

To reduce the cost of the scheme, the sampling frame was changed in August 2007 in England and Northern Ireland to collect one sample per month for units with a prevalence below 25% and five samples per month from units above 25%. Further amendments to the sampling and testing frame occurred to improve the efficiency of the surveillance. In April 2008, the sample result cut-off was changed to 0.10 S:P ratio and the ZAP score

categories changed to: ZAP 1 <50% of samples tested positive; ZAP 2 ≥50% and <75%; ZAP 3 ≥75%.

1.2.3 Evaluation of the use of MJ ELISA in Salmonella surveillance in pigs

Sensitivity and specificity of the MJ ELISA

The selection of antigenic markers, which relate to a range of *Salmonella* serovars, used in coating the ELISA plate is a factor affecting the sample test results. As the distribution of *Salmonella* serotypes throughout the world is heterogeneous, a testing method utilising one type of coating agent and validated in one country may not be suitable for all countries (van Der Heijden, 2001; Farzan, Friendship and Dewey, 2007). ELISAs may have differential abilities to detect infection by different serovars, and it is unknown if pigs infected with multiple serovars have significantly different infection characteristics and seroprevalence kinetics in comparison to pigs with a single serovar (van Winsen et al., 2001; Funk et al., 2005).

The ELISA used in the ZAP scheme detects antibodies to the majority of *Salmonella* serovars most prevalent in porcine infections, especially those associated with human foodborne salmonellosis (Chow et al., 2004). These include O-antigen 1, 4, 5, 6, 7, and 12 and group B and C serotypes, which have been shown to represent approximately 90% of *Salmonella* serotypes found in Danish pigs (Farzan, Friendship and Dewey, 2007), and contain serovars Typhimurium (group B), Heidelberg (B), Newport (C) and Braenderup (C). However, *S.* Enteritidis (a group D) which is "a major human pathogen and the number one serotype worldwide" is not detected by the ELISA and two other serovars that were in the top 10 human serovars, *S.* Hadar and *S.* Blockley, are likely to be poorly detected (Davies et al., 2003). A more recently compiled list of human serovars showed that *S.* Hadar and *S.* Blockley were no longer in the top 10 but were replaced by *S.* Newport, *S.* Kentucky and S. Typhi (serogroups C2, C3 and D respectively) which would also be likely to be poorly detected (VLA, 2010). A different ELISA kit, Herdchek ELISA designed by IDEXX laboratories, has been shown to detect B, C1 and also the D *Salmonella* serogroups, which may improve on *Salmonella* serosurveillance (Ballagi, Camitz and Holmquist, 2003). However, the antigenic markers selected for the ZAP ELISA were selected to provide a reasonable sensitivity and specificity of detecting the main serovars detected in pigs, and expanding the test to cover additional serovars may affect the test sensitivity and specificity. Data from surveillance activities in Great Britain indicate that the groups represented by the test should theoretically cover 77% of the pig salmonellas detected by the 2003 abattoir study and 86% of salmonellas detected in 2008 by routine Veterinary Laboratories Agency surveillance (Milnes et al., 2008; VLA, 2010).

As serological tests only detect a subset of known serovars they may miss new and emerging serovars outside the antigenic detection range. A further problem with assessing the results of an ELISA is the potential for false positives caused by the use of injectable vaccine (EFSA, 2004). However, at present, no *Salmonella* vaccine has been licensed for widespread use on pigs in Great Britain.

Recent infection may be a reason for false negatives to the ELISA, as IgM is the first immunoglobulin to appear after *Salmonella* infection and the "anti-IgG" secondary conjugated antibody, which is included in IDEXX (Holland) cannot bind to IgM, resulting in false negatives. Salmotype (Germany) includes an "anti-immunoglobulin" secondary antibody and can therefore detect both IgM and IgG (Farzan, Friendship and Dewey, 2007; Mejia et al., 2005). Additionally, a Canadian study of 40 pig farms, to determine the ability of ELISAs to assess *Salmonella* status, hypothesised that false-positives could be caused by cross-reaction to antibodies produced against other bacteria e.g. *Yersinia enterocolitica* (Farzan, Friendship and Dewey, 2007).

An international ring trial of 12 laboratories in 11 countries (France, Belgium, Denmark, England, Netherlands, Scotland, Ireland, Sweden, Germany, Australia and US) evaluated the ability of six "in-house" ELISAs and six commercial kits to detect *Salmonella*-antibodies from 47 sera. The study found that "the specificity of most ELISAs was satisfactory, but relatively large differences were found between the sensitivities of the tests" (van der Heijden, 2001). Also, two laboratories using the same test kit showed differences in sensitivity, possibly because of the use of different cut-offs and the use of a modification of the commercial version of the kit by one lab. Continual changes in methodology and interpretation are being made in many of these kits, which may also affect harmonisation between test labs (van der Heijden, 2001). Standardisation is important as approximately 10% of the samples in a study gave results within 10% of the cut-off values, suggesting that a slight variation in the conditions under which the tests are performed might produce a substantial change in results for individual sera (Mejia et al., 2005).

Factors affecting the level of detectable antibodies in MJ samples

A high level of antibodies detected by the ELISA may indicate a recent *Salmonella* infection. However, no standard can be used to accurately estimate the infectious dose and date of the infection, as immune reactions vary for each individual and are affected by many other factors, such as stress and other immune system challenges. Antibodies will not be detectable immediately after an initial infection and in experimental studies in horses it took around a week for antibodies to be detectable after injection of tetanus toxin, and between 10-20 days for the concentration to reach a peak before declining (Tizard, 2004). For weaner pigs (20-25kg live weight, approximately 3-10 weeks old),

antibodies to a new pathogen are usually detectable seven days after infection with a peak found at approximately 30 days (Nielsen et al., 1995). The peak of antibodies for an initial reaction is relatively small, whereas after second and subsequent infections the response occurs within 2-3 days and a much larger amount of antibody is produced, with a slower decline in concentration (Tizard, 2004). This faster response is due to lymphocytes (T and B immune cells) primed against *Salmonella* being present. An animal can maintain a protective immunity if it is subjected to a constant low level challenge by *Salmonella*. However, if not, then the level of antibodies can decline below the level of detection by the MJ ELISA in approximately two months i.e. in the finishing period before being sent to slaughter (Burch, 2004). A risk assessment model qualified the average time from exposure to a serological response that will test MJ positive as 58 days and the response will stay over the 0.25 MJ cut-off for the duration of 69.7 days (Hill et al., 2008).

Stress can dampen the immune system's response by down-regulating the central nervous system signal pathways (Tizard, 2004). For example, early weaning in piglets reduces the production of interleukin-2 molecules which help in creating antigen-specific T-cells (T-cells responsible for cell-mediated immune responses). Another common cause of stress in pigs can be the disruption of the social hierarchy of a group of pigs. Pigs establish social structure via fighting and once a structure is established levels of fighting and stress are reduced. When pigs are mixed or when new pigs are added to an existing group, then the levels of stress are increased (Tizard, 2004). An experimental study showed how the practice of feed withdrawal prior to movement to an abattoir significantly raised serum cortisol levels and the number of *S.* Typhimurium present in the ileum, ileum contents and colon, than in controls, which could pose a risk of hide contamination of carcasses in the abattoir (Verbrugghe et al., 2011). However, stress and subsequent immunosuppression caused by transit to slaughter and feed withdrawal would not affect the MJ ELISA result collected at the abattoir as antibodies persist for a number of days and there would be no immediate effect on the antibodies already circulating in the body.

Suitability of using meat juice ELISA for pig farm surveillance: Culture versus Serology

The MJ ELISA was chosen for *Salmonella* surveillance as it is cost-effective, quick, and does not require specialised microbiological skills (Proux et al., 2000; Bohaychuk et al., 2005). The test can also be automated to further reduce costs, as a study has shown a good agreement (0.9 kappa) between results from sera from 80 pigs for a laboratory using an automated system and another using a manual method (Chow et al., 2004). Serological tests also benefit from detecting subclinically infected animals and are not reliant on the animal shedding *Salmonella* at the time of sampling (Farzan, Friendship and Dewey, 2007). Culturing techniques may also not be sensitive enough to pick up

small numbers of *Salmonella* shed by subclinically infected pigs, resulting in an underestimation of prevalence (Sibley et al., 2003; Farzan, Friendship and Dewey, 2007). However, a small Canadian study comparing the detection of *Salmonella* by an ELISA and by microbiological culturing for faeces collected from subclinically-infected pigs showed that the ELISA only identified *Salmonella* in 29 (6 positive results and 23 suspicious) of the 67 tested pigs, whereas a PCR detected 41 (Sibley et al., 2003).

Whether the ELISA produces reliable and comparable results was analysed by Chow (Chow, et al., 2004), who compared a mix-ELISA results against faecal culture results using reference sera from five participating laboratories in Europe and North America. The results showed very good to excellent agreement of test sensitivity and specificity (between 88.5% and 97.5%) from the five countries. In a Danish study of 160 pig herds, evaluating tests of samples collected at the abattoir, a strong correlation was shown between herd MJ serology (categorised into groups of 10% prevalence) and the prevalence of *Salmonella* by culture from three samples taken at the abattoir: caecal-content, pharynx, and carcass surface (Sorensen et al., 2004). However, for caecal lymph nodes, no linear association was found with ELISA results, which could be because infection was either recent or in the distant past, and the level of antibodies was below the detection threshold. In another Danish study, the association between pig seroprevalence and bacteriological testing of pen samples was shown between submissions from 1,248 herds. However, the classification of herds into three categories by seroprevalence did not fully predict the prevalence of *Salmonella* from pen samples and information on whether a herd's surveillance serological status was rising or falling improved the prediction (Christensen et al., 1999).

A comparison between blood serology and faecal culture samples collected on farms in the US and Canada showed a good correlation at the herd level in two longitudinal studies, but the Canadian study showed that seroprevalence varied over the three visits to the 90 finisher farms visited over a five month period and a visit level correlation was not as effective (Funk, Harris and Davies, 2005; Rajic et al., 2007). The US study, which examined 49 groups of pre-market age pigs, also indicated that a higher OD cut-off (≥40%) was more closely related to faecal prevalence. However, in a randomised abattoir study in GB, there was poor correlation between positive MJ ELISA results and caecal carriage of *Salmonella* from 2,509 pig samples (Davies et al., 2001). Using the 40% OD cut-off value applied by Danish surveillance, the test results gave a sensitivity of 0.289 and specificity of 0.890, whereas a 10% OD cut-off level (standard for research purposes) increased sensitivity to 0.515 but specificity decreased to 0.691.

The alternatives to serology, such as microbiological culture, are costly, labour-intensive, time consuming, and require many laboratory resources, which makes them unsuitable

for testing large numbers of samples for large-scale surveillance (Chow et al., 2004; Bohaychuk et al., 2005; Farzan et al., 2007). However, culture samples can be pooled to reduce cost and would identify a wider range of serovars and would allow for their individual identification (Davies et al., 2001). This is a large issue when interpreting ELISA results in terms of risk to humans, "as herds with a moderate prevalence of virulent zoonotic serovars are not always identified as problem herds whereas herds with high prevalence of *S.* Derby, which is rarely involved in human disease, may be categorized as high risk" (Davies et al., 2001).

It should be noted that the ability of the results of culture tests of samples collected from the abattoir, to represent prevalence on the farm, could be compromised as infection could have occurred in transit or in the lairage. The transporter and abattoir lairage also provide areas where mixing of pigs from other batches occurs and there is a risk of infection from environmental sources, which affects the ability of abattoir results to represent the situation on a farm (Berends et al., 1996; Gebreyes et al., 2004). The duration of transport or time in lairage would not be long enough to stimulate a detectable antibody response to the infection and so serological tests are unaffected. A study in Northern Ireland, looking at caecal, carcass and serology results from 513 pigs sent to slaughter, found a *Salmonella* prevalence of 31.4% in caecal samples, 40% in carcass swabs but serology was only 11.5% positive, which the authors concluded may have indicated a high degree of recent infection possibly acquired in transit or in lairage (McDowell et al., 2007). Proux's experimental study (Proux et al., 2001) showed how efficiently nose-to-nose contact can spread *Salmonella* infection and how *Salmonella* could be carried in dust or water droplets, proving that a natural airborne route of infection exists. The airborne route within an abattoir lairage would mean that even if batches of pigs did not have direct contact they could still infect other groups. These findings suggest that farm level prevalence estimated from culturing could be lower than levels detected at the abattoir.

Suitability of using meat juice ELISA for pig farm surveillance: meat juice v serum

Comparisons between ELISA S:P results for both serum and MJ collected from slaughtered pigs from 20 commercial finishing farms showed a statistically significant correlation (0.77, $P<0.001$). However, variability of the correlation amongst farms was relatively large (0.50-0.90) (Davies et al., 2003). This may be due to dilution rates or factors such as stress and hydration affecting MJ ELISA results. Similar results were shown in another study of experimentally infected pigs, with MJ samples showing lower but correlated results compared with the serum samples, indicating that MJ can be considered a dilution of serum (Nielsen et al., 1998). MJ samples are preferable for use in abattoir surveillance as serum from clearly identified pigs will not normally be readily available *post-mortem.*

Suitability of using meat juice ELISA for pig farm surveillance: farm v abattoir

A number of studies have looked at the difference between collecting samples from a farm compared with samples collected at an abattoir from the same source farm or same animal. It has been shown that it is not appropriate to use abattoir results to predict individual pig level prevalence, as a large change in results from farm to abattoir has been shown (Davies, 2000; Beloeil et al., 2003). However, a study has shown that on a farm-level basis, when comparing culture samples, abattoir lymph node results were similar but slightly higher than faecal samples collected at the farm (11.7% to 14.9%) (Bahnson et al., 2005). The difference in results is thought to be because pigs experience greater stress in transit and through lack of food, which depresses pig immunity. Van der Wolf (van der Wolf et al., 2001) showed that serological abattoir results could be used to effectively classify farms into high or low prevalence and that farms classified as high prevalence were relatively stable over time and did not change herd status unless a major change to herd management happened. Another consideration of using abattoir-based surveillance is that sampling only covers healthy finisher pigs rather than all age groups and sick pigs that could be sampled on farm. However, sampling live pigs on farms would be more costly and may have ethical considerations e.g. if blood serum were collected. Testing finisher pigs is also considered more important, as they represent a closer link to human risk though pig meat.

1.2.4 Conclusion

Salmonella is an important pathogen with relatively high prevalences in both pigs and people. The importance of pig *Salmonella* was highlighted by studies showing the potential genetic links and transmission routes that attribute human salmonellosis cases to infection from pig sources.

The MJ ELISA is an appropriate test to use for *Salmonella* surveillance due to low cost, low requirements of operator ability and fast test speed. The use of the ELISA also benefits from its ability to detect latently infected pigs. The ELISA has shown an appropriate ability to represent the degree of *Salmonella* infection on the source farm but correlations with caecal culture results from individual pigs have been mixed.

The ELISA, however, suffers from a number of deficiencies which should be taken into account when analysing its results. The Sample:Positive ratio is effected by individual pig characteristics, as well as stress and other factors, which hampers the ability to compare the results between pigs. The ELISA detects ~70-90% of serotypes present in pigs, and may not detect new and emerging serotypes that are not from within the same O-group/ antigen range. The use of MJ ELISA results collected from abattoirs is biased, as the tested population are healthy finisher pigs and do not represent sick pigs, nursery farms or breeding pigs. In addition, the ELISA tests for previous infection and so it would be difficult to relate results to factors that occurred at a specific time point. There can also be problems with standardisation of the test between laboratories, which may influence how representative results from other laboratories/ countries are to the ZAP scheme.

1.2.5 References

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1.3 Risk factors for *Salmonella* in pigs

1.3.1 Method

A search was originally carried out through two internet literature databases (PubMed and Web of Knowledge) using the terms "*Salmonella*", "pig", "control", "intervention", "epidemiology" and "risk factor", to search for appropriate English-language journals that would describe animal or farm level factors that had been found to affect the prevalence and spread of pig *Salmonella* (Table 1.1). The local *Salmonella* Procite reference database held at CERA was also searched for journal or conference papers on factors associated with pig *Salmonella*. All papers from categories 8 to 11 (search combinations of pig, *Salmonella* and either control, risk factor, intervention and epidemiology) were amalgamated and downloaded to a Procite database. Duplicate records were removed from the database and the titles were manually scanned to remove non-relevant papers, such as studies related to human infection from pig meat. The abstracts of the remaining 215 papers were then assessed to determine whether each paper was relevant.

The selected papers were not limited to studies conducted in the United Kingdom or Great Britain, as a great deal of work has been carried out in Denmark, US and the Netherlands. However, the search was limited to countries with similar farm systems to the UK, for example, papers relating to backyard pigs in Asia or Africa were omitted. The final assessment concentrated on finding papers related to non-specific *Salmonella* or *Salmonella* Typhimurium, which is the primary zoonotic *Salmonella* detected from pigs (Chapter 1.2), as study results relating to other serovars may not directly correspond to *S.* Typhimurium due to differences in antibiotic resistance, ability to colonise pigs and other behaviour. However, papers have been included from other serovars if they were of particular interest and relevant to UK pigs. The remaining 87 papers were collected in electronic or hardcopy and were read in full. The references cited by these papers were also checked to locate any further papers missing from the preliminary list.

Table 1.1: Search terms and results from a literature search on *Salmonella* infection in pigs and its control (27/07/10).

No.	Term	PubMed "Hits"	Web of Knowledge "Hits"
1	Pig or porcine or hog or swine	238,666	>100,000
2	*Salmonella*	67,078	>100,000
3	No.1 & 2	2,392	6,550
4	Control	2,262,290	>100,000
5	Risk factor	670,326	>100,000
6	Intervention	236,257	>100,000
7	Epidemiology	1,296,450	>100,000
8	No.1 & 2 & 4	603	1,776
9	No.1 & 2 & 5	120	61
10	No.1 & 2 & 6	33	68
11	No. 1 & 2 & 7	550	1,212

1.3.2 Results: Factors associated with *Salmonella* Infection

Biosecurity

The role of biosecurity in the control of *Salmonella* is twofold: in stopping the introduction of new types of *Salmonella* onto a naïve farm (bio-exclusion); and in stopping the circulation of *Salmonella* between pens and buildings within an infected farm (bio-management). Biosecurity controls on pig farms usually includes measures to limit the risk of staff or farm visitors introducing *Salmonella* from other farms or other groups of pigs via contaminated equipment, clothing or boots. Other controls recognise that deliveries of potentially infected animals or contaminated feed/bedding provides a risk from introducing *Salmonella*. Bio-management is needed to limit *Salmonella* transmission from wild animals, other animals on the farms (especially poultry) and also to remove contaminated farm waste away from pigs. The key aspects of biosecurity are discussed below, with further comment on feed introduction and managing farm waste covered in the Feed and Cleaning and Disinfection sections respectively.

International pig disease experts participating in an opinion workshop agreed that introducing pigs onto a farm was the most likely cause of pig infection on the farm (Stark et al., 2002). It has also been shown that pigs from 359 finishing herds in five European countries who recruited from more than three supplier herds had three-times higher odds of testing seropositive than pigs in herds which bred their own replacement stock or recruited from a maximum of three supplier herds (Lo Fo Wong et al., 2004). The importation of pigs from other farms can cause a large peak in *Salmonella* infections as

they introduce novel strains or serovars to a farm which could affect naïve pigs. Also, the introduced pigs may enter buildings that have residual environmental contamination of strains to which they are naïve. Quarantine can be used to segregate newly introduced pigs, so that the effect of any spike in *Salmonella* shedding caused by infection of the farms residual strains is limited. Pigs may be stressed by transit, which can cause *Salmonella* to shed in higher concentrations (chapter 1.2.3), and quarantine allows time for the pigs to de-stress.

A Danish study used molecular evidence in a three-year longitudinal study to show how *Salmonella* Typhimurium DT104 was spread between 14 cattle and pig herds (Langvad et al., 2006). The study concurred that the trading of live animals was related to the spread of *Salmonella* but also that transmission was caused by person contacts, the sharing of equipment and contaminated slurry. A recommendation was made that tools and machinery should be thoroughly cleaned and disinfected before being brought onto a farm. Reducing the ability of visitors or staff to introduce *Salmonella* to a pig building may be assisted by having building-specific footwear and clothing or other such visitor controls, although the evidence of the effectiveness of these factors appeared to be contradictory, with studies both supporting and refuting these ideas. A French longitudinal study of 89 farrow-to-finisher farms found that the use of farm-specific clothing, and fencing around the farm perimeter, were protective factors for *Salmonella* prevalence as detected by antibodies in blood samples (Beloeil et al., 2007). Providing visitors and staff with access to toilets and hand-washing facilities was associated with a decreased *Salmonella* prevalence in a longitudinal study of two three-site production pig farms in a high prevalence region of the US (Funk, Davies and Gebreyes, 2001). However, the use of building-specific clothing and footwear was not found to be a significant protective factor in a large Dutch study of herd seroprevalence (van der Wolf et al., 2001c). The use of showers for people entering and exiting the farm, to stop visitors bringing infection onto the premises, was actually found to increase risk in a longitudinal study of 89 Canadian farms when compared to farms that just supplied visitors with boots and overalls (Rajic et al., 2007). The multivariable modelling results from these studies may have been confused by the different sampling or testing methodologies, with faecal samples and bacteriology tests used in the Canadian and US studies, whereas blood serology results collected from two abattoirs were presented in the Dutch study. The very small study population and selection of farms from a high prevalence area in the US study may make the results hard to generalise to other populations of pig farms, whereas the Canadian and Dutch studies used much larger populations. However, the findings may also indicate that examining these individual control points has little value, when a combination of these factors is needed for effective control. Information may also have been needed on whether the precautions that were in place were actually effectively used on the farm.

Rodents and other wildlife on the farm can also introduce *Salmonella* and help maintain infection on pig and poultry farms (Henzler and Opitz, 1992; Muirhead, 1993). A large cross-sectional study of 113 Spanish finishing and 74 sow units showed significant protective associations between the use of bird proof nets on finisher units, and the use of a rodent control programme in the sow units (Mejia et al., 2006). Wildlife and pets (insects, cats, dogs, rodents, and wild birds) in the vicinity of eight cattle and five pig farms in Denmark were only found to be infected at periods when the production animals were positive, indicating a close association between infectivity of the wildlife and farm animals on a farm (Skov et al., 2008b). A study of six Scottish pig farms showed that both indoor and outdoor farms had hundreds of visiting wild birds, attracted to the pig feed, and that 7.5 % of wild bird faeces tested contained either *S.* Typhimurium or *S.* Reading (QMS, 2010). These studies show how wildlife can become reservoirs of *Salmonella* and play a role in the persistence of *Salmonella* in pig holdings, or how wildlife movement, such as the movement of birds, could spread infection within farms and to other farms and present a risk to *Salmonella* being introduced to a farm. An effective wildlife control system is important to remove animals via trapping or baiting, and deterring wildlife from entering farm buildings by using bird nets and rodent-secure feed bins.

Keeping pigs outdoors is a growing practice in Great Britain. Outdoor farms usually rear piglets outdoors but finish pigs before slaughter indoors. The proportion of the pig industry kept outdoors has grown from around 5% of breeding sows outdoors in the 1960's to ~40% sows kept outdoors at present (FAWC, 1996; BPEx personal communication 2010), but the lack of a 'walled' environment can cause problems with disease control. In a US study comparing the serological results from 616 samples from outdoor, antimicrobial-free farms and conventional indoor-reared farms, a significantly higher seroprevalence of *Salmonella* was detected in the outdoor herds than in the indoor herds, with 54% samples positive compared to 39% respectively (Gebreyes et al., 2008). Farms that produce pigs outdoors may find improving the biosecurity of the herds more difficult as the factors discussed above, such as wildlife control, are less controllable and the pigs are under continuous exposure from environmental sources, such as contaminated soil (Jensen et al., 2006). Improved cleaning and disinfection of the feeding and drinker equipment may reduce the effect of wildlife contaminating equipment, and regularly moving outdoor production to new uncontaminated sites may reduce pig prevalence.

A further route of infection for within-farm spread to an uninfected pen of pigs is from pigs in other pens within the same building. Experimental studies have shown that transmission of *Salmonella* is possible by airborne and nose-to-nose routes. Pigs may transmit *Salmonella* via sneezing and nasal discharge (Schwartz, 1999) and in experimental studies of airborne transmission that precluded sneezing pigs, pigs were still

infected over a short distance (0.8 metres), although this may have been via airflow contaminating the pen environment rather than direct transmission (Proux et al., 2001; Oliveira, Carvalho and Garcia, 2006; Oliveira et al., 2007). An interesting finding was that this route may be serovar dependent as *S.* Typhimurium was spread by airborne routes but *S.* Agona was not detected in the target pigs (Oliveira et al., 2007). It was also determined that a dose of 10^3 colony forming units (cfu) of *S.* Typhimurium was too small to stimulate an immune response by the airborne route and a dose of 10^6 cfu, a dosage found to induce infection orally, was needed (Proux et al., 2001). These findings suggest that solid walls, rather than slatted or fenestrated walls, are preferable to separate pigs within the same building.

Cleaning and disinfection

A lack of farm hygiene was shown to have a large effect on whether a pig was infected with *Salmonella* in a Dutch risk assessment model (Berends et al., 1996). The presence of residual *Salmonella* contamination of the floor and pen partitions in the fattening rooms was a risk factor and frequent removal of sow dung during the lactation period was protective (Beloeil et al., 2004; Beloeil et al., 2007). The cleaning and disinfection of pens works well at reducing environmental *Salmonella* levels but has not been found to be a key control factor, as *Salmonella* can still be detected in the environment after cleaning and disinfection (Erdman et al., 2005) or the reduction is inconclusive (Schmidt et al., 2004).

The choice of disinfectants may be key to a successful cleaning and disinfection programme, as Thomson (Thomson, Bell and Rafferty, 2007) found that seven different disinfectants, under controlled experimental conditions, had little effect on *S.* Typhimurium and *S.* Derby. The results also showed how disinfectant effectiveness was hampered by the presence of organic matter content. Another study using a further six chemical disinfectants showed that sodium hypochlorite, phenol and peracetic acid were the most effective against *S.* Typhimurium and were the most resistant to the effect of organic matter (Kich et al., 2004). Both studies agreed that the exposure time to the disinfectant was very important to efficacy and Kich (Kich et al., 2004) showed the effectiveness of the three named disinfectants after five minutes of contact. The results suggest that a thorough cleaning process is important before the application of a disinfectant suitable at inactivating *Salmonella*. The practicality of using disinfectants effectively on farms may be complicated by other on-farm factors and these experimental studies may have little relevance in practice. Farmers can struggle to maintain a standard concentration and application rate and so the use of disinfectant can be very different from that used in experimental studies.

The effectiveness of cleaning and disinfection methods from on-farm studies has had little investigation and results have been varied. A study assessing cleaning regimes and the use of two commonly used farm disinfectants (Virkon S and Vetguard) for removing Gram-negative enteric bacteria (as a proxy for *Salmonella*) found that the effects of cleaning and disinfection were highly variable (Rycroft, 2005). Virkon S active ingredients are peroxygen compounds, surfactant, organic acids, and inorganic buffer, whereas Vetguard is a Quaternary Ammonium Compound/TriButyl Tin Oxide Blend. Large differences in the bacterial counts were detected from the different accommodation houses (with varying flooring types) and from the different sampling areas within the pens. In general, hot washing of pens was no more effective than cold; there was little difference between the effects of Virkon S and Vetguard; and total viable counts

remained high. The study identified the drinkers and the corners of the pen as the two high risk areas that contained high levels of microorganisms and may be particularly hard to clean. In contrast, a Canadian cross-sectional study of 80 finisher farms showed that disinfection and cold water washing was actually a risk factor for *Salmonella* shedding, which possibly highlights the ineffectiveness of poorly applied disinfectants in unclean farm environments and the risk that washing may actually spread *Salmonella* (Poljak et al., 2008).

Studies have found that slatted floor systems are effective at reducing *Salmonella* infections (Nollet et al., 2004) on a farm as most of the faeces are removed from the pig vicinity and so the faecal-oral route of infection, reported as an especially important transmission route by EFSA (2005), is reduced. It is likely that the effectiveness of this system is also reliant on the pit below the floor being emptied regularly or at least between batches of pigs (Beloeil et al., 2004). Free-range finishers have been found to have a higher seroprevalence than conventional finishers (van der Wolf et al., 2001a). This may have been due to free-range pigs being bedded on solid floors with straw, which could increase the exposure of a pig to faeces than the typical use of slats for conventional finishers. However, the free-range pigs were also weaned later than conventional pigs and would also have been exposed at pre-weaning to an outdoors environment that would potentially have a greater degree of environmental contamination.

Utilising a batch (all-in, all-out) system, rather than a continuous system, allows the farmer to be able to have a greater influence on controlling *Salmonella* infection on the farm. The system allows for the complete cleaning and disinfection of the pig houses and the emptying of the pit below the slatted floor between batches (Tielen et al., 1997; Lund, 2003; Lo Fo Wong et al., 2004). Farzan (Farzan et al., 2006) showed that a continuous flow pig production system more than doubled the risk of finding *Salmonella* in individual pigs than a batch production system, which may indicate the risk associated with farms and buildings that are continuously occupied and subsequent persistence and transfer of contamination. As noted earlier, if the introduction of pigs onto the farm is one the largest sources of new infection on a farm, then using a batch system allows the farmer to stop new pigs bringing *Salmonella* in to currently housed pigs and vice versa. Although batch systems, along with cleaning and disinfection have been shown to reduce the occurrence of *Salmonella* (Erdman et al., 2005), it did not appear to eradicate it. In another study the omission of disinfection after pressure washing a compartment as part of an all-in/all-out procedure, was associated with a lower *Salmonella* seroprevalence (van der Wolf et al., 2001c).

Feed/ water

Many forms of feed and water have been thought to influence the level of *Salmonella* infection, although many findings are limited to single studies and are not corroborated by other studies that have assessed the impact of that feed type. In this section, the only findings discussed are those where feed or water have been shown to be important to *Salmonella* in a number of studies.

Salmonella is inhibited and killed by a highly acidic environment. *Salmonella* growth is affected by an acidic pH by slowing protein and DNA synthesis. An acidic environment also favours Gram-positive bacteria allowing them to out-compete *Salmonella* and other Gram-negative bacteria for resources. The competitive exclusion of *Salmonella* can also be assisted by the bacteria producing antibacterial substances or stimulating the pig's immune system. An acidic environment can be caused by acid added directly to feed or water or by adding pre-fermented by-products (e.g. whey) (van der Wolf et al., 2001c; Creus et al., 2007). By-products are normally fermented or acidified to preserve them, they are cheap, and feeding them to pigs is an effective way of disposing of them (van der Wolf et al., 1999). Feeding a complete liquid feed system with fermented by-products allows low prevalence herds to retain that status for longer periods (van der Wolf et al., 2001c; Poljak et al., 2008). However, some studies looking at the effect of fermented feed on *Salmonella*, showed no change in shedding between a case and control group, indicating that *Salmonella* may be able to bypass the stomach via the tonsils (van Winsen et al., 2001; van Winsen et al., 2002). The studies may have been affected by an unknown factor which caused a reduction of *Salmonella* in both groups.

Organic acids, such as formic, acetic, and fumaric, can also be provided to the pigs in drinking water at concentrations below a pH of 4.2 and have been shown to reduce *Salmonella* infections. An identified problem with this practice was that in some water systems, the pipes rapidly rusted and fungal growths formed in the pipelines, which caused blockages in the nipple drinkers (van der Wolf et al., 2001b; van der Heijden et al., 2005). An observational study of the water quality of 54 Scottish farms showed that the drinking water pH (which ranged from 6 to 8) was associated with *Salmonella*, with lower pH associated with lower seroprevalence (QMS, 2009). The study also found that on farms with very low seroprevalence, the water pH had a large range and so it was hypothesised that the pH of the water was less important to *Salmonella* control on farms with a low prevalence. However, this study was not subject to full scientific analysis and these findings may have been confounded by a number of other factors that affect *Salmonella*.

Another route of creating an acid gut environment is to use coarse ground meal feed which ferments in the gut, causing a proliferation of lactic acid producing bacteria which

lower the pH of the gut and contribute to competitive exclusion of *Salmonella*. Coarse ground feed also has a higher water binding capacity than pelleted meal, and can create a higher starch content in the content of the intestines which favours the growth of gram-positive bacteria rather (Lo Fo Wong et al., 2004; Papenbrock et al., 2005). Although pelleted feeds were originally found to reduce *Salmonella* infection (Edel et al., 1967; Edel et al., 1970; Edel et al., 1974), more recent studies, and a recent European Food Safety Authority opinion workshop (Leontides, Grafanakis and Genigeorgis, 2003; Lo Fo Wong et al., 2004; EFSA, 2010; EFSA, 2011), have found that a non-pelleted feed, whether dry meal or wet, was associated with a lower risk of *Salmonella* infection and that pelleted feed given to sows or finishers was a significant risk factor (Kranker, Dahl and Wingstrand, 2001). Non-pelleted feed is, however, less efficient for feed conversion and so effects a farm's financial performance.

The composition of feed has also been assessed to determine the amount of wheat or barley that should be added. Increasing the level of barley in pelleted finisher feed was shown to significantly decrease *Salmonella* and the number of gastric lesions, but reduced pig feed conversion productivity, in a Danish nutrition report, although the full details of this study were not apparent and do not appear to have been published elsewhere (Jorgensen, 2003). Another report, communicated at a pig conference, discussed how heat-treated pelleted feed effectively kills *Salmonella* and stops feed-related transmission onto farms, but the feed creates a suitable microenvironment in the gut for *Salmonella* growth (Kelliher, 2002). The addition of at least 25% non-heat treated barley to coarse ground meal improved gut health by creating a highly acidic environment and stimulating proliferation of competitive acid-producing bacteria that inhibited *Salmonella* growth. These results indicate that barley content may play a role in *Salmonella* control and that a blend of wheat and barley is needed in feed to balance the positive effects of barley with pig performance, but more evidence from a statistically robust intervention study is needed.

Liquid feeding systems, which involve the soaking of dry feed (normally meal formations) in water for several hours before feeding, causes a natural fermentation process, which results in the growth of lactic-acid producing bacteria and yeasts. The fermentation process lowers the pH of the feed and improved the feed conversion rate when compared to dry feed, although interestingly pigs preferred dry feed when given the choice (Brooks et al., 1996), which may indicate a potential welfare issue. Liquid feeding was associated with a decreased risk of a pig being culture-positive for *Salmonella* when samples from 20 liquid feeding farms were compared to 61 dry feeding Canadian farms (Farzan et al., 2006). Similar results have been shown by the feeding of fermented liquid feed or wet feed, in comparison with dry feed, in two large European studies (van der Wolf et al., 1999; Beloeil et al., 2004). However, in another study a significant difference was not

shown between liquid feed and dry feed, but this may have been biased by the very few farms in the study using wet feeding (Leontides, Grafanakis and Genigeorgis, 2003).

Feed additives, such as Potassium diformate (KDF), have also been shown to reduce *Salmonella* by positively influencing the intestinal flora (Papenbrock et al., 2005). This study also noted that KDF added to coarse feed reduced the *Salmonella* shedding rate, shortened the shedding period and reduced the translocation of *Salmonella* within infected piglets. The use of experimental chlorate preparations in drinking water also showed a significant reduction of caecal *Salmonella* concentrations of 60 weaned pigs and 18 finishers (Anderson et al., 2004).

Feed brought onto the farm is a less prominent source of infection in the UK as most feeds are heat-treated to kill off bacteria, but feed can become contaminated during transit or if not kept in clean feed bins that are wildlife protected (Cooke, 1997). It is important to ensure that the feed materials brought onto a farm come from monitored sources and have been found to be *Salmonella*-free. Additionally, the use of homemix has been found to be protective, but this may be due to the difference in feed type (pellet size, water binding capacity) rather than due to the risk of importation of *Salmonella* onto the farm through feed (VLA, 2006; Dahl, 2008).

Herd size

A number of European studies have shown a significant relationship between greater herd size and increased *Salmonella* faecal pat sample prevalence and serum seroprevalence (Farzan et al., 2006; EFSA, 2011). Samples from large herds (>5000 pigs slaughtered per annum) were more likely to be seropositive than samples from small herds (Mousing et al., 1997) and there was an increased risk of seropositivity in pigs on a farm when the herd size was doubled (Kranker, Dahl and Wingstrand, 2001). A large Spanish study found that herd size was a risk factor for the seroprevalence of finishing units, as was the presence of other farmed species, especially poultry (Mejia et al., 2006). The study also detected that a specific risk factor to sows was the number of sows on the farm. A similar finding was obtained from an abattoir-based research study of caecal contents, with large herds, producing more than 2600 slaughter pigs per year, more likely to be *Salmonella* positive than small herds with an annual production of 500 to 550 slaughter pigs (Baggesen et al., 1996). Furthermore, a Canadian cross-sectional study showed that herd size had a linear relationship with logged odds of being *Salmonella*-positive (Poljak et al., 2008). The reason that larger herds have a higher prevalence of *Salmonella* than small may be that larger herds need a larger number of herds supplying them, thus increasing the chance of importing pigs from a high prevalence farm (see biosecurity section).

Contradictory evidence has been found in other studies showing that herd size was not a significant factor (Lo Fo Wong et al., 2004) or that a higher risk was detected in small to moderate herd size (<800 finishing pigs), as larger farms may have better cleaning and disinfection standards than smaller farms (van der Wolf et al., 2001c). This variability of results from studies was assessed by a Danish project (Carstensen and Christensen, 1998). A random effects model was used to compare herd size with *Salmonella* seroprevalence, whilst accounting for other farm level factors. Herd size was found to be significantly associated with seroprevalence, but this may have been an artefact of the powerful study rather than a true effect. The large within herd and between herd variances detected by the models showed that herd size was not a key influencing factor and that other, more important variables, may have explained these large variances. It has been proposed that the differences between study results may also be due to the definition of herd size, as some studies looked at the number of pigs currently on the farm, whilst others looked at the number of pigs sent to slaughter in a given period (year or month).

Spatial factors

A British review paper concluded that farm location and local spread are important factors in farm biosecurity (Pritchard, Dennis and Waddilove, 2005). The type, number and density of pig units in a 2km radius was judged as crucial, as was the distance to slaughterhouses, slurry lagoons, refuse tips and roads used by pig transporters. Local spread is a problem between farms with no apparent links, where communicable diseases might transfer between neighbouring farms. An indication of spatial differences in *Salmonella* prevalence was shown by a large abattoir prevalence study that detected that samples from pig farms in the North East of England were more at risk of being positive than from other areas of England and Wales (Milnes et al., 2009).

It has been proposed that spatial structure is common in observations of biological applications, such as disease prevalence, as measurements at close locations have a greater tendency to be similar than those taken further apart (Diggle, 2002). Diggle explains that an association with *Salmonella* prevalence could be made up of "first order" effects, which are values specified at the locations where they are measured (e.g. the difference in the average number of cases of disease infection per km^2 in two countries), and "second order" effects, which are interactions between values at different spatial locations (e.g. correlation of disease occurrence between farms within a 10km vicinity of a waste plant). Both first and second order effects can be associations with the clustering of disease in space. The relationship of first order effects with a spatial clustering effect may be because the variables themselves have a spatial structure e.g. clusters of farms, such as those in East England, have a higher chance of being breeder-finisher farms or having another covariate that might add to the spatial structure of *Salmonella* prevalence. The

spatial structure may also be dependent on a transmission mechanism, although this would be hard to detect from spatial analysis as more information would be needed on the ordering of events i.e. spatio-temporal analysis.

The spatial relationships and trends of *Salmonella* prevalence results between pig farms has been uncovered using spatial analysis of serology results from the British *Salmonella* surveillance scheme (ZAP/ZNCP). An initial report used K-function analysis and variogram analysis, from the field of geostatistics, to identify geographically localised anomalies of *Salmonella* infection, from two years of surveillance data (Clough et al., 2009). The study concentrated on the three regions with the highest density of pig farm population, and spatial heterogeneity was detected in one of the regions (East of England rather than Yorkshire and Humber or the South West). Spatial heterogeneity, or spatial dependence, highlighted by these tests, can be defined as either spatially close farms being more likely to have similar results than distant farms, or that farms with elevated *Salmonella* results are more closely clustered in space than would be expected by chance. K-function analysis did not show any significant clustering but the variograms highlighted some spatial dependence over shorter distances, up to around 20km. A further study adapted the variogram spatial analysis to account for a number of other variables (herd type, temporal trends) in a study of four years of data from two regions, East England and Yorkshire and Humber (Sanderson, 2005; Clough et al., 2007). An elevated spatial effect was detected in East England rather than Yorkshire and Humber, possibly due to the higher degree of outdoor herds in East England (Fowler, 2003), which may be at an increased risk of local spread and thus infection from neighbouring farms. 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 in *Salmonella* levels.

Studies that have examined spatial clustering in other countries have shown mixed results. A Canadian study found no relationship between *Salmonella* results and the location of a farm or the density of farms within an area (Poljak et al., 2008). However, two studies utilising the serology results from the Danish *Salmonella* surveillance scheme did show the importance of spatial factors. A study of PFGE and plasmid type results of multi-resistant *S.* Typhimurium DT104 isolates from seven years of surveillance data showed that the horizontal spread of infection between farms was important and that the proximity of farms in space and time to an infected farm increased the risk of being infected (Skov et al., 2008a). The second Danish study utilised spatially adaptive kernel estimation to allow the whole of Denmark to be analysed, as well as regional trends, as this novel technique provided a method for the analysis to use a more focused resolution in high pig farm population areas and a lower resolution in sparse areas (Benschop et al., 2008a). Previously, a fixed resolution was used for this type of analysis, which limited

studies to areas of high farm density or could only utilise a low resolution for the whole country. The results showed differences between areas in the risk of being a case farm, as determined by >40% of samples *Salmonella*-seropositive by the Danish serological surveillance test. This might have been due to the differences of farm management trends between regions/islands or regional trends in the importation of feed and pigs.

Spatial relationships may be country specific and the results found in other countries may not correspond to the British pig farm situation. Spatial factors may be of particular interest in Britain due to the structure of pig farming, with regional trends in terms of farm type and whether pigs are kept indoors or outdoors, and with high density pockets of herds (>10 herds per 100 hectares of farmed land) in the East and North East of England and North East Scotland (Fowler, 2003; Pritchard, Dennis and Waddilove, 2005).

Temporal modelling and weather/ seasonality

Seasonal differences have been shown by a number of studies in a range of European countries. However, the exact seasonal peaks and troughs seem to differ both within and between countries. In Northern Ireland, a small abattoir-based study of 513 pig carcasses found that there was a higher odds of carcass contamination from April to June and a lower odds from October to December (McDowell et al., 2007). In Canada, a study of abattoir surveillance data from all livestock species over 11 years, showed a peak in *Salmonella* in pigs from August to September (Guerin et al., 2005). The duration of temporal clusters was also examined and it was hypothesised that a cluster of *Salmonella* cases over a short period of time may indicate point source infections, whereas a longer duration could be caused by increased prevalence or increased farm-to-farm transmission. Temporal effects were found to be significant in a four year study of *Salmonella* surveillance serology results in two regions of England, with both 6 and 12 month cycles found to be associated with the outcome, and peaks of seroprevalence in late September and October and troughs in May and June (Sanderson, 2005; Clough et al., 2007). The authors discussed that these trends could be influenced by the seasonal prevalence of other diseases (possibly respiratory diseases) and temperature fluctuations.

A seasonal pattern in *Salmonella* occurrence was shown by the analysis of samples from the Danish *Salmonella* surveillance scheme of slaughtered pigs, with lower seroprevalence in summer than in winter (Christensen and Rudemo, 1998), although a later study of a dataset spanning 1995-2000 found a two peaked annual cycle with spring and autumn peaks (Hald and Andersen, 2001). Furthermore, a statistical review of the surveillance system using data from 1995-2005, found no reliable seasonal trends that would require altering the frequency of sampling of pigs in different seasons (Benschop et al., 2008b). This may indicate that seasonality is not consistent between years in the

same country and instead, the detected patterns may be related to anomalies of weather conditions, such as peaks in rainfall that differ between years.

Environmental temperature (winter and spring seasons, increased temperature variability, and below median high temperature the day of sampling) were associated with elevated *Salmonella* prevalence in pig farms in the US (Funk, Davies and Gebreyes, 2001) and it has been hypothesised that higher air temperatures may increase pig stress, which boosts the shedding of *Salmonella* (Hald and Andersen, 2001). Temperature and weather conditions would also effect the survival of *Salmonella* in the environment, as shown by an experimental study of slurry contaminated with *S.* Typhimurium where the bacteria survived for 85 days in spring and winter but only 26 days in summer (Placha et al., 2001).

Pig age

The interactions of different age groups of pigs may indicate possible routes of infection within a farm. The role that sows play in pig-to-pig transmission has been investigated in Belgium and the study found that, during lactation, piglets were less at risk of *Salmonella* infection, so it is possible that maternal immunity provides a protective effect (Nollet et al., 2005). The genetic strains of *Salmonella* in the sows were similar to those found in the nursery and in finishing pigs, indicating that sows may also play a significant role in indirect transmission on the farm. Free range farms may be at particular risk of this transmission as on Dutch free range farms, pigs are on average weaned later, which may increase the risk of sow-to-piglet transmission (van der Wolf et al., 2001a).

In another study of the circulation of *Salmonella* on a farm, if *Salmonella* was isolated from weaners there was a greater chance of high seroprevalence in finishers (Kranker et al., 2001). It has been predicted from a transmission model, that carefully selecting weaners, and removing pigs that tested positive for *Salmonella* promptly from the herd, were the best ways to control *Salmonella* (Ivanek et al., 2004). However, the early infection of pigs may be protective due to improving the pig's immune system to that particular strain. The dynamics of infection within a group of pigs suggests that if only a few pigs are infected at any one time then *Salmonella* can be maintained in group, whereas if more pigs are infected then the infection rapidly reduces via herd immunity (Wales, Cook and Davies, 2011). Where serovars are present in the environment of post-weaning accommodation that differ from the pre-weaning areas then pigs would be immunologically naïve and at risk of infection when moving between accommodations. Segregating pigs from others in clean accommodation was shown to protect pigs in a small study, with all segregated pigs testing *Salmonella* negative at slaughter, whereas some unsegregated pigs were positive (Dahl et al. 1997). As well as the concern of pigs being exposed to different *Salmonella* strains present on the farm, the introduction of a

novel strain, commonly from the introduction of new pigs, causes a rapid proliferation through pigs with naïve immunities. This has been shown by the rapid emergence of monophasic *S.* Typhimurium which has caused large rises in prevalence on farms that had been historically low prevalence.

When pigs were moved from the nursery to a finishing site, the prevalence of *Salmonella* infection in the pigs increased but the serovar was the same as that found at the nursery. This may indicate that infection had been caused by increased shedding by the moved group caused by increased handling and transport, rather than new infection from the finishing site (Davies, Funk and Morrow, 1999). The effect of stress has also been shown by the mixing of pigs from different groups. Pigs are usually mixed at set periods, after weaning and before slaughter, into groups of pigs of similar age and weight so that they can be fed and managed in a similar manner. A small, controlled US study showed a significant increase in *S.* Typhimurium in weaned pigs that were mixed and described changes to behaviour, with less eating or rooting (Callaway et al., 2006). An experimental study designed to show the effect of mixing pigs on the efficacy of vaccination, using a comprehensive set of responses (including cortisol concentration and cytokine production), showed that mixing causes stress and an impairment of the immune system, particularly in gilts (de Groot et al., 2001).

Farm type

Commercial pig farms in Britain are broadly categorised into three groups, breeding farms that produce piglets and raise them to the age of weaners or growers; finisher farms, that solely 'finish' the feeding and development of pigs before sending them to slaughter; and breeder-finisher farms, that combine the roles of the previous two. The results from the British surveillance scheme showed a lower seroprevalence of *Salmonella* in breeder-finisher herds when compared to finishers (Sanderson, 2005; Clough et al., 2007). However, this scheme only sampled pigs from abattoirs, and breeding units which do not send pigs to slaughter were therefore not included. One reason for this finding could be that finisher farms import pigs from multiple sources which increases the chance of novel types of *Salmonella* being introduced into the farm environment to infect the next batch of pigs. Another explanation may be that the difference is caused by the stress of transporting weaned pigs to the finisher farm. It is possible that breeder-finisher farms have higher standards of stockmanship, hygiene and biosecurity, due to the need to protect piglets and their breeding stock, but this has not been identified in British studies.

Treatments

Prebiotics and probiotics affect the gut flora and these have been assessed for their effect on *Salmonella*. Probiotics are living microorganisms that may affect *Salmonella* by competing for nutrients or producing inhibitory substances, whereas prebiotics are

indigestible carbohydrates that stimulate the growth and activity of beneficial bacteria in the gut. However, the specific types of treatments have shown different effects on *Salmonella* presence. The use of *Bacillus spp* as a probiotic was tested in an experimental study which showed significant anti-inflammatory affects and reduced invasion of cells by *S.* Typhimurium (Aperce et al., 2010). A Canadian case-control study measured the effect of a number of treatments to groups of 10 inoculated pigs. It was noted that flavomycin (an antibiotic used in this study as a prebiotic treatment) reduced the concentration of *S.* Typhimurium in the lymph nodes but did not reduce gut concentrations or significantly reduce shedding (Letellier et al., 2000). The study also suggested that although prebiotics and probiotics may reduce the colonisation of *Salmonella* or reduce shedding, when used together they appeared to act as antagonists and did not provide benefit.

The use of a live attenuated *S.* Typhimurium vaccine has shown significant beneficial effects on reducing *Salmonella* infection (Springer et al., 2001). A further study also showed a reduction in tissue colonisation and that 90% of vaccinated piglets did not show clinical disease, whereas all placebo piglets presented with symptoms (Roesler et al., 2004). Currently a live *S.* Typhimurium vaccine has only been accepted across the European Union for use in poultry, although a vaccine has been licensed for use in pigs in Germany. The use of live *S.* Typhimurium vaccines can be problematic, with issues concerning the efficacy on farms and the costs of vaccination, as well as impacting on serological surveillance, as vaccinated pigs will produce an immune response to the live vaccine and the herds will show a high seroprevalence. As vaccines are serovar specific, the use of other vaccine strains may provide only limited protection to serovars important to human health, such as *S.* Typhimurium. However, a live attenuated *S.* Choleraesuis vaccine, available in the US, decreased bacteria in mesenteric lymph nodes and significantly reduced *S.* Typhimurium presence in the ileum (Letellier et al., 2000).

The use of antibiotics has also been shown to have a relationship with *Salmonella* prevalence. A Dutch risk factor study showed that pigs fed Tylosin, a broad spectrum antibiotic, used either as a growth promoter or therapeutically at a higher dose, were associated with a higher seroprevalence (van der Wolf et al., 2001c). A combination of chlortetracycline, procaine penicillin and sulphamethazine antibiotics used at fattening as growth promoters were associated with a four times higher risk of *Salmonella* sero-positivity than the use of an approved growth promoter (e.g. Tylosin) or a probiotic (Leontides, Grafanakis and Genigeorgis, 2003). The use of broad-spectrum antibiotics has also been identified as an important risk factor for infections with *Salmonella* (Berends et al., 1996).

The effectiveness of other antibiotics has been mixed. Fluoroquinolone treatment, along with optimised hygiene procedures, lowered but did not eradicate *S.* Typhimurium (Roesler et al., 2005). The persistent use of fluoroquinolone was deemed expensive and, considering that the largest treatment effect in the study came from the disinfection of the pig building, fluoroquinolone use was not considered an effective or efficient control measure. Other studies have found that antibiotics may reduce the shedding of *Salmonella* (Laval et al., 1992), but were not found to reduce prevalence in sick or recovered animals with enterocolitis (Wilcock and Schwarz, 1992), and that preventive treatment with Enrofloxacin did not eliminate *Salmonella* infection (Dahl et al., 1996).

The use of antimicrobials often increases susceptibility to *Salmonella* infection (van der Wolf and Peperkamp, 2001) and the overuse of antibiotics can positively select for *Salmonella* with antibiotic resistance, which is a further concern to human health. The use of antimicrobials for therapy or for growth promotion is also widely discouraged as they disrupt gut flora, affecting the competitive exclusion that may already exist, which has a beneficial effect on reducing *Salmonella* shedding (Genovese et al., 2003). In 2005, an EU-wide ban of antimicrobial growth promoters was implemented to try and reduce antibiotic resistance in bacteria.

Co-infection

The presence of a number of other infections has been shown to be associated with the presence of *Salmonella* or shown to increase the shedding of *Salmonella*. For example, a Dutch risk factor study presented that a previous diagnosis of clinical *Salmonella* infection in the herd, or herds which had more than 16% of livers with milk spots, were associated with a higher *Salmonella* seroprevalence (van der Wolf et al., 2001c). An association with milk spots was also detected by abattoir surveillance data from Britain, which may indicate that the migratory larvae of *Ascaris suum* worms which cause milk spots, penetrate the gut lining, causing lesions which can facilitate the invasion of *Salmonella* (Smith et al., 2011).

A risk factor study of 105 French farrow-to-finish farms showed that *Lawsonia intracellularis* seroconversion and PRRS (Porcine Reproductive and Respiratory Syndrome virus) presence were risk factors for *Salmonella* shedding (Beloeil et al., 2004). PRRS and *S.* Choleraesuis have been shown to be synergistic and co-infected animals were more likely to develop disease and shed *Salmonella* in higher numbers and for a longer period (Wills et al., 2000). This result was in contrast to an earlier experimental study which failed to show that PRRS enhanced infection by *S.* Choleraesuis in pigs (Cooper et al., 1995). The dose or methodology differences, or small sample sizes, may have affected these studies and the finding of an association between PRRS and

Salmonella seroprevalence from a randomised risk factor study was perhaps more important (Beloeil et al., 2007).

As well as PRRS, *Salmonella* has also been shown to be associated with the presence of wasting diseases present in the individual pigs or present on the farm. A number of small-scale Asian studies have shown a significant co-existence between pigs with Postweaning Multisystemic Wasting Syndrome (PMWS) and *Salmonella*. In Japan, 12 of a total of 14 pigs with *Salmonella* from four farms also presented with PMWS and 12 with PRRS, and in Korea an outbreak of 37 cases of salmonellosis were associated with PMWS, with 22 intestinal pig samples (59%) testing positive for the porcine circovirus type 2 (PCV-2) that causes PMWS (Ha et al., 2005; Murakami et al., 2006). It was hypothesised that these conditions may dampen the immune system and assist the infection by *Salmonella*. However, a UK case-control study of 145 farms showed that PMWS and Porcine Dermatitis Nephropathy Syndrome (PDNS) were not significantly associated with other infectious conditions, although *Salmonella* was isolated more frequently on case farms (Cook et al., 2001).

An association between *Salmonella* and pneumonia has also been examined, and in a study of 153 necropsied pigs with *S.* Choleraesuis, 99 of 109 presented with pneumonia in the absence of any other pathogen (Turk et al., 1992). A study comparing *Salmonella* seroprevalence and health conditions detected at the abattoir, detected a significant association between farms with high prevalences of *Salmonella* and Enzootic Pneumonia-like lesions (Smith et al., 2011). It has been proposed that respiratory diseases are stress factors that make pigs more susceptible to *Salmonella*, and the control of these factors would help lower *Salmonella* infection. Respiratory disease may also increase aerial transmission through coughing and sneezing. However, another plausible reason for these associations between these conditions could be the sharing of risk factors, such as bedding or ventilation conditions of pig buildings.

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Chapter 2: Analysis of routinely collected data

2.1 Introduction

Bacteria belonging to the genus *Salmonella* are gram-negative enterobacteria, most of which are capable of colonising and infecting a wide range of hosts. *Salmonella* infection in pigs can cause a range of clinical signs, from scouring to fever, septicaemia and death, although infection is often sub-clinical. At the time when this research was started, 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) (Milnes et al., 2005). Additionally, in a EU Baseline survey completed in 2007, *Salmonella* was isolated from 21.2% of mediastinal lymph node samples from UK pigs at slaughter (EFSA, 2008). *Salmonella* is also an important foodborne pathogen for the human population, with 13,213 laboratory confirmed cases of salmonellosis in the UK identified in 2007 (Defra, 2007). Around 13% of these human cases were infected with the serovar *S.* Typhimurium, which was also the predominant type detected in samples from pigs (VLA, 2007).

Many studies have tried to ascertain the factors that influence *Salmonella* prevalence and identify on-farm controls, to reduce the *Salmonella* burden in pigs. Recent studies in Great Britain have detected associations with factors such as: herd size; outdoor rearing of pigs; flooring type; and farm location (VLA, 2004; Pritchard et al., 2005). These findings have been supported by European, Canadian and US studies (Funk, Davies and Nichols, 2001, Nollet et al., 2004; Farzan et al., 2006). However, some of these studies were limited to small and potentially unrepresentative subsets of the pig farm population whilst others may not have had sufficient statistical power to detect modest associations between *Salmonella* infection and putative risk factors.

The pig industry has been proactive in developing Quality Assurance Schemes (QAS) to monitor farm practice, and these schemes collect a large amount of data on herd details and management practices and cover a large proportion of the pig farms in the UK. In June 2002, the British Pig Executive introduced the Zoonoses Action Plan (ZAP, now called the Zoonoses National Control Plan) *Salmonella* monitoring programme. This programme ran in conjunction with QA schemes to estimate the burden of *Salmonella* in pigs sent to slaughter by testing meat juice (MJ) samples for antibodies against Group B and C_1 *Salmonella* in a mix-ELISA system (Armstrong, 2003). A positive MJ ELISA result was assumed to represent prior infection but it was recognised that positive pigs were not necessarily infected when slaughtered. Farms that had a prevalence of more than 50% MJ ELISA positive pigs were required to implement an action plan or face eventual loss of their Quality Assured status and it was hoped that this threat would motivate all pig

farmers to develop *Salmonella* control plans. The scheme was based on a design by the Danish pig industry that had contributed to a reduced *Salmonella* prevalence after the introduction of strict financial penalties for high prevalence herds (Nielsen et al., 2001). Due to the human health impact of pig *Salmonella*, the ZAP scheme was linked to the Food Standards Agency's initiative to meet the target of reducing *Salmonella* in pigs by 50% by 2010 (FSA, 2009).

To be able to provide strong evidence for the associations between the explanatory factors, identified by the literature review (Chapter 1.3), and *Salmonella* prevalence, a study was needed that would cover a large and representational cross-section of UK pig farms. A large study population would provide statistical power that would allow for even weak associations to be detected, which are important to *Salmonella* control if they are present on a large number of farms. The study population was required to be representative of the commercial UK pig industry so that any findings could inform the actions of the industry as a whole in combating *Salmonella* prevalence. Data from pig QAS were identified as sources of farm information for this very reason.

A large proportion of UK pig farms are part of a QAS, and anecdotal evidence suggests that ~50% of all the pig holdings in the UK are members and that these farms cover ~90% of the pig population, as the remaining pig premises are typically small holdings and semi-professional herds. Each farm that is a member of a QAS has information collected on a regular basis (usually during quarterly visits by an auditor) to ensure that the farm is meeting certain health, management and welfare standards set by that QAS. These data from the QAS were collected and assessed to see if they would be suitable for risk factor analysis. This assessment was completed in two stages: a description and comparison between each QAS dataset, described in Chapter 2.2; and then an evaluation of the representativeness of the data and usefulness for epidemiological analysis, using standard methods, described in Chapter 2.3.

2.2 Description of QAS data

The three main QAS were contacted and the aims of the study were explained to the scheme organisers. Each scheme was asked to provide documents detailing the information that was collected from farms by them, and also a dataset of their most recent member records. The data were analysed for content, missing values and transcription errors, to evaluate the quality of the data within each scheme. This evaluation was to check the suitability of the data for use in a multivariable regression model, where records with missing data in variables selected in the model are omitted from the model population. The data were also assessed to determine which factors were collected by all three schemes, and which variables would require recoding to allow them to be compared

and analysed across the schemes. An appraisal was also made on the usefulness of the *Salmonella* seroprevalence surveillance data, collected by the ZAP/ ZNCP scheme, and how these results could be connected by farm identifiers to the QAS records. A review of the similarities of the data collected and the size and data quality of each dataset is listed below, with a full list of the questions asked by each QAS in appendix A.

A review of the data collected by each QAS is provided below:

QAS name	Quality Meat Scotland	Assured British Pigs	Genesis Quality Assurance
Abbreviation	QMS	ABP	GQA
Web address	www.qmscotland.co.uk	www.assuredpigs.co.uk	www.genesisqa.com
Date data supplied	20/12/07	19/12/07	18/07/07
Farm information	CPH, postcode and slapmarks	Membership ID, herdmark, CPH and slapmarks	Membership ID and herdmark
Level of data	Farm level	Farm level	Farm level, plus enclosure level information for unique breeding, farrowing and finishing enclosures
Members	293	1,827	848
Audit records (period)	299 (25/04/05-02/02/07)	3,186 (02/06/05-24/09/07)	1,409 (14/05/04-17/07/07)
Data issues	1. The QMS scheme collected the smallest dataset and had the most amount of missing data e.g. 51 (17%) records did not have any pigs recorded as being present. 2. Data on the use of feed companies, abattoirs and weaners suppliers were free text fields that required re-assortment to extract each company, abattoir or farm into a single record. 3. The scheme records did not contain travel times and distances from farm holdings to abattoirs.	1. 173 members were located in Northern Ireland, or on the Irish Republic border, which required a different geographical coordinate system to locate the position of the farm. Some of these farms had connections to farms, vets and other farming businesses in Eire for which no further data was recorded.	1. 28 members were located in Northern Ireland, or on the Irish Republic border, which required a different geographical coordinate system to locate the position of the farm. Some of these farms had connections to farms, vets and other farming businesses in Eire for which no further data is recorded.

2.2.1 Generic QAS data issues

A number of members in each scheme had more than one physical location recorded, as identified by either: a separate site ID, suffixes to the membership ID or more than one postcode linked to a member. In QMS, 14 members had multiple postcodes and records were provided for each individual postcode. ABP grouped data from multiple units under one membership ID if the units were within 5km, and 258 members had multiple sites under the same membership ID. GQA identified farms with multiple sites by adding suffixes to the membership ID to identify the 64 farms with multiple sites but all data were aggregated to the membership level.

All three datasets contained records with null (missing) values for questions and it is unknown whether these represented questions that were not recorded; were not known by the farmer; or were a negative answer (e.g. zero sows kept or no bedding used). The definition of pig categories was not recorded in a standard manner by the three QAS. Some definitions were also related to the weight or age of the pigs, which was also not standard between the schemes. To account for this, the pig numbers were summarised under three categories: number of sows; number of finishers; and total herdsize.

Identifying information had been recorded without data validation methods (such as input masks which ensure that the correct information is entered in the correct format) which may have created multiple records for the same member, as transcription errors would have created a new unique farm identifier.

2.2.2 Zoonoses Action Plan/ Zoonoses National Control Plan (ZAP/ZNCP) data

The *Salmonella* surveillance scheme dataset that was provided by BPEx recorded sample results from 20/06/02 to 25/09/08 and contained 127,351 records. However, 7,241 (5.7%) were missing sample:positive ratio, although the binary result for the ELISA was present, and 10,194 (8.0%) had zero or negative ratio results. The serology records included the following farm identifiers: herdmark, slapmark, QAS membership ID and postcode. However, all four identifiers were only present for 51,768 (7.4%) records.

2.2.3 Combination of the datasets and analysis

Due to the non-standard recording of farm identifiers in the QAS and *Salmonella* Surveillance scheme, it was decided to connect the records in the datasets in stages. This was attempted so that all identifying information could be used to try and verify which samples corresponded to which farm, without creating incorrect links or omitting samples that matched all but one identifier, possibly due to transcription error.

2.3 An Analysis of Quality Assurance and Zoonoses Action Plan Data from Pig Herds in the United Kingdom

2.3.1 Introduction

Information from the ZAP and QA schemes were merged and the data assessed for suitability for epidemiological analysis. Evidence for supporting the association of a factor with the increase or decrease of an outcome (usually a disease or presence of a pathogenic organism) within a population can be provided by epidemiological analysis. Epidemiology has incorporated statistical modelling, which allows for the creation of a 'model' from the information collected from a study of an outcome. The epidemiological model allows for a comparison of the effect of each explanatory variable and discrimination of the statistical significance of factors in relation to the outcome. Statistical models are a key component of an epidemiologist's armoury, as large and complex datasets can be summarised more readily and multiple explanatory variables can be assessed simultaneously.

Regression analysis is used to explore the relationship between a dependent (or outcome) variable, such as the presence of a bacteria in the faeces of an animal, and one or more independent (explanatory) variables (e.g. whether the animal had close contact with another infected animal). Linear regression is appropriate for outcome variables such as weight, that are continuous (quantitative) and have a Normal distribution (Chapter 3.4). It is not appropriate for binary outcome variables or for modelling the risk or prevalence of disease. For a binary outcomes, such as whether a herd is infected with a particular organism, logistic regression can be used (Chapter 2.3.3) (Greenland, 2008).

For easy interpretation of model outputs, a model coefficient or odds ratio (the exponential of the coefficient value) is displayed for each explanatory variable retained in the model. The value for a factor that is protective in relation to the outcome variable (e.g. a factor that reduces risk) will be represented by either a negative coefficient or an odds ratio less than one. Conversely, a factor associated with increased risk will be represented by a positive coefficient or an odds ratio greater than one. In general, the larger the coefficient or odds ratio for an explanatory variable, the larger the associated effect on the outcome. However, for continuous variables the coefficient or odds ratio represents the coefficient/ odds ratio for a unit change in the continuous variable e.g. an odds ratio of 2.0 would indicate that for every increase of the continuous variable, then the odds ratio would increase by two.

A test for an association between a single explanatory variable and the outcome is called a univariable analysis, but analysing multiple explanatory variables simultaneously can be achieved by carrying out multivariable regression. For multivariable models, a stepwise selection can be used to select and deselect explanatory variables to reduce a model to only

those that are significantly associated (as set by a determined *P*-value) with the outcome, whilst assessing for the variance accounted for by the other explanatory variables included in the model. The level of significance typically set in a model is a *P*-value of 0.05, which is that there is a 95% chance that it is correct to reject the null hypothesis that the association between the model variable and the outcome was produced by chance alone (Rothman, Greenland and Lash, 2008). In a regression model, the *P*-value is obtained by comparing the calculated statistic (z-value in a logistic regression and t-value in a linear regression) with a Normal distribution. The statistic is the coefficient divided by the standard error, If the *P*-value is significant, the 95% confidence interval for the coefficient does not cross zero (or one for odds ratios) e.g. 95% confidence intervals of 1.15-2.35 rather than 0.82-1.18 for odds ratio.

The analysis reported in this chapter describes how the data were used for preliminary analyses to examine relationships between the prevalence of MJ ELISA positive pigs and farm characteristics on a large population of pig farms. This analysis would help determine the suitability of using updates of this information for further analyses and to guide the direction of further analyses.

2.3.2 Materials and Methods

Creation of a combined QAS dataset

Datasets were supplied in 2007 by three pig assurance schemes covering the UK: Assured British Pigs (ABP); Genesis Quality Assurance (GQA); and Quality Meat Scotland (QMS). The datasets differed greatly in format and size, ranging from a single record per holding to a longitudinal dataset held in a series of subtables, with multiple entries per holding. Each dataset was assessed to determine how to consolidate all the data from the QAS into a single table and how to combine the records with the ZAP data.

The criteria for selecting explanatory variables for use in our analysis was that: the data had to be comparable across all three schemes; the variables had to be biologically plausible risk or protective factors for *Salmonella* infection; and the variables must not have a high proportion of missing values, as this would reduce the dataset available for multivariable analysis. These criteria omitted a large number of variables such as: whether teeth were clipped or tails docked; whether antibiotics and enzymes were used; whether feed was restricted or to appetite for the different age categories; and information on the ventilation of the pig houses.

The most recent record for each pig holding was selected from the datasets. Data in the ABP and GQA schemes were contained in subtables, and not all records could be linked to data corresponding to the same date from all of the subtables, due to missing data. The majority of these holdings could be linked to records from the same date, but 11% of the records

were linked to at least one subtable collected a year from the most recent date and 7% had to be linked to a subtable up to two years from the most recent date.

As the data for each scheme were collected and recorded in different ways, data were recoded to allow them to be compared across the schemes. In the ABP and GQA schemes, different categories of sows and pigs were recorded (e.g. in-pig sows, maiden gilts, pigs under 30 kg). These were recoded to match the variables collected in QMS. The number of sows was generated by combining: maiden gilts; in-pig gilts; in-pig sows; suckling sows; and other sows. The number of finishers was created by combining the variables 'feeders <30kg' and 'feeders >30kg'. Records with missing values for the number of pigs of a certain type were coded as zero if the number of any other pig type had been recorded for that holding. The total number of pigs was then created based on the number of pigs given in each pig category.

Data collected on the types of feed used were coded into three binary (yes or no) variables for whether: wet feeds were fed to finishers; compound feeds were fed to finishers; or homemix was used on the farm. Pellet and meal answers from ABP and GQA were combined as "compound feed" to match that recorded by the QMS scheme. Individual variables on whether any specific floor types, and bedding, were used for finisher pigs on that holding. A variable was also created to record whether or not all the pigs were kept indoors or whether any stage of the production was outdoors.

Postcode was used for each holding to locate a map reference (X & Y coordinates) in the GIS software ArcGIS 9.1 (ESRI), and these coordinates were used to identify the NUTS (Nomenclature of Units for Territorial Statistics) region for that holding. NUTS regions are commonly used in the analysis of spatial data as the boundaries are more stable and less subject to change over time than counties. For Northern Irish holdings, as Ireland uses a different coordinate system, the multimap website (www.Multimap.com) was used to collect an Ordnance Survey reference for each postcode, which was then converted into X & Y coordinates.

The distance between each holding was calculated and the number of other pig holdings within 3km and 10km calculated (the standard outbreak protection/ surveillance zone distances used in the UK). Variables for temporal trends and seasonal effects were also designed by adding sine and cosine terms for 3, 6 and 12 month periods to create quarterly, half-yearly and yearly cycles.

ZAP sample testing

Small pieces of muscle were removed from pigs at the abattoir. The samples were placed in a meat juice tube which was frozen and then thawed to collect the MJ fluid. The MJ sample

was tested by a mix-ELISA serological test to detect antibodies to *Salmonella* (Nielsen et al., 1998). Three samples were collected from randomly selected carcasses in every batch of pigs sent to slaughter (Armstrong, 2003; BPEx personal communication, 2010).

Connection to ZAP data

The holdings from the QAS were linked by holding identifiers to all unique samples collected by the ZAP scheme, within a time period of up to a year after the QAS record date. Due to errors and inconsistencies in the herdmarks provided in the QAS and ZAP schemes, additional identifying information and data checking procedures had to be used to ensure that the linked information was correct. For example, many records had missing herdmarks or the references were recorded with typing errors (e.g. 1C and IC recorded instead of 10) or with spaces in the ZAP herdmark between characters which did not appear in the QAS herdmark. The additional identifying information (certifier membership reference and postcode) was not consistently recorded by the three QAS or the ZAP programme and so some matching errors occurred when a) holdings had limited identifying information; b) a holding had moved between schemes, or c) a ZAP sample herdmark and membership reference linked to different holdings. To resolve these problems ZAP samples with duplicate records were matched to the holding with the record date closest to the sampling date. Once the datasets for each individual QAS had been verified and checked for duplicate samples, all three schemes were combined into a single dataset (see Table 2.1 for the final dataset).

Table 2.1: Explanatory variables in the final combined dataset.

Scheme	No. of sows
Country	No. of finishers
NUTS region	Total no. of pigs
Abattoir ID from where sample collected	Any finishers housed on full slats
X & Y coordinates	Any finishers housed on part slats
No. of farms within 3km	Any finishers housed on bedding
No. of farms within 10km	Any finishers housed on solid floor
3 month temporal trend	Any finishers fed wet feed
6 month temporal trend	Any finishers fed compound feed
12 month temporal trend	Homemix feed used
All pigs kept indoors	

Data Analysis

A positive sample, identified by the MJ ELISA cut-off value of 0.25, was included as a binary outcome in the logistic regression analysis. A multivariable logistic regression was completed in STATA 10 (Stata corp., college station, Tx) to model associations between exposure factors (management factors, herdsize, region etc) and the binary MJ ELISA outcome. One key assumption in binary logistic regression is that observations are independent of each

other. This assumption is violated if there are relationships between explanatory variables, where the inclusion of one variable influences another, for example a person's height and weight are not independent. Violations of the assumption of independence of observations may result in incorrect statistical inferences due to biased standard errors. Modelling records that are from groupings where we might expect some similarity in terms of outcome also violates the independence assumption. A robust cluster function was used to account for the assumption that sample results from the same farm would be more similar than those from different farms. This function uses the identifier of each animal or farm in the example to adjust the standard errors to a more robust variance estimate (Rogers, 1993; Williams, 2000). Clustering may also have occurred at the slaughter batch, farm company and abattoir level; however, these potential sources of clustering were not tested as data for these factors was less reliably recorded.

A univariable screening stage was used at the start of the analysis, where factors yielding a univariable *P*-value of over 0.25 were excluded from further analysis. Once the factors to be included in the model had been selected, (those with a *P*-value of ≤0.25), a backwards stepwise selection was conducted. A backwards stepwise selection begins with all explanatory variables present in the model. The least significant variable (as determined by the highest *P*-value and model fit) was removed at each step, until only those factors with a *P*-value under 0.05 were included. Records with missing data for the explanatory variables were dropped from the model. Once selection has been completed, each individual dropped explanatory variable was re-introduced to the model to check whether they could improve the fit of the model and to assess for potential confounding.

Model fit was tested by using a Hosmer-Lemeshow's goodness of fit test (estat gof function in STATA) to compare models, this is a modification of the Pearson Chi^2 statistic for use with logistic models only where there are many covariate patterns. The Wald's Chi^2 test and Akaike Information Criterion (AIC) were also examined. A significant Wald's Chi^2 test under 0.05 *P*-value indicates that the model as a whole was statistically significant. The AIC (Akaike, 1974) was used to compare the model fit for models of the same number of records, where a smaller AIC indicates an improved model fit. A further observation of model fit was made by checking for the appearance of large standard errors, which are indications of collinearity or model instability.

All holdings with available map coordinates were plotted onto a map of the UK using ArcGIS 9.1 (ESRI, Redlands, Calif., USA) to show the distribution of holdings and the spread of farms for each QAS. For individual NUTS regions, a case-control K-function analysis, which tests the null hypothesis of an equivalent degree of clustering in high prevalence and low prevalence holdings against an alternative of a differential clustering mechanism in the two groups (Diggle and Chetwynd, 1991) was completed using the Splancs library (Rowlingson

and Diggle, 1993) in the statistical package R 2.7.1 (R Development Core Team, Vienna, Austria). Data were examined to see whether higher prevalence holdings (those with more than 25% of samples positive) were more clustered in space than the other holdings. A descriptive analysis of the QA information for farms within each individual NUTS region was also completed with univariable logistic regression, used to compare the answers of a single region against all the remaining regions, to investigative whether there were regional differences in farm management.

2.3.3 Results

The final dataset of holdings that linked to at least one ZAP sample contained 1,535 holdings, 767 from ABP, 570 from GQA and 198 from QMS. A total of 45,557 samples were linked to these holdings, with a mean of 30 (1-370) samples per holding. The map (Figure 2.1) shows the location of the 1,415 QA scheme holdings that could be matched to map references. ABP farms were spread throughout England and Northern Ireland, GQA farms were more concentrated in Yorkshire and East England, and QMS only had farms from within Scotland.

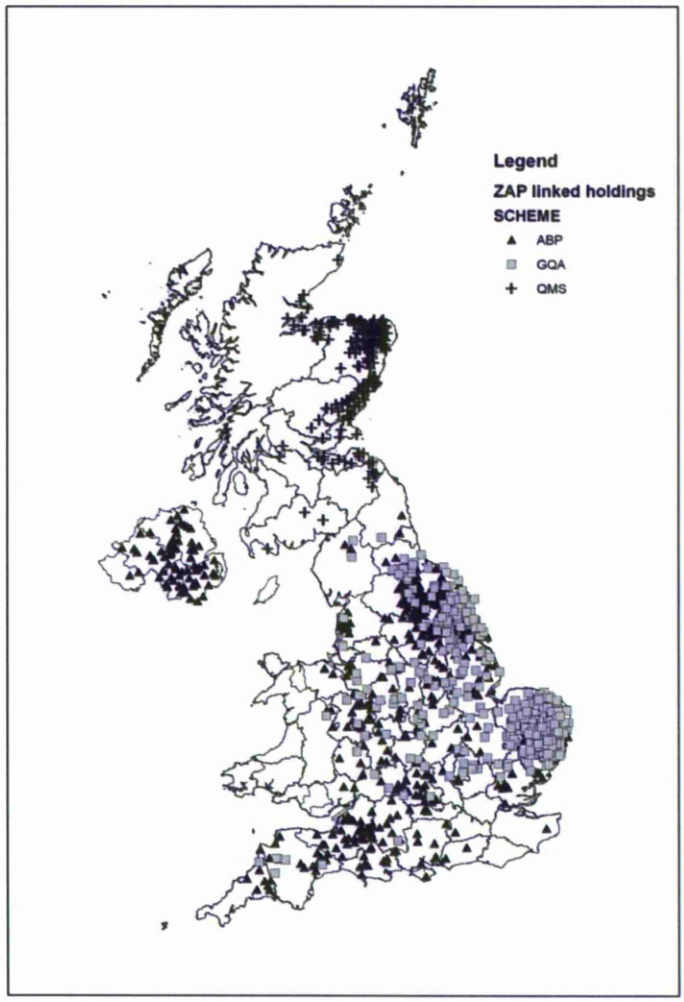

Figure 2.1: Map of the UK showing the position of pig farms in relation to their Quality Assurance scheme.

The descriptive analysis of the differences between farm management in different NUTS regions showed that there were distinctions between the regions of East of England, the South East, Yorkshire and the Humber, Scotland and Northern Ireland (Table 2.2). The differences between the other regions were less marked (not shown).

The significant results (*P*-value <0.05) of the analysis of the regional pig farm demographics showed that farms from Scotland and Northern Ireland had on average more pigs (total pig number and number of finishers), whereas farms from Yorkshire and the Humber and East of England had the least (Table 2.2). There was no significant difference in the number of sows between the regions. Farms in the East of England and Yorkshire and the Humber had more pig farms within a 10km radius. East of England and Northern Irish farms had a high density of farms within 3km, whereas farms in the South East had very low densities at either radius.

Factors relating to the use of flooring, housing and feeding were also found to be different between the regions (Table 2.2). In particular, holdings in Northern Ireland were managed differently, as wet feeding was more frequently practised in Northern Ireland and units were more likely to have at least one finisher building with fully or partly slatted floor without bedding, than in the other regions. More farms in Northern Ireland, and also Scotland, used homemixed rations (38.8% and 36.9% respectively) than the other regions. All holdings in Northern Ireland also recorded that all pigs were kept indoors, whereas more than half (55%) of holdings in South East England had some pigs kept outdoors. The results also show that finishers in Yorkshire and the Humber, and East and South East England were most likely to have been kept in pens with a solid floor and with bedding.

Table 2.2: Regional differences of the farm density, herd size and meat juice ELISA results of Pig QA scheme member farms.

Variable name	Yorkshire and the Humber		East of England		South East England		Scotland		Northern Ireland	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Farms within 3 km	2.6	0-12	4.1	0-21	0.2	0-2	1.4	0-11	3.4	0-11
Farms within 10 km	21.3	0-52	27.6	0-73	1.5	0-5	11.4	0-45	6.8	0-16
No. of sows	159	0-1,620	186	0-31,322	273	0-1,423	146	0-1,200	339	0-4,807
No. of finishers	1,950	0-10,000	1,779	0-17,000	2,707	0-11,976	3,910	0-30,000	2,937	0-45,050
Total no. of pigs	2,112	0-10,000	1,968	0-32,738	2,993	100-11,976	4,057	0-30,000	3,280	0-49,864
% of farms with:-										
Any full slats for finishers	37.6%		8.3%		29.7%		20.7%		80.4%	
Any part slats for finishers	22.6%		3.0%		37.8%		16.3%		36.2%	
Any solid floor for finishers	55.7%		85.5%		67.6%		10.3%		3.6%	
Any bedding for finishers	58.5%		87.5%		67.6%		68.0%		3.6%	
All indoor production	79.1%		90.9%		45.2%		86.2%		100.0%	
Use home-mixing	10.2%		9.9%		23.8%		36.9%		38.8%	
Use wet feed for finishers	4.2%		4.3%		4.8%		8.9%		43.2%	
No. samples	8,357		14,340		381		13,942		1,118	
% positive	42.0		31.0		33.6		9.8		20.1	
No. of farms	382		394		42		203		139	

The results of the multivariable logistic model indicated that a summarised region variable (Figure 2.2), and whether finisher pigs used any solid flooring, were the only variables that entered our final model. The summarised region variable joined geographically close regions that had similar farm management as shown by the univariable regional analysis. This indicated that samples taken from farms in Yorkshire and the Humber, and those that used solid flooring, were associated with a significantly higher risk of being positive (Table 2.3).

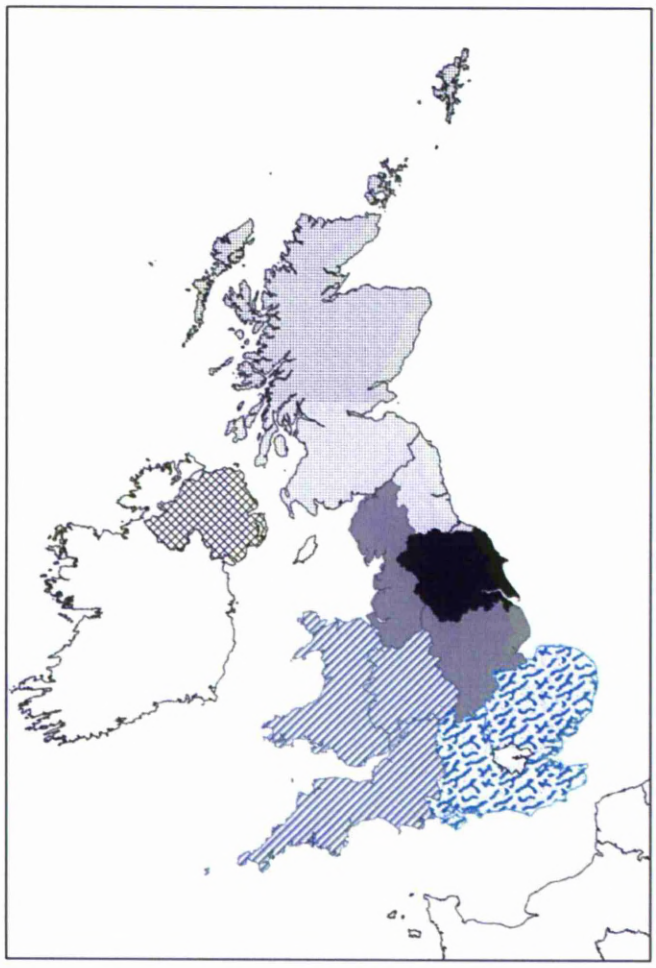

Figure 2.2: Map to indicate the grouping of neighbouring regions with similar pig farm management for use in multivariable analysis.

Table 2.3: Results of a multivariate logistic regression of pig Quality Assurance data and meat juice ELISA positive/ negative results, adjusted for the clustering of samples from each holding (1,333 holdings and 40,536 samples)

Variable	Level	# farms	# samples	Odds ratio (95% CI)	*P*-value
Region	Yorkshire and the Humber	359	8,014	1.00	-
	East Midlands & North West England	115	2,405	0.40 (0.26-0.62)	<0.001
	East & South East England	340	12,765	0.55 (0.44-0.68)	<0.001
	Scotland & North East England	224	14,391	0.16 (0.12-0.21)	<0.001
	Northern Ireland	144	1,316	0.34 (0.23-0.52)	<0.001
	Wales & South West England & West Midlands	151	1,645	0.24 (0.15-0.38)	<0.001
Finisher pigs – any solid flooring	No	650	20,280	1.00	-
	Yes	683	20,256	1.29 (1.06-1.57)	0.01

Figure 2.3 shows the difference in K-hat (the estimate of value K) between high (more than 25% of samples positive) and low prevalence farms (equating to a measure of the excess clustering in high- over low-prevalence farms) plotted against the distance between holdings. Significant excess clustering is indicated by the solid line being outside the simulation envelopes, which are created under a null hypothesis of an equivalent degree of clustering in both groups. The analysis showed that high prevalence farms were more clustered in space at just above the 3km mark, than low prevalence farms, in Yorkshire and the Humber (Figure 2.3). However, no clustering was found in the other regions, including the other region of high pig farm density, East of England (Figure 2.4).

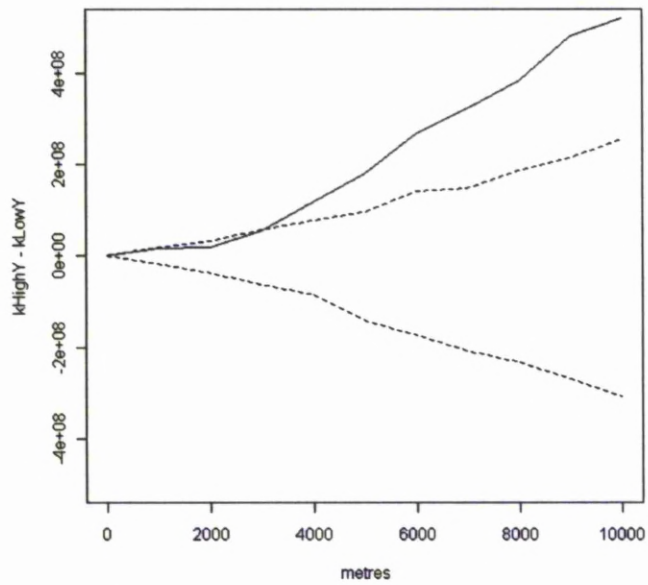

Figure 2.3: Plot of the difference in K-hat between high and low *Salmonella* prevalence pig farms in Yorkshire and the Humber and associated simulation envelopes (dotted lines).

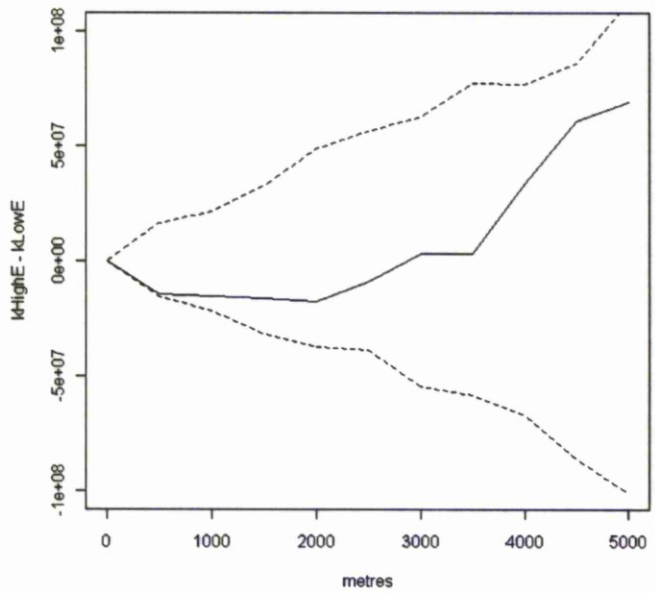

Figure 2.4: Plot of the difference in K-hat between high and low *Salmonella* prevalence pig farms in the East of England and associated simulation envelopes (dotted lines).

2.3.4 Discussion

The created dataset contained a large number of farms, dispersed throughout the pig farming areas of the UK. Although this dataset covered a large population, it only included farms that were QAS registered and sending pigs to slaughter and so this may have introduced some bias when extrapolating the findings to the whole UK pig population. For example, the findings would not provide evidence for *Salmonella* control for QAS registered specialist pig breeding holdings. However, it was hypothesised that the finisher pig farm population was of more interest to controlling human infection due to the closer temporal proximity between factors on finisher farms that might influence the pigs *Salmonella* status before it is slaughtered and enters the food chain. The author also believes that the population was very close to the total number of commercial producers found in all regions included in this study and so any finding from this study would be relevant for and representative of the UK pig industry.

The epidemiological analysis showed that the housing of finishing pigs on solid floors was associated with an increased risk of MJ ELISA positive samples in this study. This type of housing has been identified as a risk factor in previous studies (Nollet et al., 2004). Solid floor housing systems in the UK commonly use a push-through manure scrape system which can be responsible for spreading contaminated material from one part of the building to another, whereas slatted flooring is more effective at removing faeces from the pig's vicinity, and so assists to control a route of transmission of infection between pigs. Geographical

region was also included in our final model as a variable associated with MJ ELISA results, demonstrating that pigs from certain areas are more at risk of infection than others. As indicated by the univariable analysis, region represents differences in housing systems and pig farm management. The inclusion of region in the model, rather than the other factors, may indicate that these have been dropped due to collinearity with the region variable and that they did not entirely explain the variation caused by the region variable. Region could also be an indicator of other production practices; producers from the same area being exposed to the same disease control advice; producers using the same veterinary practices and feeding companies; or differences in regional weather conditions over the year. The use of postcode to generate coordinates may have produced some error, as the postal address of the farm may be different to where the pigs are kept. The grouping of the NUTS regions by similar locations and management may also have removed specific regional differences.

The exploration of the regional management differences shows that the high prevalence regions were more likely to contain fewer pigs per farm; have a greater number of farms within 10km; use solid flooring rather than slatted floor; and were less likely to use wet feeding and homemixing than low prevalence regions. However, there are some discrepancies between the results of the individual high prevalence regions and also between the low prevalence regions. The results for whether all pigs were kept indoors may also have been biased by whether a region had a higher percentage of specialist finisher farms in our study population, as breeding herds within the region would not have been present in the ZAP database. This is suggestive that the regional effect cannot be attributed to a single factor but relates either to a combination of factors or to regional varying factors that were not available in this analysis.

Variables seeking to describe the spatial clustering of farms did not enter the final multivariable model, but the K-function analysis shows that high prevalence holdings were significantly more clustered in space than low prevalence farms in Yorkshire and the Humber. The significant clustering in only a single region suggests evidence for either a regionally specific contagious mechanism, or for underlying, locally varying, risk factors within the region. Local spreading of *Salmonella* could occur through animal movements; when sourcing animals from a nearby breeding farm or from local farms linked to the same integrated company, where finisher farms at the bottom of the breeding pyramid are affected by the *Salmonella* status of the incoming pigs from the company's nucleus and multiplier breeding herds.

Due to the selection criteria, a large number of variables were dropped but the criteria ensured that the dataset contained holdings from all three schemes and that the multivariable model population was not greatly reduced in size by removing those records with missing data for a selected variable. The dataset may also have suffered from some

over matching of samples to farms, as some holdings had very little identifying information and so the linkage between holdings and samples could not be validated. In this respect, we would expect that these database problems would have added statistical noise to the analysis, hampering the detection of those factors associated with the prevalence of MJ ELISA positive pigs and reducing the power and the strength of the associations detected.

The use of the MJ ELISA may have introduced some problems for this type of analysis as it records the serological response to an earlier *Salmonella* infection and does not determine how recent the infection was. This may have explained why no seasonal trends were included in the final multivariable model, where it had been found to be significant in other studies (Christensen and Rudemo, 1998; Placha et al., 2001). The inclusion of ZAP records for each farm from more than year may improve the detection of temporal trends.

The study reported here shows the types of analysis possible with the data available and that a statistically powerful analysis is possible that would detect even weak associations, due to the large sample size. The utilisation of routinely collected information also provides a cost-effective route to large scale demographical data to be fed into risk assessments and help generate outputs that are representative of the UK pig industry. These types of analyses would provide the scientific evidence to help select factors that could control *Salmonella* on pig farms and shape national control plans. For example, if risk factors vary by region then control measures could be customised for each region. However, these types of data were not collected specifically for epidemiological analysis and so they provided problems due to the quality of the data. The errors in the collection of identifying information made it difficult to match QAS holdings to ZAP samples. The large amount of missing data, up to 70-80% in some instances, and the differences in which variables were collected by the three QAS, meant that many variables could not be analysed without compromising the number of holdings present in the dataset.

If increased effort was made to standardise the collection of a larger selection of variables and if stringent validation procedures were used, especially in the recording of holding identifiers, the range and quality of the dataset could be improved. The inspection of the data from the QAS also highlighted that other variables known to be significantly associated with *Salmonella* infection, identified through a literature review (Chapter 1.3), were missing and could be additionally collected by the schemes. Although this data analysis benefited from a very large study population, to provide a true estimate of the risk of the associated factors, a multivariable analysis would be needed that included substantially more of the variables that had been previously identified. This would enable covariance between variables to be analysed, as well as identifying the true effect of a variable whilst controlling for the variance of other significant variables. With these enhancements the schemes would provide vital,

updateable information to epidemiologists and risk analysts to allow them to carry out complex analyses with high statistical power and confidence.

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Chapter 3: Analysis of associations between *Salmonella* seroprevalence and explanatory factors, collected from questionnaires and Quality Assurance schemes

3.1 Introduction

Salmonella enterica is a zoonosis and can be carried by livestock raised for food production. Human salmonellosis is characterized by diarrhoea and infection can be through foodborne routes (O'Brien, 2005). The importance of pigs as vectors of *Salmonella* has been shown by a large abattoir study where the prevalence of *Salmonella* in pig caecal samples, collected in Great Britain, was high (23.4%), when compared to both cattle and sheep (1.4% and 1.1% respectively) (Milnes et al., 2007). In a European Union baseline survey in 2007, a similar level (21.2%) of *Salmonella* was isolated from mediastinal lymph node samples from United Kingdom (UK) pigs at slaughter (EFSA, 2008). Infection in pigs can cause a range of clinical signs, from scouring to fever and death, but is often sub-clinical and so, is difficult for farmers to monitor and detect. Although it is unknown how many cases of human salmonellosis are attributed to eating pig products, of the 13,213 laboratory confirmed cases in the UK identified in 2007, 13% were related to the serovar *S.* Typhimurium, which is the predominant type detected in samples from UK pigs (Defra, 2007; VLA, 2007).

Many studies have tried to ascertain the factors that influence *Salmonella* prevalence, and identify on-farm control measures to reduce the *Salmonella* burden in pigs. Recent studies in the UK have highlighted associations with factors such as herd size; outdoor rearing of pigs; flooring type; and farm location (VLA, 2004; Pritchard et al., 2005; Smith et al., 2011). These findings have been supported by European, Canadian and American studies (Funk, Davies and Gebreyes, 2001; Nollet et al., 2004; Farzan et al., 2006). Seasonal peaks and troughs of *Salmonella* prevalence have been identified by studies, with a two-peaked annual cycle apparent, which may be related to meteorological conditions such as environmental temperature (Funk, Davies and Gebreyes, 2001; Hald and Andersen, 2001). However, a number of the studies above were limited to a small and potentially unrepresentative subset of the pig farm population, which may not have had sufficient statistical power to detect modest associations between *Salmonella* infection and putative risk factors. Other studies analysed only a small number of variables and so may have missed more important risk factors or not estimated the true effect of a variable by accounting for the potentially confounding effect of other variables.

Schemes are present in the UK that routinely collect data on *Salmonella* in pigs and farm management characteristics, from a large number of farms. In June 2002, the UK Zoonoses Action Plan (ZAP, now called the Zoonoses National Control Plan (ZNCP)) 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 (Armstrong, 2003). The scheme was based on a design by the Danish pig industry that had contributed to a reduced *Salmonella* prevalence (Nielsen et al., 2001). The QAS routinely collect details on the structure and management of pig farms to ensure a level of health and welfare standards are met.

The data collected by the QAS were found to be useful for epidemiological analysis, and with a coverage that was largely representative of the UK finisher pig farm population, although it lacked many of the variables associated with *Salmonella* identified from a literature review (Chapters 1.3 and 2.3, Smith et al., 2011). In light of these findings, a purpose-built postal questionnaire (Chapter 3.2 and appendix A) was designed to supplement the QAS information. The returned questionnaires were linked to data to allow for temporal and spatial analysis, and the data were transformed where necessary to allow epidemiological analysis (Chapter 3.3). Finally, a full epidemiological analysis was completed on the dataset (Chapter 3.4).

3.2 Design of supplementary questions form

A postal questionnaire was designed to supplement the information collected by the three Quality Assurance schemes (QAS) with additional data on potential risk factors for *Salmonella* infection. The design of the questionnaire was originally based on all the data gaps in the QAS data as indicated from the *Salmonella* risk factor literature review (Chapter 1.3). A summary of the data gaps are given in Table 3.1.

Table 3.1: List of on-farm risk and protective factors identified by previous pig *Salmonella* studies, not collected by the Quality Assurance schemes.

Risk factor	Protective factor
Biosecurity	
Herds recruiting from >3 supplier herds	Breed their own replacement stock or recruit from a maximum of three supplier herds
Wildlife presence	
Number of visitors, sharing of equipment	
Nose to nose contact with neighbouring pigs	
Cleaning & disinfection	
	Cleaning regime
	Use of disinfectants
	Batch production system
	Emptying the pit below the slatted floor after batch
Feed/ water	
	Coarsely ground feed
	Liquid feed containing fermented by-products
Contaminated feed	
	Feed and water additives
	Barley/ wheat concentrations
Weather/ seasonality	
Air temperature	
Pig age	
Salmonella status of sow	*Salmonella* status of sow
Weaning age	
Mixing of pigs	
Treatments	
	Use of prebiotics or probiotics
Use of antibiotics and antibiotic growth promoters	
	Salmonella Vaccine use
Co-infection	
Lawsonia	
Porcine Respiratory & Reproductive Syndrome Virus (PRRS)	
Milk spot liver	
Previous diagnosis of clinical *Salmonella* infection in the herd	
Postweaning Multisystemic Wasting Syndrome (PMWS)	
Pneumonia	

The list of factors was sent to a number of epidemiologists and pig experts at the VLA to elicit the following:

1) Whether these variables could be collected from any other sources of data that had already been collected by previous VLA projects;
2) What the optimum size of a questionnaire should be, so as not to overburden the farmer and risk under representing the pig farm population through poor participation rates;
3) Which factors were of high or low priority for collection in relation to *Salmonella* control;
4) Whether any factors of interest had been left out of the list and whether grey literature sources, such as unpublished work, could provide evidence of their effect.

This review was used to produce the original list of factors that fitted four sides of A4 paper (the optimal size suggested that would maximize the data collected but not hamper farmer participation). The review also highlighted important factors for *Salmonella* control that would have been very difficult to collect from a postal study e.g. the use of disinfectants, as most farmers would not easily remember or would not record their specific type, concentration and usage. Other factors were ranked as low priority as they would not assist in finding differences between farms, as the practice or management factor would be too widespread. For example, most farmers share equipment and so it would be difficult to analyse the potential risk of sharing equipment. The final panel of important factors for which questions would be needed was decided as the following:

- Feed, especially use of acidified feed and water;
- Flooring;
- Movements of animals;
- Number of young pigs and total number of pigs. It was decided to ask for the number of pig places on the farm as the QAS dataset asked for the number of pigs currently on the farm and this may not have been representational of the herd over a 12 month period;
- Number of other farmed animals on site;
- Mixing of young pigs, and mixing of sick animals with others;
- Feed mills used;
- Types of water source and types of drinker;
- When and how are pig houses cleaned and disinfected;
- Wildlife presence;
- Vehicles and personnel who visit the farm;
- Herd health status;
- Use of antibiotics, growth promoters, probiotics, prebiotics and vaccines;
- Type of farm enterprise and production system;
- Number of herds used to buy stock from and the number of movements onto the farm.

Information on the actual movements between pig farms could be collected from the Animal Movement Licence System (AMLS). Meteorological data, to indicate weather condition difference between regions and between seasons, would be collected directly from the Met Office. Colleagues at Liverpool University provided information on the suitability of the data for network analysis.

Previous *Salmonella* and pig farm studies (OZ0323, An integrated risk-based approach to the control of *Salmonella* in UK pig farms; ED1006, FIATEST; FZ2014, Use of routine data to investigate risk factors for *Salmonella* spp. infection to pigs; OZ0316, Epidemiology studies of *Salmonella* in pigs and control by intervention) were examined for successful ways of wording questions. Otherwise, new questions were designed with input from VLA pig experts. To refine the draft questionnaire, it was decided that to ensure the size of the questionnaire did not become overly large, questions of wildlife and pest control were removed, as it was shown from previous studies that the quality of data would be low, especially when collected from a postal questionnaire.

Finally, the standard confidentiality notice was added to the questionnaire along with a notes section so that farmers could add any other relevant comments. The questionnaire was then submitted to Defra's form design unit to assess its burden on the farm and veterinary population and to ensure the quality of the questionnaire was acceptable.

3.2.1 Piloting of questionnaire

The draft questionnaire was sent to a total of five pig farm personnel, two commercial pig farmers recommended by a colleague and three pig farm workers from the VLA animal services unit. Each participant was asked to complete the questionnaire and to complete a feedback form (appendix B).

The results of the feedback form showed that the questionnaire took a mean average of 15.8 minutes to complete (range 10-25). Both commercial farmers, but none of the three pig farm workers, needed to consult their records (both paper and computer) to answer the questions. This pointed out that feed (Q2.3), pig deliveries (Q2.4) and 4.2 and 4.3 (ailments) may require the farmer to refer back to his records. However, the time required to check the records was only five minutes and the total time taken was still within the boundaries that experts had advised would not dissuade farmers from completing the form. Positive comments were also received from the participants that stated that the form was easy to complete and the wording very clear.

The pilot participants suggested a number of improvements to the form:

- For Q2.7, it was suggested that "between batches" and "every 5 months at batch end" should be added as options for the cleaning of drinking system in finisher housing and so the range of answers was changed to: ☐ Between batches ☐ Every other batch ☐ Quarterly ☐ Biannually ☐ Annually or less
- The presence of cats and dogs should be removed from Q1.5 as these are ubiquitous on farms and the results would be of no use. After assessing data from other recent pig farm studies, this was accepted and the two rows were removed from the question table.
- It was also noted that one farmer forgot to add the letters in front of the assurance scheme number at the start of the form and so the form was adjusted to highlight that the full assurance scheme membership identifier was required.

3.3 Collection of questionnaire data and preliminary analyses

3.3.1 Collection of questionnaires

Once the questionnaire had been finalised (Chapter 3.2), the three QAS were approached for their permission for the questionnaire to be completed by their members. It was agreed that the questionnaires would be sent to a list of private vets, supplied by the QAS, who completed the quarterly visits to the members. The questionnaires were sent in June 2007, along with a letter and supporting information (appendices C-E) explaining the aims of the study and asking for the vets to complete the questionnaire with each scheme member at their next quarterly visit. The completed questionnaires were sent to CERA in the supplied reply-paid envelopes, and the data were entered into a Microsoft Access database by administrative staff.

The initial return of questionnaires from the ABP and GQA schemes was poor, and 25 vets, who were linked to six or more members and had not returned a questionnaire, were contacted by telephone to encourage them to participate in the project. By 25th January 2008, a total of 104 questionnaires had been returned and so, in agreement with the QAS, it was decided that a letter and a questionnaire would be sent directly to the members who had not yet completed one (appendix F). This was not required for the QMS scheme, where a single representative of the scheme took responsibility for organising and collecting the questionnaires at audit visits. To further improve participation from all three schemes, an inconvenience payment of £20 was paid to each member for the completion of the form. Private vets and farmers who had already completed questionnaires were also sent the inconvenience fee. The large pig farm companies in the UK were contacted individually to ask for their consent to contact their farmers (appendix G). All of the companies agreed for the forms to be sent out directly to their farmers, although two companies asked for the forms to be sent to their company vet for them to collect the information.

A total of 671 questionnaires were received, 104 by vets during the initial period (6th June 2007-25th January 2008) and 567 direct from farmers or company staff (22nd February 2008-29th October 2008). The identifying information supplied on the questionnaires was used to link each member to up to four years of their most recent *Salmonella* surveillance sample results. A total of 566 members were linked to surveillance records from an updated dataset of serology records, the remaining 105 were possibly either breeder farms that had mistakenly completed a questionnaire or farms with membership identifiers that were incorrectly transcribed and did not correspond to any surveillance samples.

The 566 questionnaires used for analysis came from all three QAS, and when compared against the full QAS member population recorded in the schemes data extract, this

represented 20% of GQA members (171 members), 29% from QMS (90) and 16% from ABP (305). The study population had a broadly similar distribution of farms in each NUTS region (Table 3.2), although with less farms recruited from East of England and more from Scotland and the South West. This may have been due to the difference in recruitment in Scotland where a local QAS representative was involved with enrolling farms, whereas East of England may have had a reduced population, as it is believed that more specialist breeder farms come from there. The mean herdsize of the study population was higher than the mean of the QAS population, where data was available (QAS = 2,200 (n=2,967), study = 2,621 (n=427), T-test P=0.004), which may indicate that a number of smaller herds in the QAS population did not participate in the study. Otherwise, it was believed that the study population was representative of the QAS members.

Table 3.2: Comparison of the distribution of pig holdings by NUTS region for a studied population and the population of Quality Assurance Scheme (QAS) members from which they were enrolled. Four study population farms did not provide sufficient data to be linked to a NUTS region and were omitted.

NUTS region	Study population	% of study total	QAS population	% of QAS population total
East Midlands	33	5.9%	397	8.1%
East of England	106	18.9%	1,400	28.6%
Northern Ireland	43	7.7%	249	5.1%
North East	6	1.1%	96	2.0%
North West	17	3.0%	190	3.9%
Scotland	93	16.5%	319	6.5%
South East	23	4.1%	319	6.5%
South West	63	11.2%	394	8.0%
Wales	6	1.1%	42	0.9%
West Midlands	33	5.9%	318	6.5%
Yorkshire and The Humber	139	24.7%	1,177	24.0%
Total	562		4,901	

3.3.2 Gathering of data for spatio-temporal analysis and data transformation

The use of four years of *Salmonella* seroprevalence test results was decided upon to provide a suitable dataset for the assessment of temporal trends and trends in meteorological factors, supplied as monthly regional averages by the Met Office. Rather than using a particular cut-off point to determine whether a meat juice sample was positive, as had been used in previous studies (van der wolf 2001; Gebreyes et al., 2008; Benschop et al., 2008a; Smith et al., 2011), it was decided to assess the effect of modelling the sample:positive ratio directly. By using this approach, it was hoped that risk factors would be found for farms with higher levels of *Salmonella* antibody serology, which might indicate that these farms had either pigs under a greater challenge of *Salmonella* or more recent infections.

A Box-Cox plot (Figure 3.1; Box and Cox, 1964) was used to assess whether the sample:positive ratio required transformation to ensure the model outcome would approximate normality and meet the standard modelling assumptions. The Box-Cox score of -0.101 indicated that logarithmic conversion would be the most suitable transformation. Histogram plots of each individual continuous explanatory variable were assessed by eye and by Anderson-Darling tests to check whether the variables had a normal distribution and whether data transformation was necessary. Each participating farm that could be linked to X and Y map coordinates where plotted onto a map of Great Britain and Northern Ireland, to link each farm to the corresponding NUTS region that they fell within (as described in Chapter 3.4). The map coordinates of the full QAS member population were used in Microsoft Access to measure the distance in kilometres between each holding using Pythagoras' Theorem, where the distance between two points equals the square root of the sum of the squares of the difference between the two X coordinates and the two Y coordinates. These data were then used to calculate the number of QAS member farms within three and ten kilometre radii of each participating farm (Figures 3.2 and 3.3). Three and ten kilometre radii were used as these are the standard control zones (protection and surveillance zones) used by Defra for determining farms in risk of infection from an outbreak of an infectious disease.

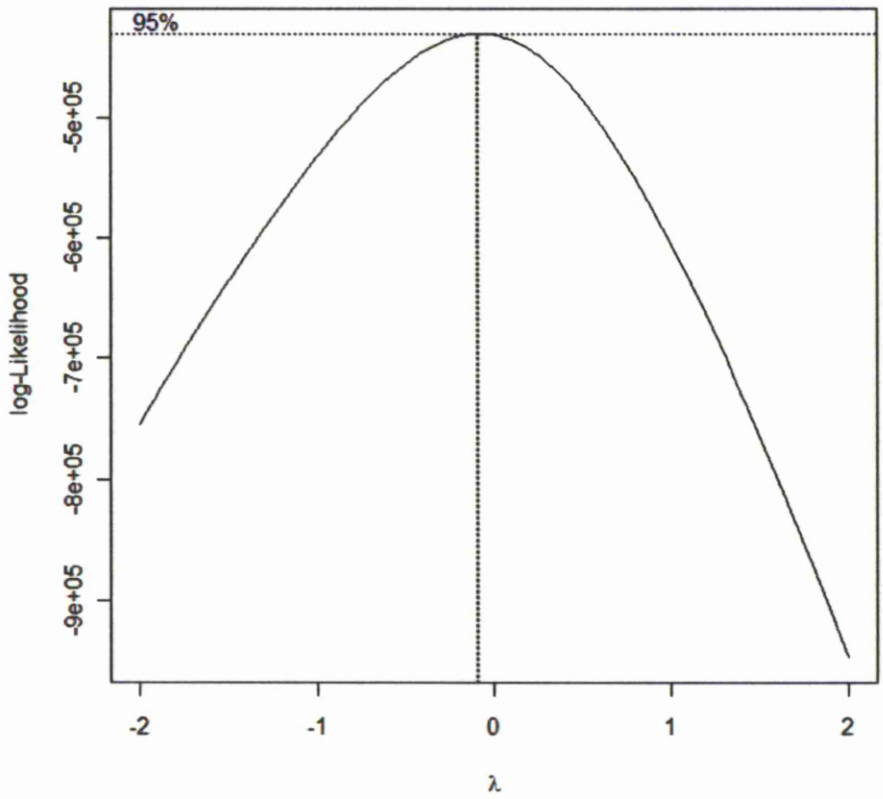

Figure 3.1: Box-Cox plot of the sample:positive ratio result of a serological test of meat juice samples collected from slaughtered pigs, to determine which type of transformation would approximate a normal distribution.

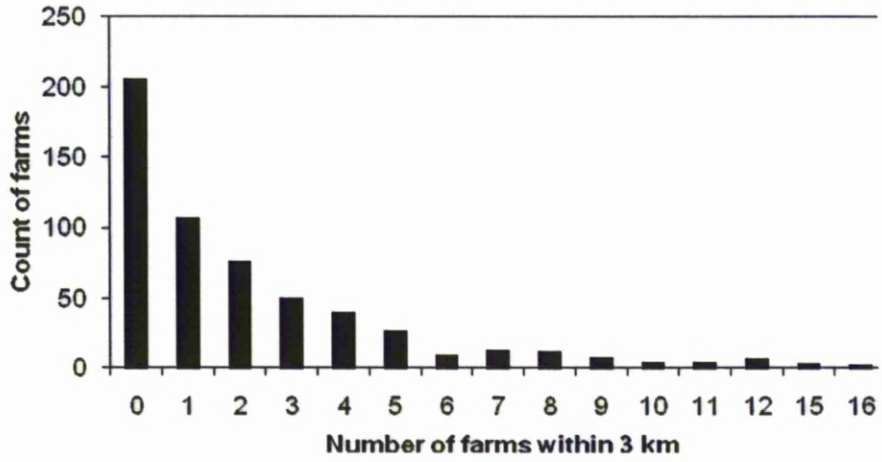

Figure 3.2: The density of Quality Assurance scheme members within three kilometres of each participating study pig farm.

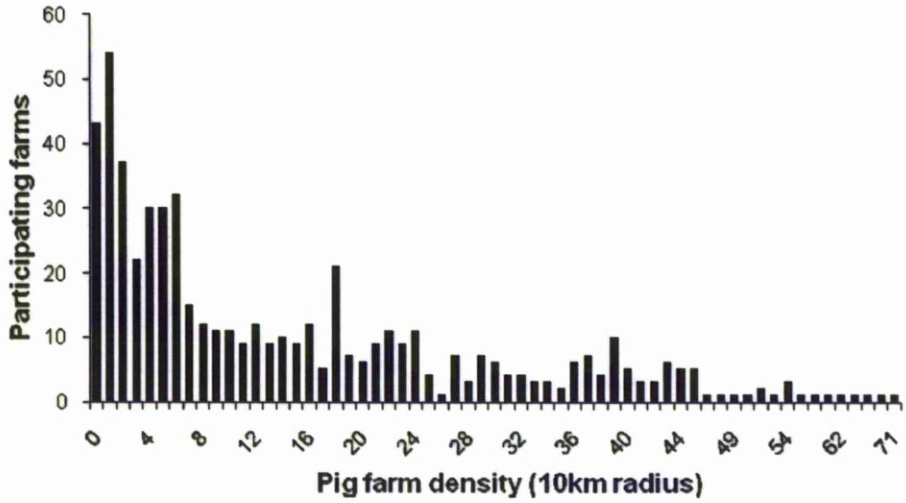

Figure 3.3: The density of Quality Assurance scheme members within ten kilometres of each participating study pig farm.

3.4 Epidemiological Analysis of meat juice ELISA results and questionnaire data to investigate farm-level risk factors for *Salmonella* infection in UK pigs

3.4.1 Introduction

This analysis reports how data from the QA and ZAP schemes were used, along with a postal questionnaire, to implement a cross-sectional study to analyse the effect of a large number of explanatory factors (biosecurity, farm demographics, meteorology) on *Salmonella* seroprevalence for QAS-registered finisher holdings, in the UK.

3.4.2 Materials and Methods

Data on explanatory factors were collected from a number of sources and combined into a single dataset, for analysis in the model. Datasets were collected from three QA schemes (Approved British Pigs (ABP); Genesis Quality Assured (GQA); and Quality Meat Scotland (QMS)) and from the ZAP scheme. The data were coded and linked to map reference coordinates according to the previous method (Smith et al., 2011). These coordinates were also used to identify the NUTS (Nomenclature of Units for Territorial Statistics) geographical region for that holding. NUTS have four subdivisions and NUTS 1, equivalent to government office regions, were used rather than other sources of clustering, such as county, as they are more stable over time and less subject to boundary changes than counties. It was also believed that the categories represent more biologically sensible categories in terms of the country's animal species population. Meteorological data of monthly regional summaries, including actual and 'anomaly' (difference from long-term averages) records, were gathered from the Met Office website (http:www.metoffice.gov.ukclimateukindex.html) and linked to the dataset by the region of farm and the month of sample collection. A supplementary questionnaire was designed to collect information on a number of covariates previously identified as significantly associated with *Salmonella* presence and rated as key to *Salmonella* presence in the UK by a number of experts. These included pig stocking levels (Farzan et al., 2006); feeding practices (Lo Fo Wong et al., 2004); housing systems (Nollet et al., 2004); biosecurity (Beloeil et al., 2004) and geographical location (Benschop et al., 2008), to supplement those routinely collected by the QAS (Table 3.3). The questionnaire was posted, along with a covering letter, to all 2,064 farms listed under the three QAS, asking for the farmer's voluntarily completion of the questionnaire, which was to be returned in a supplied envelope.

Table 3.3: Variables generated from data collected by Quality Assurance schemes and a postal questionnaire.

Variable category
NUTS 1 region
Coordinates (X, Y)
Pig farm density at 3km & 10km radii
Season of sampling
Quality Assurance Scheme
Enterprise type
Reared on contract
Production system (batch/ continuous)
Any pig production outdoor
Flooring
Number of each pig type
Other farm animal species present
Mixing of pigs
Isolation of sick pigs (freq, where)
Types of feed fed to weaners, growers, finishers and sows
Drinking system and water source
Cleaning & disinfection of pig houses and drinking system
No. pig deliveries/ collections
No. and type of other farm visitors
Delivery procedures
Boot dip usage
Health conditions present
Top 3 causes of pig mortality
Top 3 causes of pig treatment
Regional summaries of meteorological factors
Temporal cycles

The ZAP data were limited to results collected up to four years prior to the completion date of the postal questionnaire, to allow a comparison of temporal trends over a number of years. Variables for temporal trends and seasonal effects were designed by adding sinusoidal components (sine and cosine terms) for 3, 6 and 12 month periods to create quarterly, half-yearly and yearly cycles (Chatfield, 2003). These cycles may account for seasonal trends or any reduction of ELISA ratio through the years of the study population caused by the control of *Salmonella* through the ZAP scheme.

For the ZAP scheme, small pieces of muscle (from diaphragm/ neck) were removed from pigs at the abattoir and placed in meat juice (MJ) tubes which were frozen and then thawed to collect the fluid (Nielsen et al., 1998; Armstrong, 2003). The MJ sample was tested at a single UK laboratory by a mix-ELISA serological test (Guildhay VETSIGN™Kit) for a "host" response of antibodies to Group B and C_1 *Salmonella* (Nielsen and others, 1998). *Salmonella* infection in pigs produces an immune response, which includes the production of antibodies. These are detected by the ELISA from which a sample to positive ratio (ELISA ratio) was calculated, which was related to the titre of circulating antibodies (Sorensen et al.,

2004; Hill et al., 2008). Three samples were randomly collected from every batch of pigs sent to slaughter on any particular date in accordance with the sampling regime agreed on May 2003, but samples collected from late 2006 onwards were collected at a rate of five per farm per month (BPEx, personal communication, 2010). For routine surveillance, a cut-off point is applied to the ELISA ratio to provide a binary outcome but for this study the ELISA ratio was used directly to allow an analysis of a linear relationship.

Data analysis

Multivariable regression modelling of the ELISA ratio results was completed. The linear model required four assumptions to be observed: independence (described in chapter 2.3.2 data analysis); normality; heteroscedasticity/ heterogeneity; and linearity (Zuur et al., 2009). Before model selection, exploratory histograms were used to examine whether each continuous outcome and explanatory variable showed a normal distribution or whether significant outliers were present. After a model had been selected a plot of the model residuals was used to determine whether the remaining variance not explained by the significant variables showed normality and whether the final model still met this assumption. Transformation of a variable can fix non-normal distribution. A Box-Cox plot was used to verify whether the ELISA ratio results required transformation and what type of transformation was necessary to approximate normality (Box and Cox, 1964). All negative and zero ELISA ratios were coded to 0.005, which was half of the lowest recorded result, prior to transformation.

Heteroscedasticity/ heterogeneity cover the assumption that the variance of the data should be the same for each record. This was tested by plotting the standardised residuals from the final model against the fitted values to examine for a roughly even spread of residuals at each fitted value. Each explanatory variable retained in the model was compared against the model residuals by using a Bartlett test to assess for homogeneity of variances (R version 2.7.1, R Development Core Team, Vienna, Austria). However, this test is sensitive to violations of normality.

To test for linearity, the model residuals were plotted against the explanatory variables to locate any 'bowed' patterns, which would indicate non-linearity. Any identified non-linear relationships were fixed either by transforming the variables or by utilising moving to a model type that was less affected by non-linear relationships e.g. a generalised additive model.

Some additional assessment for re-coding of categorical explanatory factors was completed before starting the model, with factors with more than two levels tested to see whether they should be split into multiple dichotomous variables. For example, a variable with levels for each NUTS region was tested at the univariable level, as well as binary variables for each individual region, to see which factor was more significant/ fitted the model better. Where

biologically plausible interactions between explanatory variables were identified in the dataset, then interaction terms between these variables were also added to the model and tested along with the other variables. Relationships between the transformed ELISA outcome and the explanatory factors were analysed by mixed linear regression (STATA 10, Stata corp. LP, College Station, TX), with the farm holding identifier selected as a random effect, to allow for dependence between observations within the same premises. A random effect accounts for the effect of the correlated observations linked to a farm or animal identifier and provides estimates of either a random intercept or random coefficients for each clustered group (Venables and Ripley, 2002). As a large number of explanatory variables were to be tested, a univariable screening stage was used so that any variables that yielded a *P*-value of more than 0.25 were omitted from the multivariable model selection.

Due to the large number of factors under examination, variables were entered into the model manually using a forward stepwise method rather than backwards selection. In circumstances where there are many explanatory variables and a high degree of missing values, then a backwards stepwise selection would begin with a smaller number of records due to the missing variables being omitted, and this starting model may not be representative of the full study population. The process begins with an empty model and the variable with the lowest *P*-value on univariable analysis was entered first into the model, and each subsequent variable was then independently introduced into this model before selecting the next variable with the lowest *P*-value and repeating the process. Due to the large dataset size, a *P*-value of 0.01 was set as the significance threshold and this stepwise method continued until no further variables could be identified whose addition generated a *P*-value of less than 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.

Covariance can be assessed through an autocorrelation plot of the model residuals or collinearity tests of each pair of explanatory variables. When variables show a high degree of collinearity (>0.8), an assessment of the model fit of each inclusion of each individual variable can be used to decide upon which should be dropped from the analysis. Explanatory variables, that are perfectly collinear with variables already included in the model, are dropped automatically by STATA.

Likelihood ratio tests were used to compare models of the same population size to determine whether the included variable significantly improved the model. Likelihood ratio tests can also be used to compare models of the same population size to determine whether the included variable significantly (*P*-value<0.05) improves the model. A further parameter that may determine a problem with model fit would be the appearance of large standard errors, which are indications of collinearity or model instability. The Wald's Chi^2 test and Akaike

Information Criterion were also examined to assess model fit. A significant Wald's Chi^2 test under 0.05 *P*-value indicates that the model as a whole is statistically significant. The AIC can be used to compare the model fit for models of the same number of records, where a smaller AIC indicates an improved model fit.

The farm holding records with map references were plotted as points onto a map of the UK using ArcGIS 9.1 (ESRI, Redlands, Calif., USA).

3.4.3 Results

Between 6th June 2007 and 30th October 2008, a total of 566 questionnaires were returned and successfully linked to the ZAP database. These questionnaires consisted of 305 ABP, 171 GQA and 90 QMS registered holdings. The 554 holdings that provided the necessary information to generate map coordinates were presented on a map (Figure 3.4). This shows the distribution of participating farms around Great Britain and Northern Ireland, and the large difference in farm density between regions such as Yorkshire and the Humber and North West England (Smith et al., 2011). A X^2 comparison between the holdings present in the study population and the total QAS population indicated fewer farms in East England and more in Scotland and the South West ($P<0.05$). The holdings linked to a total of 119,906 ZAP samples, with a mean average of 224 samples per holding (range 1-1,671). Plots of the ELISA ratio results (Figures 3.5 and 3.6) indicate that a seasonal average ranged from 0.25 (autumn) to 0.22 (spring and summer) and a comparison of means showed that this was significant ($F=29.09$, $P<0.001$), and also the mean ELISA ratio differed greatly ($F=12.75$, $P<0.001$) between each year of sampling. The seasonal trend was consistent between years, with the highest mean ELISA ratio detected in autumn in all complete years, apart from 2004 where the winter season had the highest ratio. The majority of ELISA ratio results were close to zero (60.9% were below 0.10) and a Box-Cox plot verified that a logarithmic transformation was required to approximate normality.

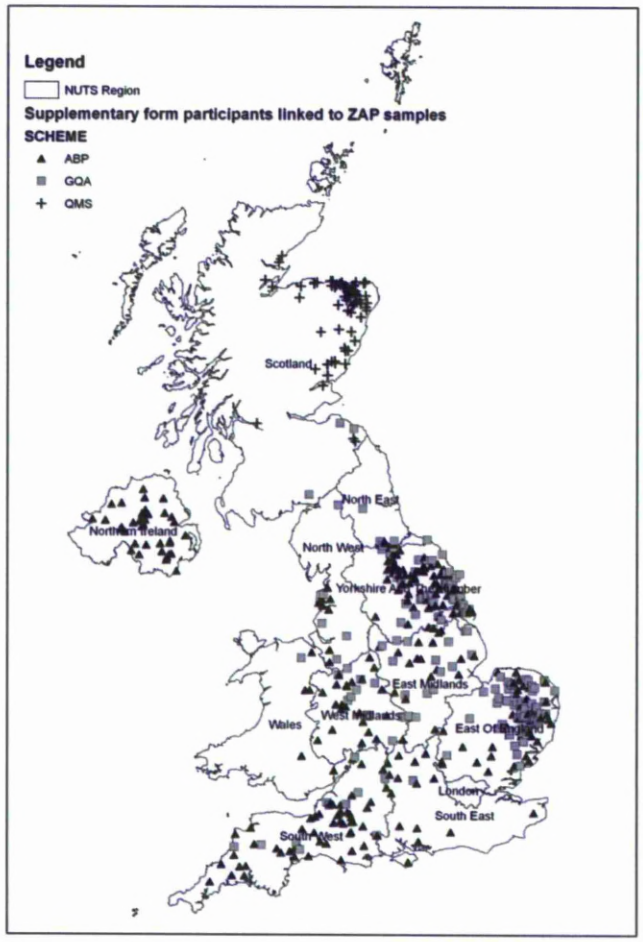

Figure 3.4: Distribution of participating pig holding locations by Quality Assurance scheme (N=554).

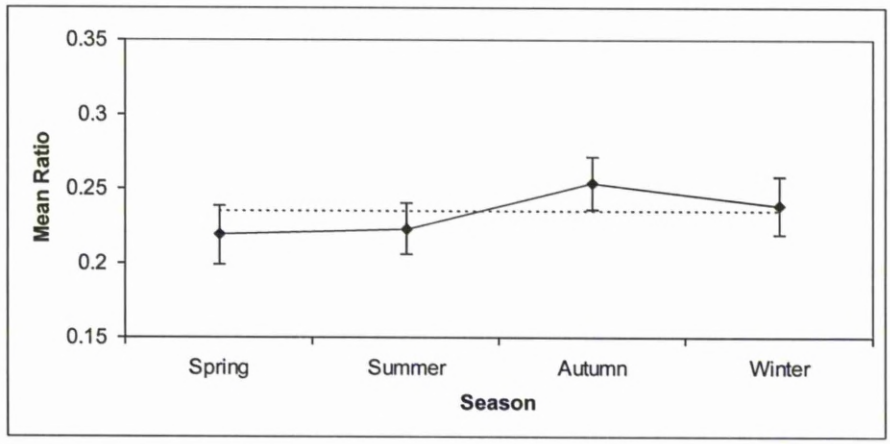

Figure 3.5: Mean meat juice ELISA ratio results, with 95% confidence intervals, by season of sampling, for 566 pig holdings. Dotted line indicates mean ELISA ratio.

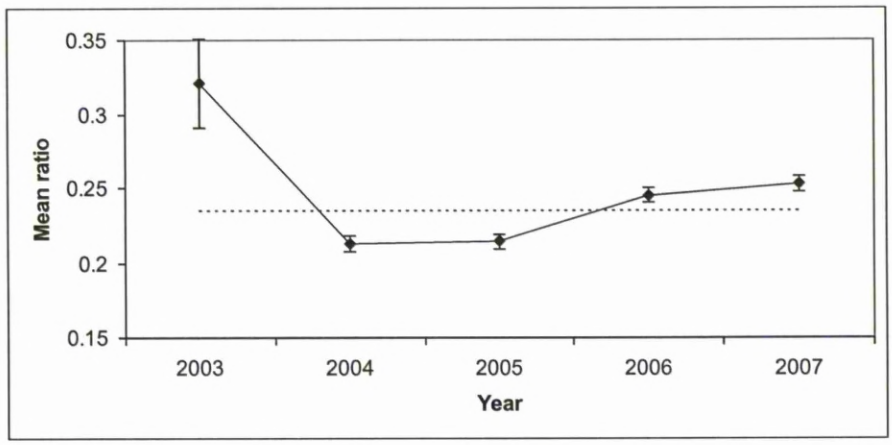

Figure 3.6: Mean meat juice ELISA ratio results, with 95% confidence intervals, by year of sampling, for 566 pig holdings. Dotted line indicates mean ELISA ratio.

The results of the linear regression model are presented in Tables 3.4 and 3.5, with Table 3.4 presenting the strongly significant variables detected from the univariable screening of the variables (full results are presented in appendix H) and Table 3.5 presenting the final variables that remained in the multivariable model.

Table 3.4: Variables strongly associated ($P<0.05$) with *Salmonella* from univariable mixed linear regression of logged meat juice ELISA ratio results collected from slaughtered pigs.

Variable	Level	Coefficient	*P*-value	No. farms
QA scheme	ABP	Baseline		305
	GQA	0.458	<0.001	171
	QMS	-0.658	<0.001	90
NUTS Region	Other	Baseline		469
	Scotland	-0.824	<0.001	93
Pig farm density within 3km radius	Continuous	0.085	<0.001	554
Pig farm density within 10km radius	Continuous	0.021	<0.001	554
Season that sample was collected from	Spring	Baseline		n/a
	Summer	-0.169	<0.001	n/a
	Autumn	-0.133	<0.001	n/a
	Winter	-0.099	<0.001	n/a
Farm enterprise - Conventional	no	Baseline		51
	yes	-0.741	<0.001	515
Farm enterprise - Freedom foods	no	Baseline		480
	yes	0.583	<0.001	86
Pigs reared on contract at farm	no	Baseline		292
	yes	0.385	<0.001	254
Cattle present on farm	no	Baseline		373
	yes	-0.282	0.001	193

Table 3.4 Cont.

Variable	Level	Coefficient	*P*-value	No. farms
Number of cattle currently present	Continuous	-0.001	0.019	537
Sheep present on farm	no	Baseline		419
	yes	-0.241	0.011	147
Cats present on farm	no	Baseline		554
	yes	0.636	0.025	12
Pigs mixed at weaner group	no	Baseline		142
	yes	-0.368	<0.001	390
Pigs mixed at other time	no	Baseline		376
	yes	0.209	0.046	114
Pigs never mixed	no	Baseline		494
	yes	0.477	<0.001	72
Weaners fed fermented feed	no	Baseline		366
	yes	-0.692	0.044	8
Weaners fed homemix	no	Baseline		285
	yes	-0.623	<0.001	89
Weaners fed concentrates	no	Baseline		128
	yes	-0.182	0.031	246
Weaners fed barley	no	Baseline		190
	yes	-0.272	0.002	184
Percentage of barley in weaner feed	Percentage	-0.014	<0.001	533
Growers fed homemix	no	Baseline		280
	yes	-0.572	<0.001	126
Growers fed wheat	no	Baseline		180
	yes	-0.302	<0.001	226
Percentage of wheat in grower feed	Percentage	-0.005	0.002	540
Growers fed barley	no	Baseline		185
	yes	-0.404	<0.001	221
Percentage of barley in grower feed	Percentage	-0.019	<0.001	545
Finishers fed fermented feed	no	Baseline		501
	yes	-0.591	0.003	25
Finishers fed homemix	no	Baseline		385
	yes	-0.539	<0.001	141
Finishers fed barley	no	Baseline		269
	yes	-0.284	0.001	257
Percentage of barley in finisher feed	Percentage	-0.014	<0.001	532
Sows fed fermented feed	no	Baseline		282
	yes	-0.899	0.009	8
Sows fed homemix	no	Baseline		187
	yes	-0.660	<0.001	103
Percentage of wheat in sow feed	Percentage	-0.006	0.012	542
Sows fed barley	no	Baseline		134
	yes	-0.440	<0.001	156
Percentage of barley in sow feed	Percentage	-0.016	<0.001	541
Pig water source: Mains	no	Baseline		188
	yes	0.218	0.014	366
Pig water source: Borehole	no	Baseline		376
	yes	-0.223	0.013	178
Any nipple drinkers used	no	Baseline		170
	yes	0.249	0.006	377

Table 3.4 Cont.

Variable	Level	Coefficient	*P*-value	No. farms
Number of pig deliveries	0-5	Baseline		343
	6-11	0.702	<0.001	83
	over 11	0.483	<0.001	132
Number of live pig collections	0	Baseline		21
	1-5	0.814	0.001	21
	6-11	0.626	0.009	74
	>11	0.570	0.007	441
Number of dead stock collections	0-5	Baseline		114
	>6	0.380	<0.001	442
Number of vermin controller visits	0	Baseline		237
	>0	0.193	0.026	295
Number of any other deliveries	0-11	Baseline		564
	>11	-1.512	0.026	2
Enzootic Pneumonia status (last 12 months)	Negative	Baseline		302
	Positive	0.187	0.026	264
PMWS status (last 12 months)	Negative	Baseline		250
	Positive	0.365	<0.001	316
PRRS status (last 12 months)	Negative	Baseline		436
	Positive	0.435	<0.001	130
Glasser's status (last 12 months)	Negative	Baseline		473
	Positive	0.316	0.005	93
Swine dysentery status (last 12 months)	Negative	Baseline		535
	Positive	-0.364	0.049	31
Clinical salmonellosis status (last 12 months)	Negative	Baseline		528
	Positive	0.562	0.001	38
No health conditions present (last 12 months)	no	Baseline		445
	yes	-0.318	0.002	121
Primary cause of pig mortality in the last 12 months	Other	Baseline		278
	Respiratory or wasting	0.510	<0.001	266
Number of sows (log. converted)	Continuous	0.036	0.030	438
Any homemix fed	no	Baseline		334
	yes	-0.548	<0.001	144
Any wet feeding	no	Baseline		448
	yes	-0.434	<0.001	65
Any compound feeding	no	Baseline		67
	yes	0.500	<0.001	446
Any solid flooring in finisher houses	no	Baseline		275
	yes	0.462	<0.001	249
Monthly maximum temperature anomaly for farm's region (oC)*	Continuous	0.023	<0.001	505
Monthly minimum temperature actual for farm's region (oC)	Continuous	0.003	0.013	505
Monthly minimum temperature anomaly for farm's region (oC)*	Continuous	0.032	<0.001	505
Monthly mean temperature anomaly for farm's region (oC)*	Continuous	0.031	<0.001	505

Table 3.4 Cont.

Variable	Level	Coefficient	*P*-value	No. farms
Monthly rainfall actual for farm's region (mm)	Continuous	0.001	<0.001	505
Monthly rainfall anomaly for farm's region (mm)*	Continuous	<0.001	0.004	505
Monthly sunshine actual for farm's region (hours)	Continuous	<0.001	<0.001	505
Monthly sunshine anomaly for farm's region (hours)*	Continuous	0.001	<0.001	505
Quarterly cycle	Cos	-0.051	<0.001	566
	Sin	-0.038	<0.001	566
Yearly cycle	Cos	-0.070	<0.001	566
	Sin	0.060	<0.001	566

*'anomaly' is the difference from long-term averages.

Thirteen variables were retained in the final model and the model population was reduced to 474 holdings, due to missing data. The identified significant risk factors were respiratory or wasting diseases as the primary causes of pig mortality; the number of other pig farms within 10 km radii; increased annual numbers of pig deliveries or dead stock collections; and increased regional sunshine, rainfall and temperature differences above long-term average related to the month of sampling. The identified protective factors were whether farms were located within Scotland; use of a conventional farming enterprise (rather than organic or other non-conventional types); homemixed feed provided to finisher pigs; and increased barley concentration in feed to grower pigs. Both annual and quarterly (seasonal) temporal cycles were also found to be significant and improved the fit of the model. The overall model had a significant Wald's Chi^2 result $P<0.001$ and a likelihood ratio test for the inclusion of the random effect was also significant ($P<0.001$). The 'season' variable was dropped from the model as it was collinear with the temporal cycles, and 'scheme' was dropped as it was perfectly collinear with region.

Table 3.5: Multivariable mixed linear regression of variables associated with logged meat juice ELISA ratio results collected from slaughtered pigs (N=109,912 samples (474 holdings)). The standard deviation of the random effect was 0.74 (0.69-0.80 (95% confidence intervals)).

Variable	Level	Coefficient	*P*-value
NUTS Region	Scotland	-0.747	<0.001
	Other	Baseline	
Pig farm density within 10km radius		0.017	<0.001
Farm enterprise	Conventional	-0.518	<0.001
	Non-conventional	Baseline	
Primary cause of pig mortality in the last 12 months	Respiratory or wasting	0.290	<0.001
	Other	Baseline	
Monthly mean temperature anomaly for farm's region (°C)*		0.024	<0.001
Monthly Rainfall actual for farm's region (mm)		0.001	<0.001
Monthly Sunshine actual for farm's region (hours)		0.001	0.001
Finishers fed homemix	Yes	-0.377	<0.001
	No	Baseline	
Percentage of barley in grower feed		-0.007	0.003
Number of pig deliveries	>11/year	0.289	0.001
	6-11/ year	0.439	<0.001
	0-5/year	Baseline	
Number of dead stock collections	>6/year	0.245	0.007
	0-6/year	Baseline	
Yearly cycle	Cos	-0.100	<0.001
	Sin	0.042	<0.001
Quarterly cycle	Cos	-0.046	<0.001
	Sin	-0.041	<0.001
Constant		-2.866	<0.001

*'anomaly' is the difference from long-term averages.

A histogram of model residuals was plotted to evaluate normality, to ensure the standard model assumptions were met (Figure 3.7). Similarly the random effect values from the model were plotted to ensure that the values fitted a normal distribution (Figure 3.8). A Bartlett test was used to test the hypothesis of homogeneity of variances between explanatory variable and residuals, and all variables were found to be significant ($P<0.001$), indicating that the assumption had been met. The assumption of independence was checked by first testing for any biologically plausible interactions e.g. temporal sinusoidal components and meteorological variables. Once the final model had been selected, then a review of collinearity tests, as provided as a model output in R, was assessed to check for any large correlations (>0.8 or <-0.8) between fixed effect coefficient estimates (Table 3.6).

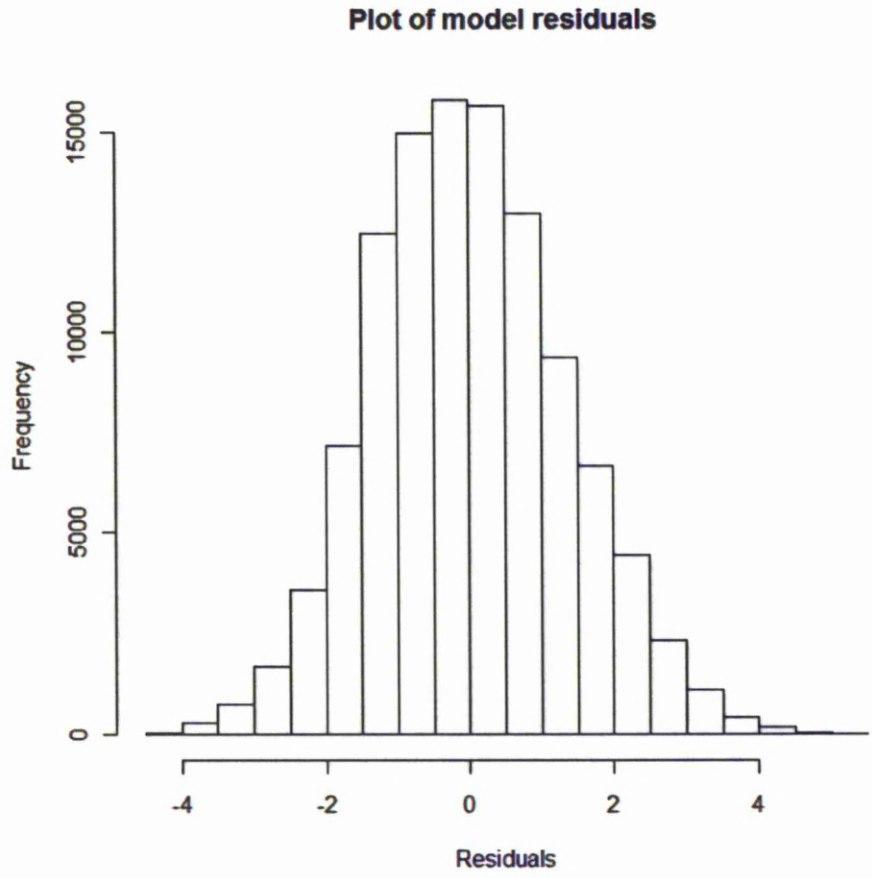

Figure 3.7: Histogram of linear mixed model residuals from a risk factor analysis of pig *Salmonella* seroprevalence.

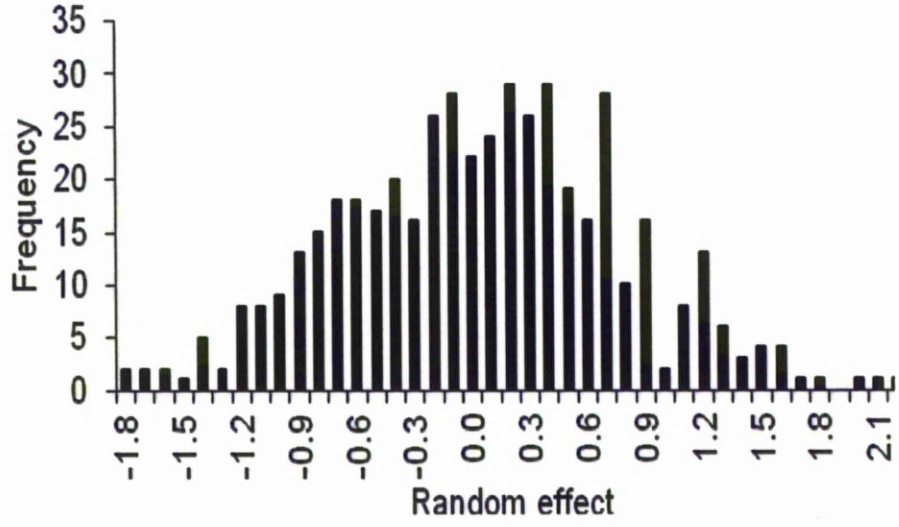

Figure 3.8: Histogram of linear mixed model random effect values from a risk factor analysis of pig *Salmonella* seroprevalence.

Table 3.6: Collinearity test results for pair-wise comparisons of variables selected in a multivariable model.

No.	Explanatory variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Region - Scotland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Pig farm density within 10km radius	0.031	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Conventional Farm enterprise	-0.092	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Percentage of barley in grower feed	-0.113	0.007	-0.077	-	-	-	-	-	-	-	-	-	-	-	-
5	Finishers fed homemix	0.203	0.128	-0.106	-0.444	-	-	-	-	-	-	-	-	-	-	-
6	Pig deliveries 6-11/year	0.138	0.019	-0.001	0.052	0.071	-	-	-	-	-	-	-	-	-	-
7	Pig deliveries >11/year	0.105	-0.043	-0.014	0.097	-0.037	0.275	-	-	-	-	-	-	-	-	-
8	>6/ year dead stock collections	-0.147	-0.097	0.042	-0.049	0.172	-0.105	-0.014	-	-	-	-	-	-	-	-
9	Wasting or respiratory primary cause of pig mortality in the last 12 months	0.200	-0.036	-0.002	0.072	-0.152	-0.133	-0.175	-0.102	-	-	-	-	-	-	-
10	Quarterly cycle cosine	-0.003	0.003	0	0.001	0.003	0.001	-0.001	-0.001	-0.003	-	-	-	-	-	-
11	Quarterly cycle sine	0.001	0	0.001	0	0	-0.001	0.001	0.002	0.002	0.007	-	-	-	-	-
12	Monthly mean temperature anomaly for farm's region	-0.009	0.001	-0.005	0	0	0.005	0.003	-0.004	0	0.085	-0.179	-	-	-	-
13	Monthly Rainfall actual for farm's region	-0.026	0.021	-0.003	-0.002	0.001	0	-0.004	0	0.002	0.087	0.064	-0.059	-	-	-
14	Monthly Sunshine actual for farm's region	0.017	0.006	0.001	0.004	-0.001	-0.002	-0.003	0	0	0.038	0.123	-0.361	0.419	-	-
15	Yearly cycle cosine	-0.015	-0.006	-0.002	-0.004	0.001	0.004	0.003	0	0.001	-0.022	-0.150	0.359	-0.319	-0.886	-
16	Yearly cycle sine	-0.008	-0.007	0.001	-0.002	0.001	-0.002	0.001	-0.001	-0.001	-0.022	-0.107	0.211	-0.400	-0.588	0.507

3.4.4 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. The geographical spread of the study holdings indicated that the population was generally representative of the Quality Assured pig farms in the UK, with similar high density clusters in Eastern England (mean average of 28 farms within 10km), Yorkshire and the Humber (21 farms) and in the North East of Scotland (11 farms) (Smith et al., 2011).

In the final model, both yearly and quarterly cycles were found to be significant and improved the final model, with the highest mean ELISA 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 (Funk, Davies and Gebreyes, 2001). Air temperature has been linked to pig stress, which in turn can increase the shedding of *Salmonella* and can lower immunity (Hald and Andresen, 2001). 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 pigs in Scotland have a lower logarithmic ELISA ratio and thus farms in Scotland have a lower seroprevalence of *Salmonella*. This may be because the farms in Scotland are more likely to use certain management procedures (e.g. all indoor production; homemixing) and, due to their geographical isolation, are more likely to purchase animals from similarly low seroprevalence Scottish farms. Anecdotal evidence suggested that the majority of commercial pig farms in Scotland belonged to the same integrated company, which benefited from the company's nucleus farm having a low prevalence of *Salmonella* and so supplied low prevalence pigs down the production pyramid.

The range of neighbouring pig farms within 10 kilometres varied greatly (from 0 to 73) and farms with a higher *Salmonella* prevalence have been shown to be more clustered in space than low prevalence farms by other studies in the UK and Denmark (Benschop et al., 2008; Clough et al., 2009). In these studies, positive farms were more congregated in space than would be expected, possibly due to local spread and transmission of disease. Location and farm density were identified by a review of UK pig *Salmonella*, which noted that the "type, number and density of pig holdings in a two kilometre radius is crucial" (Pritchard, Dennis and Waddilove, 2005).

It has been described in other studies that health conditions, especially respiratory and wasting diseases such as Porcine Reproductive and Respiratory Syndrome and

Postweaning Multisystemic Wasting Syndrome, may have interacted with *Salmonella*. This may be by lowering the immune system or increasing transmission by sneezing or shedding *Salmonella* in larger numbers and for a longer period of time, and these relationships were also identified in the model (Schwartz, 1999; Wills et al., 2000; Beloeil et al., 2004; Beloeil et al., 2007).

A larger number of pig deliveries were also shown to be a risk factor, and the introduction of pigs onto a farm was agreed to be the most likely cause of pig infection by an international expert workshop (Stark et al., 2002). A larger number of pig deliveries may indicate a larger number of suppliers, which has been shown to be a risk factor 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 (Lo Fo Wong et al., 2004). A higher number of dead stock collections might indicate that the farms have greater health problems, possibly caused by *Salmonella* or from health conditions associated with *Salmonella* infection. These factors may also be a risk simply because the increased number of vehicles entering the farm can facilitate the spread of *Salmonella*. To decrease the risk from deliveries and visitors, biosecurity measures such as wearing farm-specific clothing and footwear; the routine use of bootdips; ensuring deliveries are only made at the farm perimeter, and closing the farm to all but essential external vehicles should be utilised (Pritchard, Dennis and Waddilove, 2005; Beloeil et al., 2007).

Managing a farm as 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 outdoor production (only 5% of the conventional farms had any outdoor production in comparison with 33%), and these enterprises have been shown to have a significantly higher *Salmonella* seroprevalence in pigs (Gebreyes et al., 2008). Procedures to control *Salmonella* transmission which are used in indoor production are harder to implement outdoor and so the pigs may be at an increased risk of infection from wildlife and the environment (Jensen et al., 2006).

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* (Lo Fo Wong et al., 2004; Pappenbrock et al., 2005). The use of homemix feed was found to be protective, which had been indicated in an earlier British pig study (VLA, 2004) and the use of purchased feed, rather than that mixed on farms, was a significant risk factor for *Salmonella* in other studies at the multivariable (Benschop et al., 2008) and univariable (Rajic et al., 2007b) levels. A reason for this could be that homemixed feed is usually coarser than purchased feed, with a larger particle size, and these factors influence the growth of

competitive gut flora by affecting the acid and starch content in the gut. Purchased feed is also likely to have been pelleted, which has also been indicated as associated with a higher *Salmonella* prevalence (Lo Fo Wong et al., 2004; Leontides, Grafanakis and Genigeorgis. 2003). However, in a longitudinal study of the use of fermented feed, no significant effect was shown, indicating that *Salmonella* may be able to bypass the stomach environment via the tonsils (van Winsen et al., 2001; van Winsen et al., 2002). 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 (Kelliher, 2002; Jorgensen, 2003).

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. The cross-sectional study design also meant that we were unable to distinguish between risk factors associated with the infection or persistence of *Salmonella*. The analysis may also have identified risk factors through reverse-causation, with explanatory factors associated with *Salmonella* which have been instigated as a response to *Salmonella* presence, rather than contributing towards *Salmonella* presence. The large sample size and large number of explanatory variables may also have identified factors associated with *Salmonella* by chance, due to the large amount of statistical power, although the significance level was lowered to account for this.

Utilising a study population drawn from the QAS may have provided selection bias to 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 representative of the remaining farms. Anecdotal evidence suggests that non-assured farms are more likely to be smaller, non-conventional holdings. The non-assured population may utilise different pig management to the QAS holdings and so they may have a different set of factors that are associated with *Salmonella*. The use of a postal questionnaire may also have provided selection bias, as holdings that responded may be more aware of *Salmonella,* and *Salmonella* control, and thus more eager to assist with research. The use of a questionnaire that included questions relating to time periods may also have introduced some recall bias, and it could be theorised that a well-managed and organised farm would have been more likely to be able to use recorded information to answer the questions, whereas a disorganised farm would have been less likely to recall instances over the time period.

The use of serological samples from the ZAP study was a key component of this study, as they provided outcome data from a large number of pig farms, with a large number of samples collected from four years. The MJ ELISA is a useful screening tool for surveillance

as the test is cost-effective, quick and does not require more specialised microbiological skills (Bohaychuk et al., 2005). However, the use of serological samples and modelling the ELISA ratio directly, without conversion to a binary positive/negative outcome, may limit the interpretation of the findings when considering the infection status of pigs, as the results represent previous exposure, rather than current infection. As the ELISA ratio is an indicator of previous infection, this may have caused information bias in the temporal results. The ELISA ratio is influenced by the strength of the *Salmonella* challenge and the time since infection, but immune reactions vary amongst individuals and are affected by many other factors, such as stress. A high ELISA ratio does not necessarily coincide with a more recent infection and a high mean average ELISA ratio in the autumn does not indicate that pigs were infected in the autumn (Tizard, 2004). The ELISA benefits from detecting previous infection, even if it is subclinical, but only detects a number of known serovars with potentially differential abilities to detect infection by different serovars (Funk, Harris and Davies, 2005). However, studies have shown a significant correlation between serology results and caecal prevalence, and although farm results can fluctuate between visits/ sampling occasions, the test has been shown to be useful in identifying farms with a *Salmonella* problem (Sorensen et al., 2004; Rajic et al., 2007a).

The study provided a large and detailed risk factor analysis and examination of the spatial and temporal trends of *Salmonella* seroprevalence, with a study population large enough to detect factors with modest associations to the ELISA ratio. Large sample sizes can provide greater statistical power and provide narrower confidence intervals for estimated associations and so are more likely to detect if a significant difference is present in the data. Even though association may be weak, it may still have a significant impact on *Salmonella* presence in the study population if it is present in a large proportion of the population. Specifically, the model results suggest that measures are needed to control *Salmonella* infection on farms utilising outdoor production and to protect pigs from the effects of large variations in weather conditions and an intervention study would be required to test this finding. The model also highlighted a region of the UK that may require more intensive surveillance and control to limit the transmission of *Salmonella*. The utilisation of data collected routinely via the QAS and ZAP schemes, as well as a one-off postal questionnaire provided a cost-effective means to design and analyse a large risk factor study.

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Chapter 4: Spatio-temporal analysis of pig *Salmonella* prevalence

4.1 Introduction

Salmonella is an important zoonosis which may be carried by livestock raised for food production. In human cases, salmonellosis is usually characterized by diarrhoea and can be transmitted through foodborne routes, as well as from contact with contaminated faeces from an infected person or animals (O'Brien, 2005). The importance of pigs as reservoirs of *Salmonella* has been shown by a large abattoir study where the prevalence of *Salmonella* in pig caecal samples, collected in Great Britain, was high (23.4%, 19.9-27.3% $CI_{95\%}$), when compared to both cattle and sheep (1.4% and 1.1% respectively) (Milnes et al., 2007). In a European Union baseline survey completed in 2007, a similar prevalence (21.2%) of *Salmonella* was isolated from mediastinal lymph node samples from United Kingdom (UK) pigs at slaughter (EFSA, 2008). Although it is unknown how many cases of human salmonellosis are attributed to eating pig products, of the 13,213 laboratory confirmed cases in the UK identified in 2007, 13% were related to the serovar *S.* Typhimurium, which is the predominant type detected in samples from UK pigs (Defra, 2007; VLA, 2007). Furthermore, identical subtypes of a number of 'phagetypes, which are particularly associated with pig infection, have been detected in humans, indicating that human infection could be attributed to pig reservoirs (Kirchner et al., 2007; Kirchner et al., 2011). Infection in pigs can cause a range of clinical signs, from scouring to fever and death, but is most often sub-clinical and so, is difficult for farmers to detect and control.

A number of earlier studies have shown that the risk of pigs being previously *Salmonella* positive varies with their geographical location. Pigs in Scotland have been found to have on average a lower response to a *Salmonella* serological test and Scottish farms had a lower seroprevalence of *Salmonella* than those in England and Wales (Smith et al., 2011). The density of other pig farms within a farm's vicinity has also been shown to be associated with *Salmonella* prevalence. The range of neighbouring pig farms within 10 kilometres varied greatly in the UK (from 0 to 73) and farms with a higher *Salmonella* prevalence had been shown to be geographically more clustered than low prevalence farms (Clough et al., 2009; Smith et al., 2011). Location and farm density were also identified by a review of UK pig *Salmonella*, which noted that the "type, number and density of pig farms in a two kilometre radius is crucial" (Pritchard, Dennis and Waddilove, 2005). Possible reasons for the spatial heterogeneity of high prevalence farms could include local spread and transmission of disease, common biosecurity and management of local groups of farms, or some underlying geographically localised risk factor.

The identification of spatial heterogeneity of pig farm *Salmonella* prevalence in UK regions through K-function analysis, presented in Chapter 2, and the presence of spatial (region of

farm and farm density within a 10km radius) and temporal factors (quarterly and yearly temporal cycles, meteorological monthly summaries for each geographical region) in Chapter 3, suggested a need for further examination of the spatial and temporal structure of pig *Salmonella* seroprevalence in the UK. Access to comprehensive pig *Salmonella* surveillance data, from the Zoonoses National Control Plan (ZNCP) scheme, allowed for these factors to be evaluated over a longer period of time than that previously used, with additional spatial techniques used to further explore the initial findings. The ZNCP collected meat juice samples from slaughtered pigs for serological testing to detect *Salmonella* antibodies via an ELISA.

An analysis of temporal trends (yearly, monthly and seasonally) was completed first (Chapter 4.2), followed by the use of a number of spatial analysis methods to confirm evidence for spatial dependence in *Salmonella* status of the farms within the surveillance datasets (Chapter 4.3). Statistical modelling techniques were then used to examine any spatial dependence after the effect of the risk factors, detected in Chapter 3, had been accounted for (Chapter 4.4). Finally, a separate assessment of spatial dependence in Northern Ireland was completed, due to the physical separation of Northern Ireland from mainland Britain (Chapter 4.5).

4.2 Temporal analysis

4.2.1 Method

The dataset of sample serology results collected for the pig *Salmonella* surveillance scheme (ZNCP), from inception to the date of analysis, was described by year, month and season of sample collection using summary tables and graphs designed in MS Excel. Initially, summaries were made using the 0.25 ratio cut-off to determine a binary (positive or negative) ELISA result, and then ELISA ratio results were assessed directly for temporal patterns. Chi-squared tests for 2 x n tables were completed in Epi-Info 6 (Dean et al., 1996) to test for significant differences between different groupings of samples (e.g. comparisons between years or between seasons).

4.2.2 Results

A review of the data shows that comparable numbers of samples were collected between 2003 and 2007, peaking in 2005 (Table 4.1 and Figure 4.1). The number of samples collected in 2002 (beginning of the scheme) and in 2008 (when the dataset was closed to facilitate analysis) was incomplete, as samples were not collected from all twelve months of the year. The percentage of the samples that provided seropositive results in each year was also relatively stable, with significant ($P<0.001$) increases in the percentage of seropositives in the two incomplete years.

Table 4.1: Percentage of seropositive *Salmonella* samples from the ZNCP surveillance dataset, by year of sample collection.

Year	No. of positive samples	No. of samples	% of positives	95% Confidence Interval
2002#	11,789	43,025	27.4	27.0-27.8
2003	27,528	114,942	23.9	23.7-24.2
2004	32,265	141,910	22.7	22.5-23.0
2005	33,067	156,995	21.1	20.9-21.3
2006	34,057	140,550	24.2	24.0-24.5
2007	26,845	103,160	26.0	25.8-26.3
2008*	6,761	24,320	27.8	27.2-28.4
overall	172,312	724,902	23.8	

Data collection began on 20/06/02; *includes records up to 25/09/08.

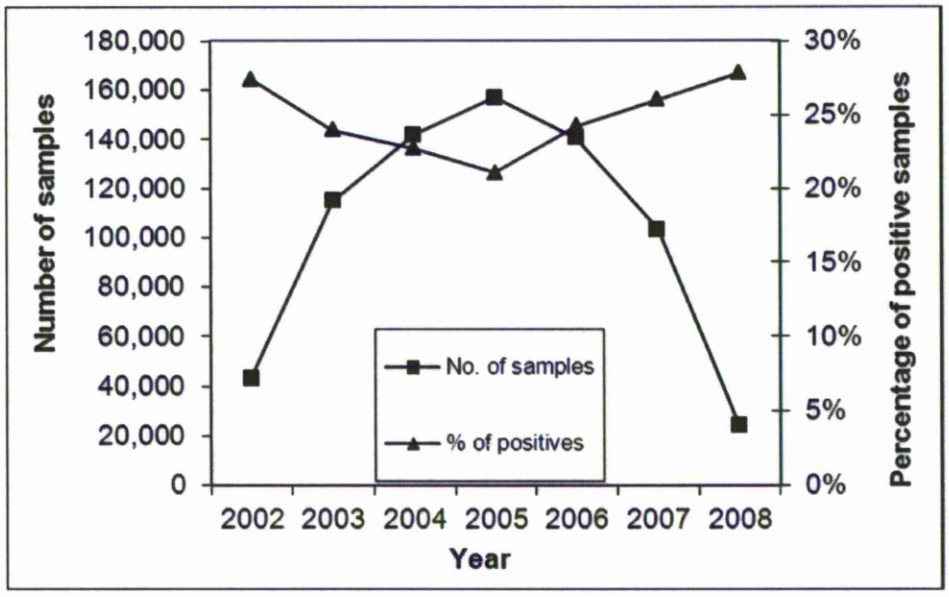

Figure 4.1: Graph to show the number of samples and percentage of positive samples for each year since the beginning of the *Salmonella* surveillance programme.

An assessment of temporal trends in the serological data by month of sample collection shows that there were fewer seropositives at the start of the year and more towards the end, which coincided with an increase in the number of samples tested (Table 4.2 and Figure 4.2). A summary of the results on a seasonal basis shows this trend more clearly, with the fewest samples collected and a significantly ($P<0.01$) lower percentage of positives in spring, and more samples and a significantly higher percentage in autumn (Table 4.3).

Table 4.2: Percentage of seropositive *Salmonella* samples from the ZNCP surveillance dataset by month of sample collection.

Month	No. of positive samples	No. of samples	% of positives
Jan (01)	13,590	59,694	22.8
Feb (02)	13,505	55,673	24.3
Mar (03)	13,409	57,250	23.4
Apr (04)	11,859	53,220	22.3
May (05)	10,786	51,013	21.1
Jun (06)	12,678	60,102	21.1
Jul (07)	17,024	73,261	23.2
Aug (08)	16,406	65,634	25.0
Sep (09)	17,157	65,493	26.2
Oct (10)	16,720	65,201	25.6
Nov (11)	16,186	63,027	25.7
Dec (12)	12,992	55,334	23.5

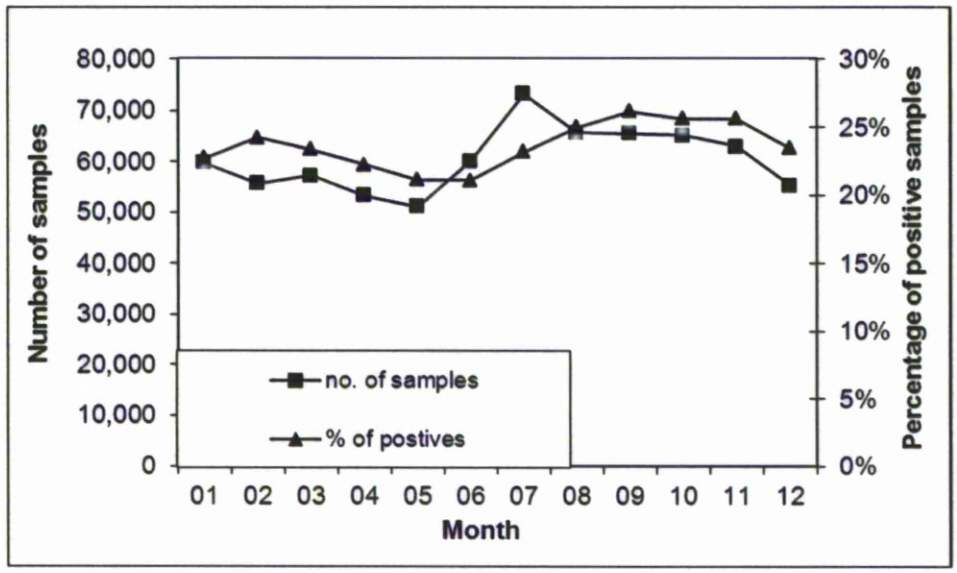

Figure 4.2: Graph to show the number of samples and percentage of seropositive samples for each month (Month 1 = January, Month 12 = December).

Table 4.3: Percentage of seropositive *Salmonella* samples from the ZNCP surveillance dataset by season of sample collection.

Season	No. of positive samples	No. of samples	% of positives
Spring (March-May)	36,054	161,483	22.3
Summer (June-August)	46,108	198,997	23.2
Autumn (September-November)	50,063	193,721	25.8
Winter (December-February)	40,087	170,701	23.5

A summary of the mean ELISA ratio (with 95% confidence intervals) by year and season, showed a dip in the mean ratio in 2004 and 2005, below the 0.25 individual sample cut-off point used by the surveillance scheme to determine positive samples (Figure 4.3). The analysis was completed on 700,506 samples as 24,396 samples, including the samples collected in 2008, did not have ELISA ratio results recorded. The reason for the missing values was unknown, although it was expected that this was a combination of data entry errors and limited data entry of the results during some periods of the scheme. The mean ELISA ratio was highest in autumn (0.27) and below the cut-off value in both spring and summer (Figure 4.4).

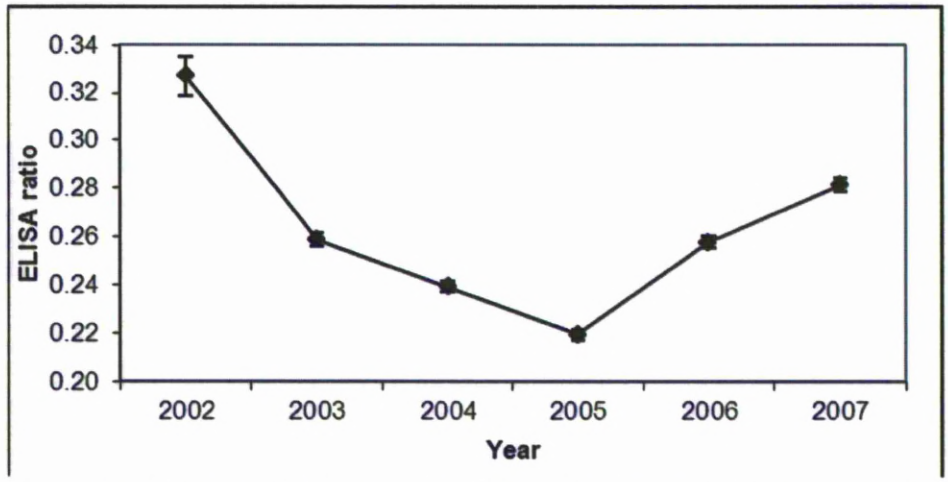

Figure 4.3: Graph to show the mean ELISA ratio for pig samples tested for *Salmonella* serology with 95% confidence intervals, by each year.

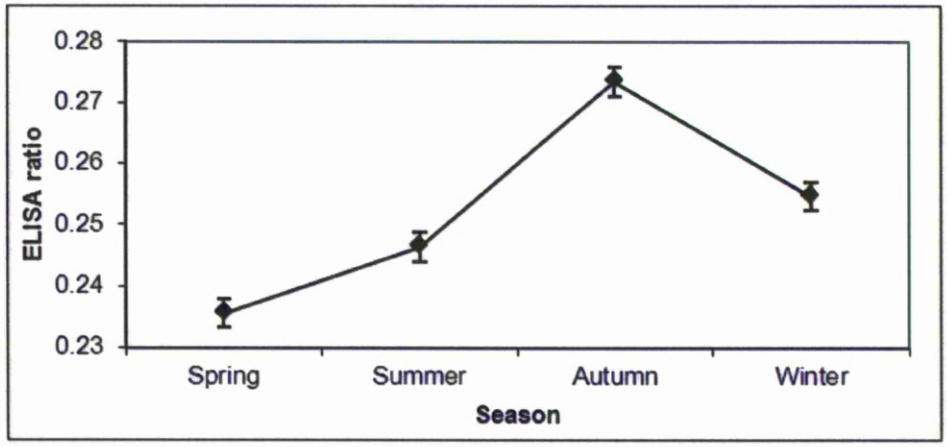

Figure 4.4: Graph to show the mean ELISA ratio for pig samples tested for *Salmonella* serology with 95% confidence intervals, by each season.

4.2.3 Discussion

The results from the full extent of the surveillance dataset concur with the findings of the risk factor model (Chapter 3; Smith, Cook and Clough, 2010) which detected significant seasonal and yearly trends. The results indicate a peak of positive serological results (both percentage of positive samples and mean ELISA ratio) in autumn and a trough in spring. This may be related to UK weather conditions, as a combination of warm weather and higher rainfall was recorded for autumn months, when compared to the average weather conditions in spring (Met Office, online data). Weather conditions have previously been found to affect pig *Salmonella* prevalence and the survival of *Salmonella* in the environment. Environmental factors, such as increased temperature variability, high air temperature and below median high temperature, have been associated with elevated *Salmonella* prevalence in pig farms in Denmark and the United States (Funk et al., 2001; Hald and Andersen, 2001). The

researchers suggest that this may highlight possible ineffective building ventilation, or causes of stress to pigs, as an explanation for the association of weather with *Salmonella* prevalence. The weather conditions may also affect *Salmonella* survival in the environment. An experimental study has shown that *Salmonella* had been found to survive in slurry for less than a third of the number of days in summer than in spring or winter (Placha et al., 2001). However, it should be noted that due to the serological nature of the tests and the influence of the individual pig's immune system, the peaks and troughs may not coincide directly with the dates of pig infection or presence of clinical signs. For example, two pigs challenged with the same quantity of *Salmonella* may continue to present with a detectable level of antibodies for a different number of days.

The seasonal pattern detected in this study was compared with that found by other studies in countries with similar weather and seasons. In Canada, a similar peak of prevalence from August to September was detected from 11 years of abattoir surveillance (Guerin et al., 2005). A review of Danish serological surveillance detected a two peaked annual cycle with spring and autumn peaks in *Salmonella* occurrence, although a more recent assessment of ten years of Danish data found no reliable seasonal trends (Hald and Andersen, 2001; Benschop et al., 2008). Comparisons between countries may not be helpful due to variation in the weather patterns and differences in pig management, such as the differing degrees of outdoor production and differences in housing systems.

The variation in results between years was also noteworthy. The increase in prevalence for both of the serological outcomes when fewer samples than average were collected in a year, and decrease in results when more samples were collected, was perplexing. A smaller number of samples would have larger confidence intervals than a greater annual number of samples, and so the result would be more prone to random variation from the true mean. However, in every year the dataset was large enough to allow for confident estimations. Utilising such a large dataset, can mean that statistically significant results are easier to detect due to the large power within the sample size and so caution should be made in over interpreting the results.

The surveillance schemes have been supported by efforts to encourage and inform farms on *Salmonella* control and so a reduction in seroprevalence over time may have been expected. However, the rise in seroprevalence after 2005 is a concern. No information has been located to inform whether any changes in the structure of sampling scheme would have affected these results, and a true difference in the annual results cannot be discounted. The fluctuation of results may have been related to the rise in other contributing factors, such as outbreaks of other diseases that increase pig susceptibility to *Salmonella* infection or the increase in outdoor rearing, or possible weather anomalies, specific to that year.

The identification of the relationships with season and year of sampling from a large national surveillance dataset provides evidence for temporal trends with *Salmonella* serological results. Although, serological results only detect historical infection, the identification of these patterns in this and other studies requires more detailed study to examine this particular issue. Understanding the mechanisms and potential causes of these temporal trends may provide information that may be key to monitoring *Salmonella* in the UK. For example, a detailed investigation may provide farmers with evidence of how to manage ventilation and heating in pig houses throughout the year to minimize pig stress, or the findings may indicate how seasonal trends could be accounted for in surveillance outputs, so as to allow for the identification of additional fluctuations in pig prevalence.

4.3 Preliminary spatial analysis

4.3.1 Introduction

Most spatial analysis has been developed from the analysis of data from the field of ecology (e.g. Ripley's K-function 1976). Statistical methods were used to determine whether there were significant spatial patterns in the characteristics at spatial point locations, and also provided improved methods to summarise and present the spatial characteristics of a dataset. When these techniques were transferred to the epidemiological field there were difficulties in their application to human or animal populations that were not randomly or orderly distributed, and which could move between locations over short time periods. Epidemiological datasets disagreed with the assumptions that the distribution of points (e.g. the position of the farms) should show:

a) isotropy, where the spatial relationship is identical in all directions, and;

b) stationarity, where the spatial relationship between two points is related to the distance between them and not the location of either of the points.

For example, the assumptions of modelling spatial patterns state that in a spatial point process the location of each point is random and there is no preferred origin or orientation of the spatial pattern. However, a number of spatial analysis techniques have been used in epidemiology, with those chosen being robust to failure to meet the assumptions or where adjustments were made to the techniques to account for those failures.

This section describes and compares a number of spatial methods used to examine the pig *Salmonella* dataset extracted from the ZNCP scheme (see Chapter 2 for a full description of this *Salmonella* surveillance scheme). Exploratory spatial analyses were completed to identify and explain any spatial heterogeneity within the data.

4.3.2 Method

Two spatial analysis methods were used to investigate the data to try and uncover any further evidence of the spatial heterogeneity in the pig farm population shown by the preliminary analysis presented in Chapter 2. The first, K-function analysis (Ripley, 1976), has been used for examining spatial clustering in a point process, where each point is a spatial location (e.g. a farm). In an epidemiological setting, this method predominantly utilises a case-control methodology to compare whether the clustering of cases in space is above that expected by random chance (Diggle and Chetwynd, 1991). However, this method limits the analysis to a binary outcome for each location, rather than analysing patterns in individual samples collected from a location. However, as shown in human epidemiology (Cuzick and Edwards, 1990; Bailey and Gatrell, 1995; Morrison et al., 1998), the method is useful for spatial analysis in veterinary epidemiology as it can adjust for a population at risk that is not

randomly distributed (e.g. Fenton et al., 2009). This is beneficial as a confounding effect can be caused in other spatial analysis types which do not plot the distribution of the entire study population (Carpenter, 2001). However, the analysis may not be robust over large geographical areas, where differences in the density of points or other potential confounders (e.g. weather conditions relevant to disease persistence or transmission) are present in areas (Diggle and Morris, 1996). This is because the analysis uses the same intensity function over the whole area and so intensity is reduced to the lowest common level, and detail is lost from the analysis in areas of higher spatial point density. For this reason the analysis is most commonly used within defined spatial areas, for example in regions where the density of farms within that area is highly homogeneous, rather than used to analyse multiple regions or country-level data. For example, an analysis of *Salmonella* prevalence in UK badgers targeted the English county of Cheshire (Wilson et al., 2003).

The output of the K-function analysis is usually a graph displaying a line of the difference (D) between the K-hat (the estimate of K) of the cases and controls, against the distance between points (e.g. distance between farm locations). This difference represents the spatial dependence of the data, which indicates the scale of clustering at each distance and also whether it is greater or less than would be explained by underlying variation in geographical density alone. Statistically significant ($P<0.05$) clustering is shown by deviation of the data line from the 95% confidence envelopes, created from simulated data with random spatial dependence.

The original method did not account for edge effects and so any apparent clustering around the edges of the geographical area under review was less likely to be detected because of the lack of knowledge of what occurs beyond the boundary, which may or may not have contributed to the point pattern (Diggle and Morris, 1996). Development of the method was completed to allow for these edge effects and also for an inclusion of a temporal dimension to be added to the analysis (Diggle and Chetwynd, 1991; Diggle et al., 1995). These developments were added to a package (Splancs; Rowlingson and Diggle, 1993) for the R statistical software, and it has been used in a number of recent veterinary epidemiology investigations in Great Britain, such as Foot and Mouth Disease (Wilesmith et al., 2003); *Salmonella* in dairy herds (Fenton et al., 2009); and *Salmonella* in pigs (Clough et al., 2009). Other R packages were also developed to account for edge effects for K-function analysis, such as spatstat. This package was used in the novel spatial analysis of diarrhoea caused by *Salmonella* and other pathogens in dogs held in animal shelter cages (Sokolow et al., 2005).

For this analysis, the study population of QA farms, which had supplied a study questionnaire in Chapter 3, was separated into cases and controls by applying a cut-off value to farms. Case farms were defined as those that had more than 21.3% (the mean

value) of the samples positives from those collected from a four year period from the *Salmonella* surveillance ZNCP scheme. The analysis was limited to those farms for which X and Y coordinates were present and for farms in mainland Britain only (see Chapter 4.5 below for details of the analysis of Northern Irish farms). As explained above, the K-function analysis is more robust within localised geographical areas, and due to the differences in the pig industry between NUTS regions (Chapter 2), these regions were analysed individually. Additional tests were completed for each country within Great Britain and a test for the whole of Britain. A K-function analysis was completed for each of these geographical units using the Splancs library in the statistical package R 2.7.1 and the results were presented along with simulation envelopes from 99 simulations, with random labelling of the spatial points as cases or controls (as described in Rowlingson and Diggle, 1993). The observed D value was then compared against the D values from the simulated random permutations in a histogram and a *P*-value generated by ranking the observed against the 99 simulations.

The study population (cases and controls) was also compared by K-function analysis against 100 Monte Carlo simulations that randomly repositioned the farms within the spatial polygon defining the area of study. This was to determine whether the K-hat line on the graph for the study population was within the upper and lower simulation envelopes, indicating that the farm locations were not significantly different to randomly positioned data (Rowlingson and Diggle, 1993). A population which is more clustered than would be expected from geographical density alone would be shown by the study population line being above the upper envelope and a regular and ordered population would be shown by the study population line being below the envelopes. A similar comparison has been made using data on multi-drug resistant *Salmonella* infection in cattle in France (Lailler et al., 2005).

The second analysis method examined was the scan statistic, first developed by Naus in 1965. This approach is different to the K-function as it does not examine for significant clustering over an entire study area but instead locates the sites of the most likely clusters that are not randomly distributed. The test compares the relative risk of being a case within an area in comparison with the risk outside of the area. The most likely main cluster of cases is identified when the relative risk is above that expected and the cluster has the maximum likelihood of representing the study population, and secondary clusters are detected in this method that do not overlap the main cluster. This methodology has meant that this test has been used in a number of situations to investigate possible areas of disease outbreaks. The test was provided by the SaTScan package (Kulldorff and Nagarwalla, 1995; Kulldorff, 1997), which requires knowledge of the population at risk and utilises either:

- a case-control methodology (Bernoulli model), an ordinal model for ordered categories of locations;

- or a Poisson-based model "where the number of events in a geographical area is Poisson-distributed, according to a known underlying population at risk" (SaTScan, 2005).

The package can also cover both spatial and temporal-spatial clusters, although the temporal-spatial analysis greatly increases the run time of the analysis. The scan statistic can adjust for changes in population size over time and can also incorporate a limited number of categorical covariates.

The test has been shown to have the power to detect localised epidemiological clusters although the power is reduced for long and narrow shaped clusters, as the test searches for circular clusters, and for where clustering occurs throughout the study region. The test has also been amended to look at different shapes of clusters (e.g. oval), although these are computationally complex. Veterinary examples of the use of the scan statistic have been shown by Pare (Pare et al., 1996), to assess the most likely spatial-temporal cluster of horse cases of *Salmonella* Krefeld in a veterinary hospital. Other examples include the assessment of acute respiratory disease in Norwegian cattle herds (Norstrom, Pfeiffer and Jarp, 2000) and the detection of the most likely temporal cluster of different *Salmonella* serotypes in dairy cattle (Sato et al., 2001).

For the detection of spatial clusters in the studied QA scheme pig farm population, both the Bernoulli (using the same case-control definitions used for the K-function analysis) and Poisson models (using the collection of positive or negative results from samples taken at each farm location) were used to locate circular clusters. As applied as standard, a maximum size for a detected cluster was set as 50% of the studied population, as when the maximum is larger than 50% the output reflects a decreased risk outside of the cluster rather than an increased risk within it (Norstrom, Pfeiffer and Jarp, 2000). The scan statistic analysis also used the same study population as described in the K-function analysis.

4.3.3 Results

The analysis of farms for which a completed study questionnaire (described in Chapter 3) was received, with the removal of the farms in Northern Ireland, included 511 British farms. The farms were linked to ZNCP samples from a four year period ranging from 2003-2008 depending on the date of enrolment into the original study (Chapter 3). The holdings were linked to a total of 119,906 ZNCP samples, with a mean average of 224 samples per holding (range 1-1,671).

The K-function analysis for the whole of Britain did not detect any significant spatial heterogeneity (Figure 4.5). The difference (D) in K-hat between case and control farms plotted against the distance between holdings, was inside of the simulation envelopes which

were created under a null hypothesis of an equivalent degree of clustering in both groups. The result was further explored by a histogram comparing the D value of the British population against the simulated datasets that were randomly labelled. This showed that the British population is central within the histogram distribution and so was not significantly different to the simulated populations (P=0.57, Figure 4.6). However, the pig farm population was found to show significant clustering in space, where the data line in Figure 4.7 was above the upper limit envelope of simulations designed on the basis of complete spatial randomness. K-function plots of each of the individual regions are shown in appendix A. Due to the small populations in some regions, substantive plots were only achieved in three regions: East England; Yorkshire and Humber; and Scotland. However, no significant clustering was detected in any of these regions.

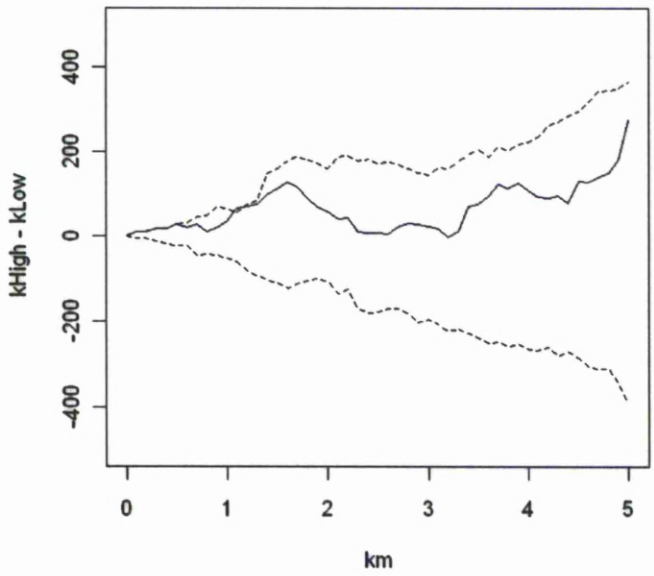

Figure 4.5: Plot of the difference (kHigh-kLow) in K-hat between high and low *Salmonella* seroprevalence pig farms in Great Britain against distance in kilometres and associated simulation envelopes determined from randomised datasets (dotted lines).

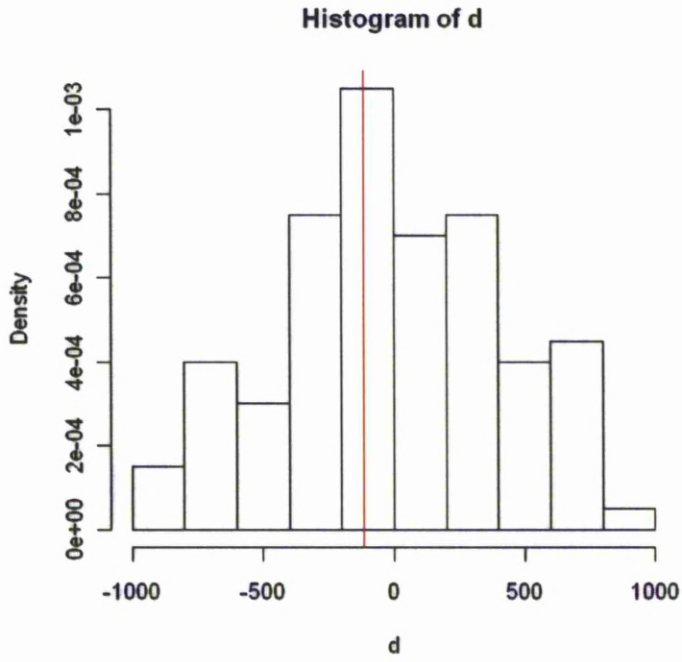

Figure 4.6: Histogram shows the range of difference between the K-hat for cases and controls (d) calculated by the 99 Monte Carlo simulation runs and the observed d as a red line.

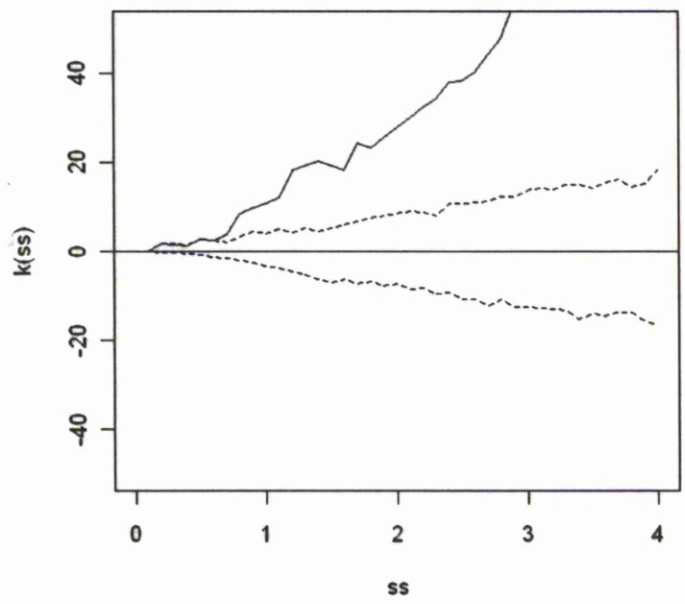

Figure 4.7: Comparison between the study population K-hat plot against 200 Monte Carlo simulations of datasets of the same size with complete spatial randomness (ss = distance in kilometres, k(ss) is the K-hat at each measure of distance).

The scan statistic analysis detected circular areas of significant clustering ($P<0.01$) by both tested methods. The Poisson model found the most likely cluster (Relative Risk=2.04, $P=0.001$) located in the Yorkshire and Humber region (Figure 4.8). The cluster contained 12,737 positive samples (111 farms) against a population of 37,140 samples, which was above the expected ratio of 1.532 samples per positive. A number of significant secondary clusters were also detected, one containing 77 farms in the East of England ($P=0.001$), although others contained much smaller number of farms: three farms in Wales and North West England ($P=0.001$), four farms in the South West and South East ($P=0.001$) and four farms in the South West ($P=0.005$). Other statistically significant clusters only covered one or two farms and so were of less specific interest to this population study, although not all case farms were included in the most likely or secondary clusters.

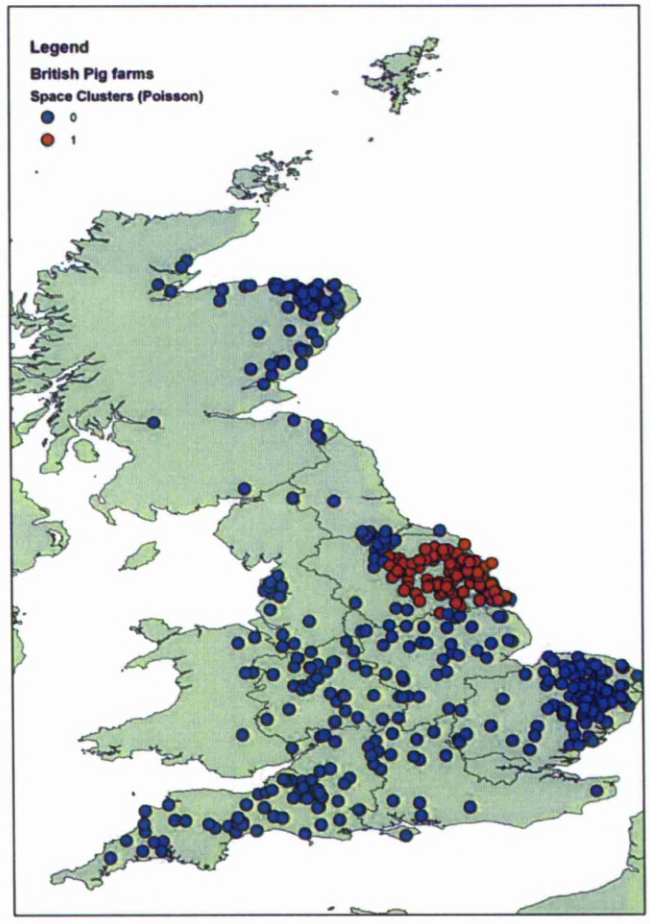

Figure 4.8: Map to show position of the most likely pig *Salmonella* spatial cluster detected by the Poisson scan statistic method.

The Bernoulli method (using a cut-off method of 21.3% samples positive to define farms and cases and controls) expanded the primary Poisson cluster to cover parts of the East Midlands and East England (Figure 4.9). The most likely cluster (Relative Risk=2.45, *P*=0.001) included 159 case farms within an area of 246 farms and no secondary clusters were detected.

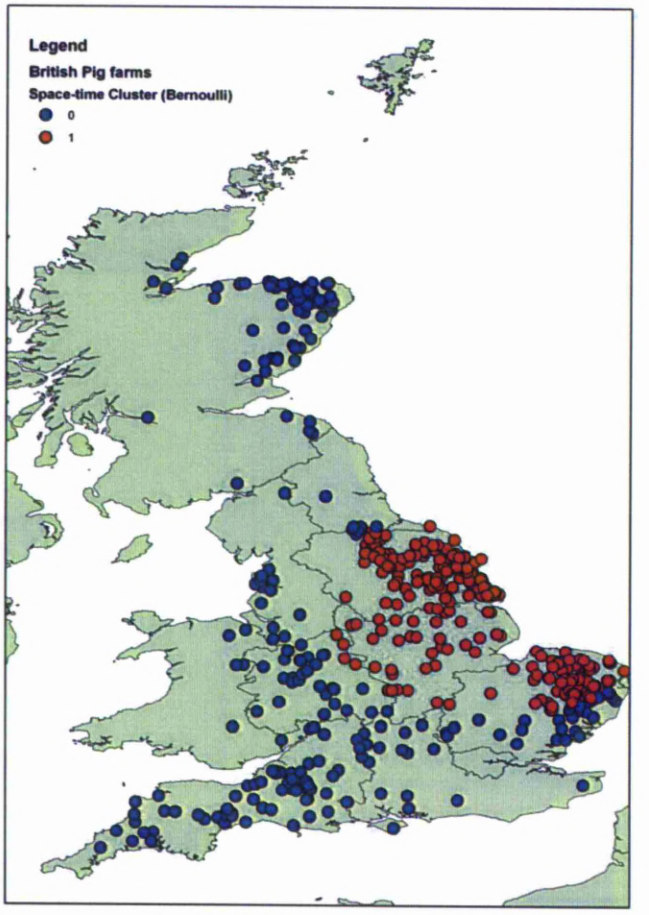

Figure 4.9: Map to show position of the most likely pig *Salmonella* spatial cluster detected by the Bernoulli scan statistic method.

4.3.4 Discussion

The K-function analysis, at either a global British level or at a regional level, did not detect any significant aggregation over and above that which would be explained by the geographical density of pig farms alone. The result indicates that cases within the study population were not more likely to be more clustered in space than control farms. This result agreed with a similar analysis of ZNCP data which focused on three regions of Britain (East England, Yorkshire and Humber and the South West) with sample data from two separate 12

month periods (year 1 - August 2002 to July 2003, year 2 - August 2003 to July 2004) (Clough et al., 2009). The previous study used all farms belonging to the ZNCP scheme within those regions (n=912) but differed from this study by classifying cases as those that had more than 65% of samples positive, which was used originally in the ZNCP scheme to define and classify high prevalence farm, whereas the study described here defined cases as having above average seroprevalence. However, a K-function analysis of the ZNCP records, collected from 2007, that could be linked to QA farms (n=1,415 farms) showed that farms with more than 25% of samples positive were more clustered in space in the Yorkshire and the Humber region, than control farms (Chapter 2; Smith et al., 2011). Although the cut-off points for defining cases were similar to the method described here, the use of only one year of data from the ZNCP scheme, rather than up to four years used in this analysis, may have meant that differences were related to a specific clustering event in that year rather than to a four year average for each farm.

The other global spatial analyses (histogram of D), examining the British population, presented an additional method of displaying that the pig farm population was not significantly different to a random allocation of cases and controls, and so, cases were not clustered in space. However, the results from the spatial points of the whole study population were clustered in space within Britain, rather than randomly or uniformly distributed. This finding was largely as expected, as the commercial pig farm population in Britain has been shown to be clustered within specific areas (North East Scotland, East and North East England) and sparsely populated in other areas (Smith et al., 2011).

The scan statistic analysis detected significant clustering by both tested methods, with the Poisson method detecting a significant cluster within the Yorkshire and Humber region, whereas the Bernoulli method most likely cluster covered Yorkshire and areas of the East Midlands and East England. In both clusters the relative risk indicated that the risk for samples from farms within the cluster was between 2 and 2.5 times more likely to be a case, than for farms outside of the cluster.

Identifying the presence of the most likely statistically significant clusters in the areas of high pig farm density in England (Yorkshire and Humber and East England) provides further evidence to previous work which showed that the clustering of farms in high farm density areas was important in England but not in Scotland (Chapters 2 and 3) and agrees with the previous identification of spatial clustering of pig *Salmonella* in these areas (Clough et al., 2009; Clough et al., unpublished data). This may highlight that spatial clustering is caused by a method of transmission that is important when the farms are congregated, with small distances between them. In Scotland, as the average prevalence has been shown to be lower than in England and Wales (Chapter 2) this 'local spread' of infection may be less important. The findings from this study may also suggest pockets of farms within these areas

that are either managed in a similar way, have the same risk factors for *Salmonella*, or are exposed to the same *Salmonella* control advice from local sources. The reason may also relate to the regional groupings of integrated pig companies, where clusters of high prevalence in finisher farms within a region are influenced by the prevalence and strains of *Salmonella* in the local nucleus and multiplier herds that supply their pigs.

The use of this study population to examine spatial heterogeneity in *Salmonella* in pig farms may not be general to all pig farms, as the population utilised QA registered farms that were part of the ZNCP scheme. The results may also be affected by not covering all pig farms within the defined regions, although it is believed that the QA dataset includes most commercial pig farms. The study population precluded specialist breeder farms and might not contain less conventional farms, such as semi-professional farms, that were not part of a QA scheme. The population also omitted farms that could not be linked to X and Y coordinates, which may have biased the population towards rural farms that did not have a postcode or an easily geo-referenced address.

The difference between the results of the two spatial tests may be down to the differences in methodology, in particular the ability of the scan statistic to locate clusters of any circular size within the whole Britain, whereas the K-function test examined a defined region which may have been split between a clustered area and a non-clustered area. This highlights the need to use multiple statistical analyses to examine spatial heterogeneity within a population, due to the differing natures of the approaches and their ability to answer different questions.

The analysis may have benefited from developing the tests further. Adding covariates to the scan statistic may have helped identify whether any specific factors could account for the clustering, and using a non-circular scanning window may have located other clusters that did not fit the circular pattern. The K-function analysis was also limited to a binary (case-control) outcome, rather than being able to account for the *Salmonella* result of each sample from a farm which would have been beneficial, as this would have accounted for the differing numbers of samples collected by each farm.

4.4 Explaining the spatial heterogeneity of pig *Salmonella* in Great Britain

4.4.1 Introduction

The identification of significant spatial heterogeneity of pig *Salmonella* prevalence in previous British studies (Chapters 2 and 4.3; Smith et al., 2011), coincided with the identification of explanatory variables, significantly associated with *Salmonella*, identified from a comprehensive analysis of a large population of UK pig farms (Chapter 2; Smith, Clough and Cook, 2010). Through the use of geostatistical modelling approaches it was hoped that the significant variables could be added to the spatial analysis of pig *Salmonella* data, to determine whether spatial heterogeneity remained and was not accounted for by the explanatory variables.

The study described here was designed to test for, and further describe, the presence of spatial heterogeneity in the serological *Salmonella* results of a large pig farm population. Initial approaches sought to determine whether there was evidence of spatial heterogeneity in the raw data. This alone would be unsurprising; such variation could potentially be explained by known *Salmonella* risk factors which may or may not be themselves spatially structured. A two stage process was used: first, a model-based approach to establish whether known and measured risk factors could explain any of the observed variation, and subsequently adding a spatial analysis to the model to determine whether any residual spatial heterogeneity remained.

4.4.2 Materials and Methods

The collection of farm data from a postal questionnaire was described in Chapter 3. The postcode of each farm that returned a questionnaire was used to locate a map reference (X and Y coordinates) in the software package ArcGIS 9.1 (ESRI). However, it should be noted that the postcode may relate to the correspondence address of the farm owner and may not necessarily coordinate with the actual location of the pigs. As some farms were listed under the same postcode, the coordinates were agitated by a random number generator, based upon a normal distribution, to ensure a random and symmetric re-location most probably close to the original coordinates. The farms were moved so that they were located under separate coordinates and would not overlap for spatial analysis, although the movement was minimal (up to 30 metres from the original). Further details on the data collected from the questionnaire have been published elsewhere (Smith, Clough and Cook, 2010).

Each questionnaire was linked to information from the ZAP scheme for that farm, limited to results collected up to four years prior to the completion date of the postal questionnaire. A

four year period was chosen to allow for the analysis of temporal trends over a number of years, for another part of this project. For the ZAP scheme, small pieces of muscle (from diaphragm/ neck) were removed from pigs at the abattoir and placed in meat juice (MJ) tubes which were frozen and then thawed to collect the fluid (Nielsen et al., 1998; Armstrong, 2003). The MJ sample was tested at a single UK laboratory by a mix-ELISA serological test (Guildhay VETSIGN™Kit) for a "host" response of antibodies to Group B and C_1 *Salmonella* (Nielsen et al., 1998). *Salmonella* infection in pigs produces an immune response, which includes the production of antibodies. These are detected by the ELISA from which a sample to positive ratio (ELISA ratio) is calculated, which is related to the titre of circulating antibodies (Sorensen et al., 2004; Hill et al., 2008). Three samples were randomly collected from every batch of pigs sent to slaughter on any particular date in accordance with the sampling regime agreed in May 2003, but samples collected from late 2006 onwards were collected at a rate of five per farm per month ((BPEx, personal communication, 2010).

For routine surveillance, a cut-off point of 0.25 is applied to the ELISA ratio to provide a binary outcome, but in order to exploit the full power of the data for this study, the results were extracted in two ways: as the raw ELISA ratio for each submitted sample; and as a binary result determined using the surveillance cut-off point. The binary result was used to classify each farm as a case or control, depending on the percentage of positive samples from the total number of samples tested for that farm. Farms with 22.2% (the mean value) or more positive samples were classified as cases, due to this higher relative seroprevalence.

Data analysis

The data were imported into the R 2.11.0 statistical package for analysis (R Development Core Team, Vienna, Austria). The data points were presented on maps of Great Britain, using the add-in package Splancs (Rowlingson and Diggle, 1993). A smoothed map of farm locations was also produced to show the regions of highest farm density within Great Britain, accounting for edge effects (using the kernel2d command).

A map was produced using the sparr package (Davies, Hazelton and Marshall, 2010) to present the log-relative risk of farms being labelled a case, and to highlight hotspots of *Salmonella* risk. The sparr package uses an edge-corrected adaptive bandwidth to provide a focused estimation of risk in areas with high numbers of farms, whilst maintaining stable estimates in areas of low farm populations. This method corrects for the potential over-smoothing in areas of low density and under-smoothing in high density areas, caused by a fixed bandwidth.

Graphical exploration (Box and Cox, 1964) confirmed that logarithmic transformation of the ELISA ratio was appropriate to transform these data to approximate normality. All negative and zero ELISA ratios were coded to 0.005, which was half of the lowest recorded result,

prior to transformation, as a crude approach to overcoming the problems associated with logarithmically converting negative and zero values. The log ELISA ratio results were analysed spatially using a geostatistical approach, by standardising residuals from a linear model and estimating a variogram, using the add-in package geoR (Ribeiro and Diggle, 2001). The variogram summarises the difference in a pair of observations as a function of their spatial separation. As such, it is a spatial analogue of the variance in more standard statistics (Clough et al., 2009). Used in this context, variograms are a useful tool for determining whether residuals, which are spatially close together, are more similar than those which are far apart; by analysing residuals in this way it could be seen whether the *Salmonella* results from geographically proximate farms are more similar than those far apart, having allowed for known risk factors (Diggle, Tawn and Moyeed, 1998; Brown et al., 2004). The distance limits of the variogram were set at 50km, which was chosen because variance estimates at large distances become less reliable, and it was the opinion that this was the appropriate limit at which spatial spread of *Salmonella* might occur. Variograms were also produced at shorter distances to further explore the results, where necessary.

A linear mixed model, with the log ELISA ratio as the outcome, was built. The unique farm identifiers were included as a random effect, to allow for likely dependence between samples from the same farm. A baseline null model was built which, aside from the farm-level random effect, included only a common mean and no risk factors. The variogram of the standardised residuals from this model were plotted against their spatial separation along with simulated spatially-uncorrelated Monte Carlo estimate envelopes, based on permutations of the data values across the locations (Rowlingson and Diggle, 1993). A lack of evidence of spatial correlation would result in the estimate of the variogram falling completely within the simulation envelopes; similarly spatial heterogeneity would be shown by departure from these envelopes. Significant heterogeneity may indicate that the farms within the area may share particular risk factors, e.g. a geographical factor such as the weather or a locally common management practice, or could highlight a particular transmission method, such as the infection of a local environmental source and subsequent infection of the local farms. A variogram was also plotted using a linear mixed model containing all the exposure variables collected by the questionnaire that were significantly associated with log ELISA ratio (Table 4.4 (Smith, Clough and Cook, 2010)), to detect whether the introduction of these variables explained any detected spatial heterogeneity in the data (Diggle, Tawn and Moyeed, 1998).

Table 4.4: List of significant ($P<0.01$) variables associated with log ELISA ratio results from pig farms, detected by a linear mixed model (for further information, please see Smith, Cook and Clough, 2010).

Variable	Level
Farm location	Scotland
	Other
Pig farm density within 10 kilometre radius	Numeric
Farm enterprise	Conventional
	Non-conventional
Primary cause of pig mortality in the last 12 months	Respiratory or wasting
	Other
Monthly mean temperature anomaly for farm's region (°C)*	Numeric
Monthly Rainfall actual for farm's region (mm)	Numeric
Monthly Sunshine actual for farm's region (hours)	Numeric
Finishers fed homemix	Yes
	No
Percentage of barley in grower feed	Numeric
Number of pig deliveries	>11/year
	6-11/ year
	0-5/year
Number of dead stock collections	>6/year
	0-6/year
Yearly temporal cycle (sine and cosine terms)	Numeric
Quarterly temporal cycle	Numeric

*'anomaly' is the difference from long-term averages.

4.4.3 Results

Of the 474 farms that returned questionnaires and remained in the final model dataset, 440 had the necessary information to generate a map reference. A kernel smoothed plot of the locations of these premises (Figure 4.10) indicates the spread of participating farms around Great Britain, with farms covering central and South West England and Eastern Scotland, with the greatest farm density in the regions of Yorkshire and the Humber, North East Scotland and East Anglia.

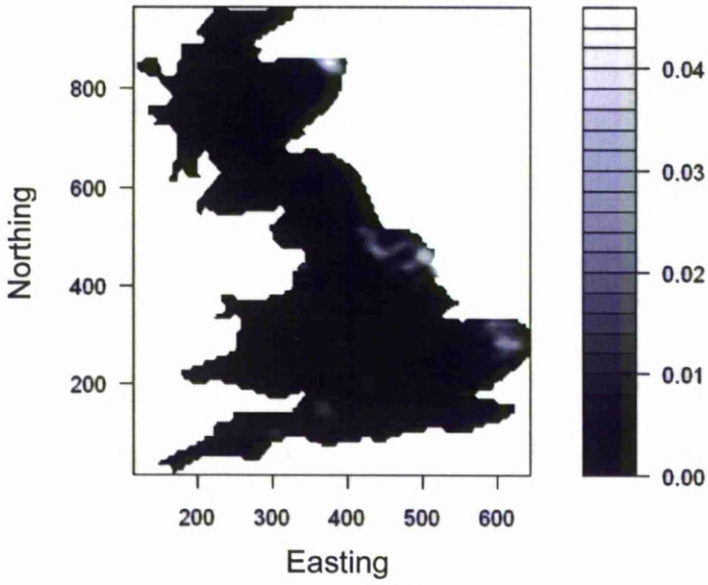

Figure 4.10: Map of Great Britain to show a smoothed distribution of the density of participating pig herds (kernel estimate). (Distance is in kilometres).

The 440 pig farms were linked to a total of 103,133 *Salmonella* serology samples over the four year period of study (mean 234 samples, range 1-1,671). According to the criteria described in the methods, 198 (45%) farms were labelled as case farms, indicating a higher than average seroprevalence. A plot of the probability of a farm being a case, using a spatially-adaptive smoothing method, shows that the highest risk was in Yorkshire (log-relative risk of 1.5), whereas farms in North East Scotland had the lowest risk (score of -4) (Figure 4.11). The map highlights an area in both Yorkshire and East Anglia that was within the thick contour bar, indicating a significantly high level of risk (*P*-value 0.05). The closeness of the 5th and 25th percentile boundaries highlights how distinct these areas were from the remainder of the geographical area.

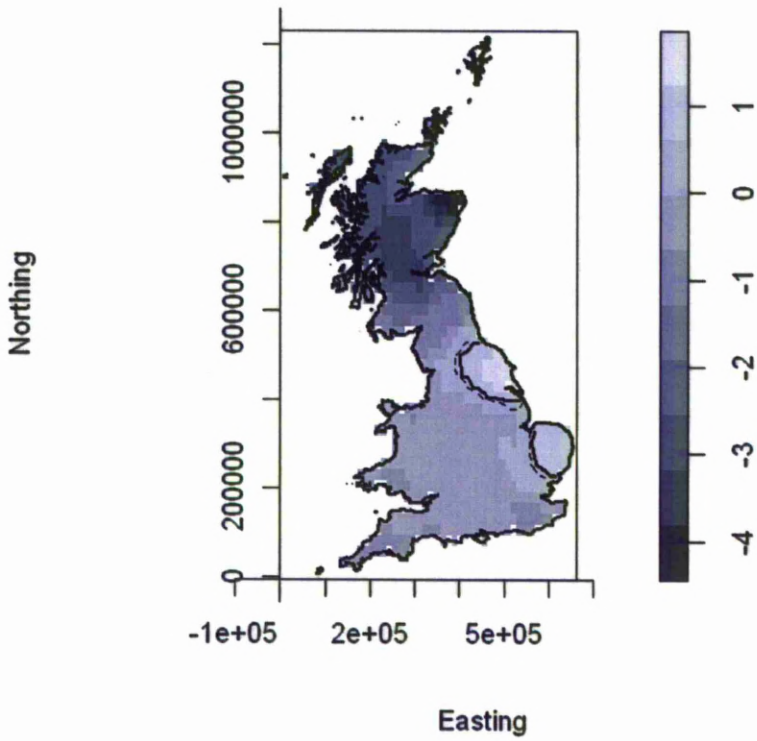

Figure 4.11: Adaptive bandwidth smoothing to show the spatial pattern of *Salmonella* risk determined by ELISA ratio results. The thick contour line represents the upper 5th percentile of risk and the dashed line indicates the upper 25th percentile. (Distance is in metres).

Figure 4.12 and 4.13 present the variogram analysis plots, using the logarithmically converted ELISA ratio for each sample whilst accounting for the similarity of results from the same farm. The estimated variogram of the residuals, from the null linear model shows evidence of spatial dependence of the log ELISA ratio up to 50km distance, indicating spatial heterogeneity of *Salmonella* concentrations (Figure 4.12). Figure 4.13 shows that once the covariates significantly associated with *Salmonella* were added to the linear mixed model, the residuals fell within the simulation envelopes equating to no evidence of spatial dependence once the risk and protective factors had been taken into account. As the covariates included a spatial variable related to the number of pig farms with a 10km radius of each farm, the variogram model was repeated but with this variable removed from the linear mixed model. The result did not change and the remaining covariates still accounted for the spatial dependence originally detected.

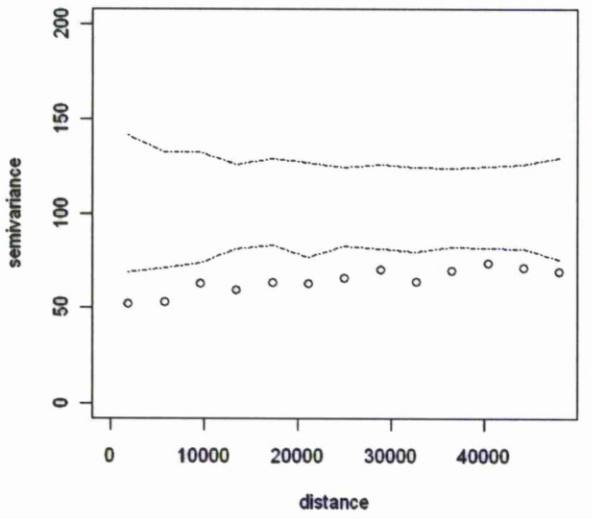

Figure 4.12: Variogram of residuals from a 'null' linear mixed model for pig farms (n=440). Distance is in metres. The standardised residuals from this model are plotted as circles against the dashed lines which represent the simulated spatially-uncorrelated Monte Carlo estimate envelopes, based on permutations of the data values across the locations.

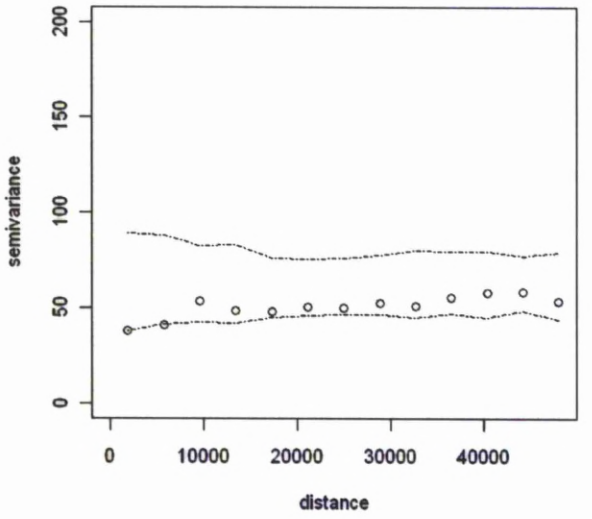

Figure 4.13: Variogram of residuals from a linear mixed model of pig farm variables significantly associated with ELISA ratio (n=440). Distance is in metres. The standardised residuals from this model are plotted as circles against the dashed lines which represent the simulated spatially-uncorrelated Monte Carlo estimate envelopes, based on permutations of the data values across the locations.

4.4.4 Discussion

The spatially-adapted smoothed plot and the unadjusted variogram analysis successfully identified spatial heterogeneity, indicating that farms that were spatially close were more likely to have similar results. However, these preliminary analyses did not take any risk factors into account. This spatial interaction was expected, as biological interactions, particularly of infectious diseases, are likely to be spatially structured, with neighbouring locations having more similar characteristics (Diggle, 2002). The two types of spatial analysis provide a complementary description of spatial dependence, with the variogram providing evidence for the significant heterogeneity at specific distances from a farm, and the smoothed risk plot identifying localised hotspots and allowing different areas to be compared. The comparison of regional areas is particularly important as the pig farm population is highly clustered in Great Britain.

A Danish study using a spatially-adaptive smoothing method, also showed regional heterogeneity of *Salmonella* on pig farms, which was hypothesised as being due to differences in management, feed and pig movements between the different regions/ islands (Benschop et al., 2008). Our findings also agree with analysis of the ZAP scheme data used previously to analyse spatial trends. An initial report using variogram analysis showed that geographically localised anomalies of *Salmonella* infection were present over short distances (up to 20km) in one of three English regions studied, East of England, rather than the Yorkshire and Humber region or the South West (Clough et al., 2009). A further study adapted the variogram spatial analysis to account for seasonality and farm enterprise type in a study of two English regions (East England and Yorkshire and the Humber), which showed an elevated spatial effect in East England, possibly due to the high proportion of outdoor herds in this area and thus increased chance of localised spread from neighbouring farms (Clough et al., unpublished data).

The introduction of the list of significantly associated factors, determined by the risk factor analysis, into the linear model used for variogram analysis, removed the significant spatial heterogeneity and no residual spatial correlation was discernible. However, the list of covariates in this study included two spatially-structured factors: high farm density at the 10km radius as a risk factor, and spatial region, where a Scottish farm location was a protective factor. This result may show that, once all covariates are accounted for, no spatial heterogeneity affects the whole of Britain and these two variables are the only significant spatial factors. Farms in Scotland may have a lower prevalence of *Salmonella* because they are more likely to use certain management procedures (e.g. homemixing, flooring types (Chapter 2)) and, due to their geographical isolation, they are more likely to purchase similarly low prevalence animals from farms within the same area as shown by a recent social network analysis (Smith, Cook and Christley, 2012). Pig farm density is an important variable as dense areas may be more at risk of localised routes of transmission. However,

as the variogram model still accounted for the spatial dependence once this variable was removed, it does not seem likely that this variable was strongly related to the spatial dependence and instead was associated with another relationship with *Salmonella* i.e. the variable was a proxy for the different pig farming regions of Britain, with similar management and advice from veterinarians within each region. The covariates also included meteorological variables, such as hours of sunshine and temperature, and these locally varying factors have been shown to be important to the survival of *Salmonella* in the environment, as well as causing stress and increased susceptibility in pigs (Hald and Andersen, 2001; Placha et al., 2001).

Utilising a study population drawn from the QA schemes may have biased the results, as although the QA schemes are believed to contain 80% of all the commercial pig farms in the UK, the remaining commercial herds and small holdings may also play an important role in the spread of *Salmonella*. The participation rate may also have introduced bias, as specific types of farms may have refused to participate. However, the map of the geographical spread of the study farms indicates that the population appears to be representative of the pig farms in the Great Britain, with high density clusters in Eastern England, Yorkshire and the Humber and in the North East of Scotland. Participation analysis in the original risk factor study (Chapter 3.3; Smith, Clough and Cook, 2010) found that the study population was broadly geographically representative of the full QA population. The mean herd size was significantly higher than the mean of the QA population, indicating that smaller farms may have been more likely to refuse participation, but it was believed that the remaining herd characteristics were representative of the QA population.

The use of serology results from the ZAP *Salmonella* surveillance scheme may have provided some bias to the study, as the results represent a historical rather than a current infection of *Salmonella*, and so any detected spatial heterogeneity may not represent a clustering of farms that had a high prevalence of infection during the same time period. The results also do not distinguish between the *Salmonella* serotypes detected and so the clusters may actually have been infections of unrelated serotypes. However, studies have shown a significant correlation between serology and caecal content results (Sorensen et al., 2004; Funk, Harris and Davies, 2005; Rajic et al., 2007). Furthermore, these studies have shown that classifying farms as categories, such as the high (case) or low (control) seroprevalence categories used to create the smoothed risk map described here, was shown to produce a stable result.

The study demonstrates how covariates identified by a previous study of the same farm population, account for the spatial dependence in farm ELISA results. This finding highlights that two spatial variables (farm density and Scottish location), as well as a number of meteorological and farm management factors account for the localised aggregation of results

i.e. neighbouring farms are more likely to have similar *Salmonella* results as they shared similar farm management and were affected by the same weather conditions. By further describing and explaining the possible reasons behind the spatial heterogeneity, this information should provide assistance to *Salmonella* control strategies as particular farms or regions can be prioritised or targeted for specific interventions. Although our study did not show any evidence of residual spatial structure, once known risk factors had been taken into account, the usefulness of these spatial analysis approaches for determining the nature of any spatial effects is again demonstrated. This analysis has also provided a useful illustration that other authors, who find apparent spatial effects, should be cautious in their interpretation of such face value effects.

4.5 Northern Ireland spatial analysis

4.5.1 Introduction

The spatial analysis of farms in Northern Ireland was completed separately from mainland Britain as it is unknown how the physical barrier of the sea would equate to a similar spatial distance over land.

4.5.2 Method

A similar set of spatial analyses as used for mainland Britain (Chapters 4.3 and 4.4) were completed for this subset of farms to evaluate whether spatial heterogeneity of *Salmonella* seroprevalence could be detected in Northern Ireland and to investigate its structure. As stated for the previous analysis, the farm population was divided into cases and controls, according to their seroprevalence (above or below the mean percentage of positive samples for the whole UK population) for K-function analysis.

4.5.3 Results

A total of 45 pig farms in the dataset were located in Northern Ireland. It was not possible to connect two farms to any samples in the ZNCP scheme but all of the remaining 43 farms provided sufficient identifying information to be able to link them to X and Y coordinates. The high prevalence farms were evenly distributed in Northern Ireland and the K-function plot (Figure 4.14) shows that the estimated values fell within the permutation envelopes and so any spatial heterogeneity was not significant. However, the scan statistic assessment, using a Poisson methodology, found significant (Relative Risk= 2.07, $P<0.001$) spatial heterogeneity in nine farms in the North of the country (Figure 4.15). Two significant ($P<0.001$) secondary clusters were also detected in the East and South West, but these only included two high seroprevalence farms each. No significant clustering was detected using a Bernoulli method.

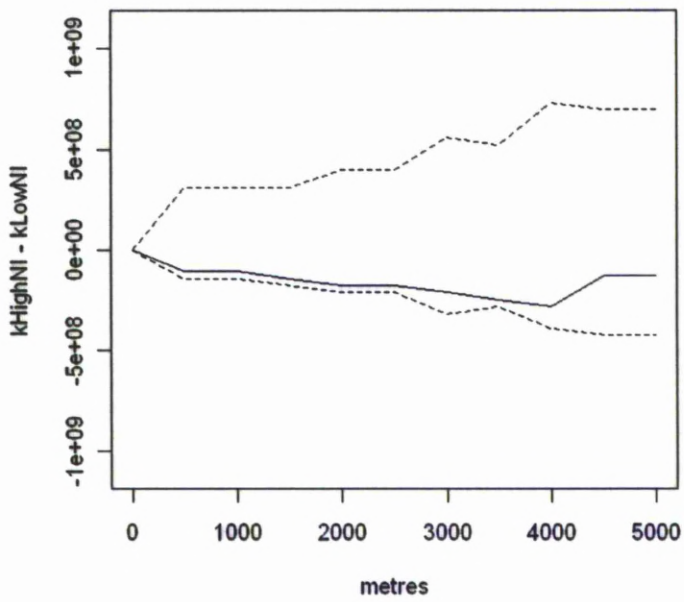

Figure 4.14: K-function plot to assess spatial heterogeneity (KHighNI-KILowNI), over distances between Northern Irish pig farms, along with confidence envelopes formed from simulations (dotted lines).

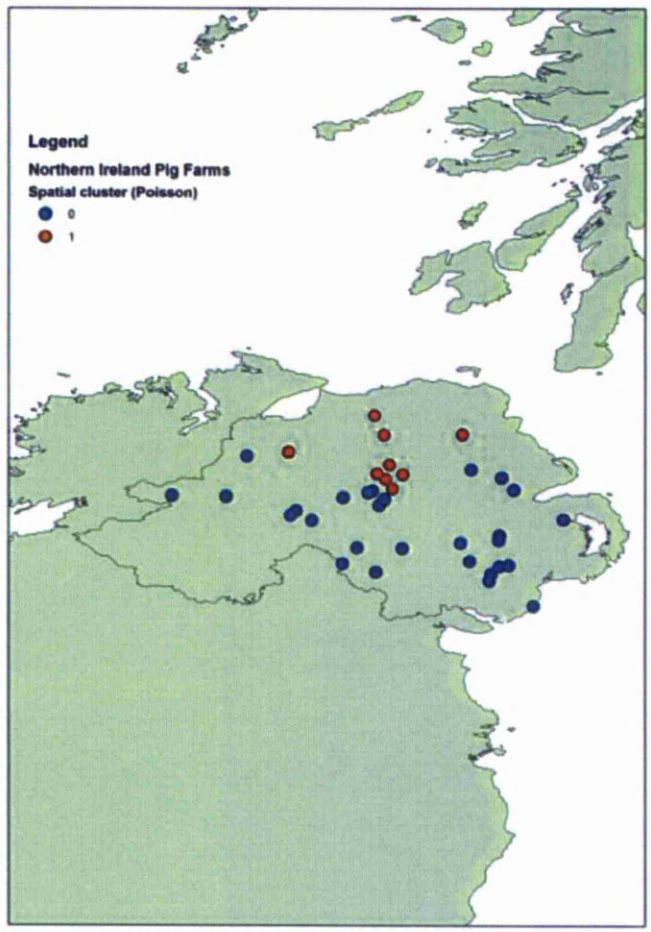

Figure 4.15: Identification of significant area of spatial heterogeneity of *Salmonella* seroprevalence results by scan statistic Poisson method assessment (43 farms).

Finally, variogram analysis was used to assess the spatial dependence of results between the Northern Irish farms. A population of 34 farms was used, as these were the farms included in the final risk factor model designed in Chapter 3. No significant spatial heterogeneity was detected in either the variogram for the null model, containing no explanatory factors, or the full risk factor model, which displays a variogram whilst accounting for the effect of the explanatory factors (Figures 4.16 and 4.17).

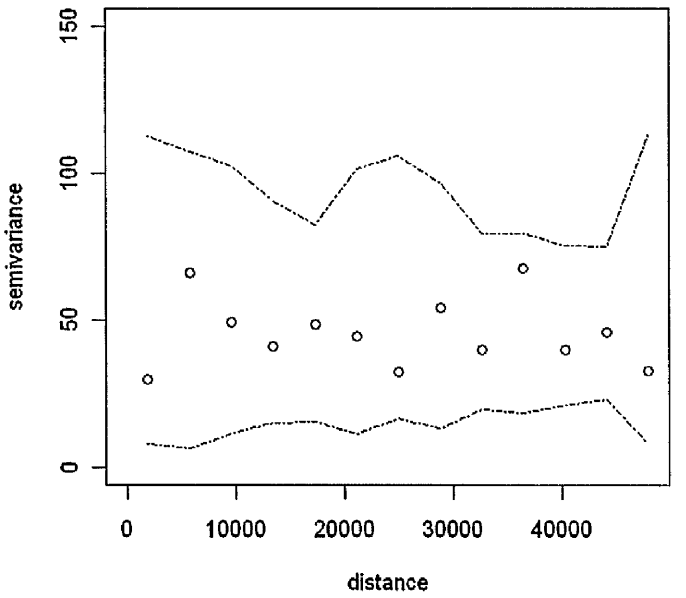

Figure 4.16: Variogram of Northern Irish pig seorprevalence by distance in metres, using a null model (34 farms).

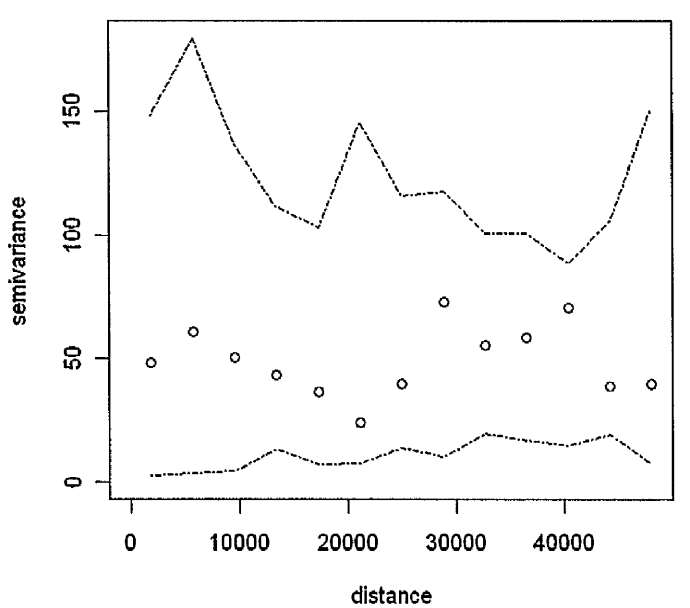

Figure 4.17: Variogram of Northern Irish pig seorprevalence by distance in metres, using a model containing significantly association factors identified by previous analysis (34 farms).

4.5.4 Discussion

No spatial heterogeneity was found when the study population was assessed by K-function or variogram analysis, and no difference was detected when the factors associated with *Salmonella* were entered into the variogram model. However, the scan statistic, which used the number of positive samples against the background of all samples by location, did show a number of small clusters of farms for which significant heterogeneity was detected. The difference in results may indicate that although no global spatial dependence was detected within Northern Ireland, there were areas of significant spatial dependence. This finding highlights the benefit of using different spatial methods to examine spatial structures, as differences may occur at a local level that cannot be detected at a global level. This may also show the difference in methodologies, as the study population may have been too small to generate the necessary power and confidence for the K-function or variogram analysis.

The identification of a number of significant clusters may highlight areas of risk caused by farms that share a local transmission route, for example, these farms may have been more likely to move pigs between each other and so a high prevalence farm would have helped to introduce a similar prevalence in farms receiving their pigs. An examination of pig farm movements may help to explain whether patterns of movements may be a risk for *Salmonella* spread either locally or nationally. The findings may also highlight pockets of farms that had similar farm management or shared similar biosecurity practices due to the farm being exposed to the same veterinary advice or attending the same farm information meetings.

The analysis could be limited by the small population of Northern Irish farms for which information was available. A small population is more prone to errors where small chance variations in the results can have large effects on the outcomes, and so these results should be viewed with caution. The study population represented roughly 20% (43/219) of the commercial scale (>50 pigs) finisher pig farms in Northern Ireland, according to the results of the agricultural census completed in 2010 (NI census http://library.nics.gov.uk/pdf/dard/2011/EBHZ.pdf). The inclusion of the farms that did not provide data to this study may have had a great impact on the spatial analysis results and it is unknown whether the subset analysed here was representative of the background population.

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Chapter 5: Network analysis of pig movements

5.1 Introduction

The epidemiological risk factor model designed in Chapter 3.4, displayed some remaining unexplained regional variation, even though a large number of explanatory variables had been tested through the model. The model also included a risk factor for the number of incoming pig movements and it was hypothesised that this regional variation could be related to the transmission of *Salmonella* via pig movements. The ability of standard risk factor analyses to fully examine the role of movements is problematic as it cannot incorporate crucial information such as the destination of those pigs, or take account of the position of each herd within the industry network structure. Therefore, a social network analysis was planned.

The commercial UK pig industry has, unlike the cattle and sheep industries, a clear structure with a small number of nucleus herds supplying a larger number of multiplier breeding farms, which in turn supply specialist finisher farms (Figure 5.1). A subset of farms combine the roles of the breeder and finisher farms and only import new breeding stock from nucleus and other breeding farms. A network analysis approach would allow specific farms, or types of farms, that are of particular importance to spreading infection, via direct and indirect methods, to be identified.

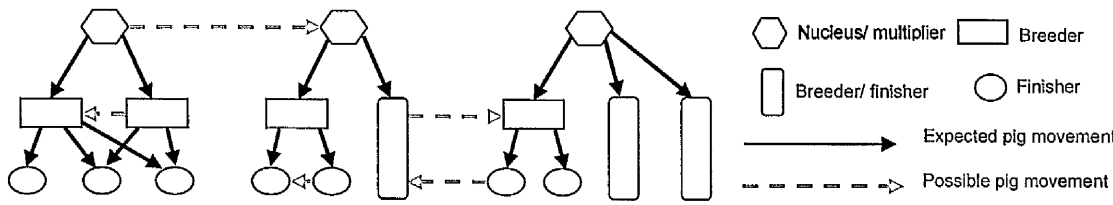

Figure 5.1: Network connections between pig farm enterprise types.

A network analysis was completed utilising the pig movement data recorded by the QA schemes, along with information on abattoir and haulier usage (Chapter 5.2). The analysis particularly focused on explaining why Scottish farms were found to have a lower seroprevalence in the risk factor model, and so comparisons between regions and QA schemes were made. A further analysis was completed to validate the recording of movements in the QA scheme records against a movement dataset collected by a Government scheme and to check the consistency and standardisation of the recording of movement records (Chapter 5.3). Finally, the network parameters were added to the risk factor model, designed in Chapter 3.4, to determine whether these were significantly associated with *Salmonella* seroprevalence and whether the inclusion of these parameters would improve the fit of the model (Chapter 5.3).

5.2 Descriptive and Social Network Analysis of pig transport data recorded by Quality Assured pig farms in the UK

5.2.1 Introduction

As with other livestock systems, the movement of pigs between farms is an important factor in the transmission of infectious diseases between farms, as incoming pigs can introduce disease to a novel and susceptible population (Ortiz-Pelaez et al., 2006). The transport of animals can increase stress in pigs, which lowers the performance of the immune system, thereby increasing susceptibility to infection. Furthermore, stress can reactivate viruses or cause infected animals to shed a greater concentration of bacteria (Thiry et al., 1987; Moberg and Mench, 2000). Transport stress may be exacerbated by the withholding of water and food (Isaacson et al., 1999; Averos et al., 2008). After transportation, stress may also be caused by newly introduced animals being mixed with other pigs, which disrupts their social hierarchy, which can increase the number of new animal infections (Tizard, 2004).

In addition to the risk of introducing infected animals to a farm, vehicular movements for animal transport and other purposes, can also be an indirect method of transmission for diseases that can survive outside of an animal (e.g. Foot and Mouth Disease). Livestock hauliers can spread contaminated fomites (e.g. mud and faeces collected on the undercarriage of vehicles) onto farm premises or onto fields neighbouring roads (Alexandersen et al., 2003). Ineffective cleaning and disinfection of haulier's vehicles between animal deliveries, and the collection of pigs from within the farm rather than at the farm gate, may contribute to the risk of infection from the haulier environment of subsequent batches of collected livestock. The number of farms using the same haulier may be an important factor in understanding how diseases can be transmitted where no evidence is apparent from farm-to-farm connections. An abattoir may also be an indirect source of disease transmission for foodborne diseases, as equipment and abattoir environment contaminated by previous batches of pigs may contaminate the carcasses of incoming pigs, which would provide a risk to human health.

Social network analysis has been used previously to provide summaries of the movement of livestock, and to improve the understanding of the interconnectivity of farms (Dubé et al., 2009; Martinez-Lopez, Perez and Sanchez-Vizcaino, 2009). These methods can help explore the potential speed and range of spread of an infectious agent. One of the aims of network analysis focuses on measurements of centrality which highlight how connected an object is within a network and how important those connections are in linking the farms. These measures have been shown to be useful in predicting the risk of becoming infected and the time to infection (Bell, Atkinson and Carlson, 1999; Corner, Pfieffer and Morris, 2003; Christley et al., 2005). Understanding the network structure can also highlight which

types of control strategy may be effective in limiting the spread within a defined population (e.g. Christley and French, 2003).

The commercial United Kingdom (UK) pig industry has a small number of nucleus herds supplying a larger number of multiplier breeding farms, which in turn supply specialist finisher farms. A subset of farms combine the roles of the breeder and finisher farms and only import new breeding stock from nucleus and other breeding farms. The industry is also spatially structured with the majority of pig farms in North-Eastern Scotland, Yorkshire and Humber and Eastern England. A network analysis approach may identify specific farms, or types of farms, that are of particular importance to spreading infection. The importance of analysing pig movements has also been shown by other studies. A panel of international pig disease experts agreed that the movement of pigs onto farms is the most likely cause of introduction of *Salmonella* infection onto a pig farm (Stärk et al., 2002). Furthermore, the number of connections between pig farms has been shown to be a particular risk; pigs sampled from herds recruiting from more than three supplier herds had three-times higher odds of testing seropositive to *Salmonella* antibodies than herds which bred their own replacement stock or used a maximum of three supplier herds (Lo Fo Wong et al., 2004).

A recent study of *Salmonella* seroprevalence, using pig abattoir surveillance data, detected significant regional variation in the UK, with lower prevalence in Scotland (Smith, Clough and Cook, 2010). It has been hypothesised that this variation might result from the relative geographic isolation of pig farms in some areas (such as Scotland), which may lead to a preference for purchasing pigs from nearby farms and this isolation restricts the flow of disease transmission. The regional differences may also be explained by a more concentrated presence of large commercial pig companies within some of the regions, possibly integrated with their own abattoirs and hauliers, as it would be expected that these companies would principally move pigs within their own company, regardless of the distances.

In this study, the network of pig movements was characterised in order to explore the potential effects of movement connections on the spread of an infectious agent, with *Salmonella* selected as an example of an important endemic disease within the UK. Data were collected from Quality Assurance (QA) schemes, which identified breeding herds used to supply weaners or finisher farms and connections to abattoirs and hauliers. QA schemes monitor farm practice, and collect data on farm structure and management, via yearly assessment visits, to ensure a level of health and welfare standards are met. The QA schemes cover different geographical areas and have slight deviations in the structure and content of the data collected and monitored. The farms that participate in these voluntary schemes generate an estimated 92% of pig meat production in Great Britain (BPEX, 2010a; SFQC, 2010). By using a network analysis of farm-to-farm movement connections, and a

descriptive analysis of the usage of hauliers and abattoirs by farms, this study aimed to test hypotheses of how pig movements in the UK may affect the prevalence of infectious disease and how movement connection characteristics may help explain the spatial heterogeneity of prevalence of *Salmonella*.

5.2.2 Method

Collection of data

Data on abattoir and haulier usage, weaner/ grower pig suppliers, and feeder units (used to finish pigs before slaughter) supplied with pigs, were collected from three QA schemes: Genesis Quality Assurance (GQA); Assured British Pigs (ABP); and Quality Meat Scotland (QMS), in 2008. The dataset covered yearly QA assessment records from 2004 to 2008, and although some farms had multiple records, only the most recent assessment for each farm was included in this study. Some differences between the data collected by the three schemes were identified. QMS, which covers the majority of pig farms in Scotland, did not record the use of hauliers or which feeder units were supplied.

Within the schemes, the data enabled the identification of linkage between premises locations (i.e. the use of an abattoir or the movement of pigs between farms), which are referred to in the text as movement connections. However, the schemes did not provide information on the number of movements in a year, the specific dates of movements or the number of pigs moved. Movement connections to abattoirs, farms or hauliers that could not be linked to a unique identifier were removed from the dataset, and so farms that were not QA scheme members were only kept in the network if they could be identified as a unique farm via other farm identifier information. A farm-to-farm network was created, with each unique farm QA membership identifier used to define a node and the presence of any pig movement connection between the farms created arcs between the nodes. Each of the arcs in the network were directed i.e. the link between farms showed the direction in which pigs were moved from one location to another.

Information on the name of the farm's company, and the map location coordinates (X and Y) of each farm, was also collected from the QA schemes. The companies were grouped into three categories, depending on the number of farms in the dataset listed under each company name, defined as: large (>50 farms); medium (5-50); and small/independent (1-4) companies. The coordinates were used to identify the NUTS (Nomenclature of Units for Territorial Statistics (EC, (2010))) region for that farm. NUTS have four subdivisions and NUTS 1, equivalent to government office regions, were used rather than other geographic boundaries (such as county) as they are more stable over time and less subject to boundary changes. It is thought that the categories represent more biologically sensible categories in

terms of the UK's animal species population and coincide with the spatial structure of pig farm density.

Map coordinates were also used to calculate Euclidian distances in kilometres between farms and abattoirs, using Pythagoras' theorem. Additional to the distance information, the ABP and GQA schemes also recorded travelling times in hours, which was validated by checking outlying travelling times against a website travel planner application (Multimap.com). If movement connections between two locations were provided with varying travel durations in the dataset, then an average duration was recorded.

Data analysis

The analysis consisted of an analysis of the network structure of farm-to-farm connections and a descriptive evaluation of the use of abattoirs and livestock hauliers. These data were handled and analysed using MS Access 2003 (Microsoft corp.), R 2.11 (R Development Core Team, Vienna, Austria) and Minitab 14 (Minitab inc.). ArcGIS 9.1 (ESRI, Redlands, Calif., USA) was used to generate a map.

Ucinet 6 (Borgatti, Everett and Freeman, 2002) was used to analyse the relationships between nodes on the basis of their arcs. Measures of centrality were used to define the importance of certain nodes and explore the heterogeneity of arcs between nodes:

- *Degree* measures the number of arcs between a single node and all other nodes in a network. The in-degree measures the number of farms which send pigs to a specific farm in the network and the out-degree measures the number of farms that receive pigs from a specific farm in the network (Dubé et al., 2009).
- *Betweenness* measures the number of shortest paths between nodes that a node is connected to, so that a betweenness score relates not only to the connectivity of a node but that of the neighbours that it was connected to (Dubé et al., 2009).
- *E-I indices* were used to investigate the tendency for connections to occur within and between groups. For this study, groups are regions, QA schemes and between company subgroups. A large positive value (up to a maximum of 1) would indicate a predominant amount of within group connections, whereas a large negative value (up to -1) would indicate many between group connections (Krackhardt and Stern,1988).
- The *clustering coefficient* measures the average proportion of connections that exist among neighbours of a farm, divided by the number of possible connections that could have existed (Dubé et al., 2009).
- The *network centralization index* measures how far a network departs from a network where all nodes have the same degree (an index of 0) (Martinez-Lopez, Perez and Sanchez-Vizcaino, 2009).

Two-tailed T-tests to compare the means of two groups, using permutation methods to assess significance level, were completed in Ucinet to compare whether network parameters were significantly higher or lower ($P<0.05$) than that expected by chance (Borgatti, Everett and Freeman, 2002). Farms that recorded no movement connections (on or off) to other farms, were excluded from the network analysis, as it was assumed that these represented farms with missing data.

The farm-to-farm network was also compared against the average characteristics of 100 randomly generated datasets, each with the same number of nodes and connections as the real network, where sending and receiving farms for each arc was chosen at random, to define whether the network structure was consistent with a hypothesised "small world network" definition. A small world network is where the majority of nodes have a small number of 'clustered' connections. By comparing the degree, average number of connecting arcs between reachable pairs (average path length) and clustering coefficient against a theoretical random contact network, the structure of the network can be identified which can help inform the types of controls that would be effective (Watts and Strogatz, 1998; Christley and French, 2003).

Data on abattoir and haulier usage were examined to identify the average number of unique connections to each location or company. The information on the region of the farm and the abattoir's region was also used to describe the range and number of regions that supply an abattoir. Summary statistics and graphical summaries were also used to examine the distance and transport duration of movement connections from farms to abattoirs. Finally, an analysis of the data on farm-to-farm movement connections, and abattoir and haulier use, was examined to compare movement connection behaviour between large, medium and small pig farm companies.

5.2.3 Results

Network analysis of farm-to-farm movement connections

A total of 2,421 unique movement connections were recorded by farms over a 12 month period, which came from the most recent QA assessment. Of these records, 2,176 (90%) had information available to calculate the transport distance and 1,719 (71%) included transport time. The movement connections were between 1,633 farms: 678 from ABP, 743 GQA and 212 QMS. A presentation of the network of farm-to-farm movement connections is displayed in Figure 5.2, which shows the interconnectivity of movement connections between and within the regions. The network diagram shows that the regions with larger number of farms (East England, Yorkshire and Humber, and Scotland), also had larger number of within region movement connections. The diagram also shows the relative

isolation of regions such as Northern Ireland and Wales that are connected to few regions, whereas many regions send pigs to the East Midlands.

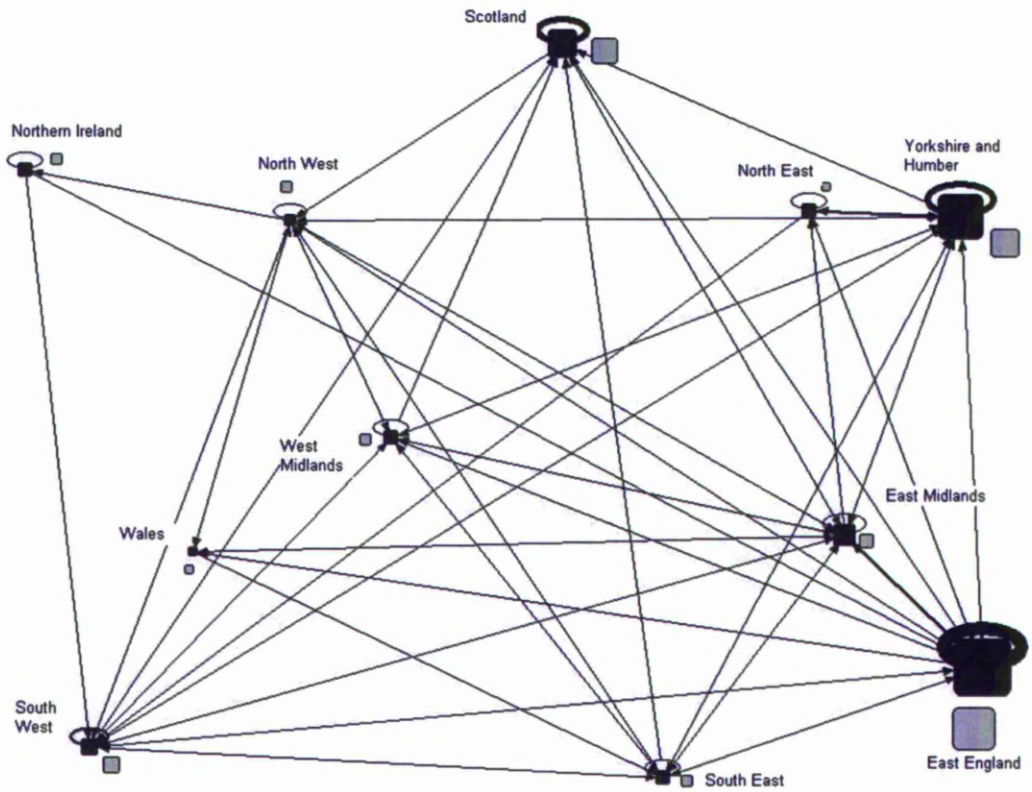

Figure 5.2: Diagram, arranged by region, of farm-to-farm movement connections of pigs within and between regions. Each region is represented by a node (square) and lines represent the movement of pigs. The width of the line indicates the number of connections and node size provides the number of farms within a region. Each region has an additional node (shaded in grey) to show the number of farms without outgoing connections. Farms with an unspecified region have been omitted.

The mean degree for farm-to-farm connections was 3, ranging from 1 to 22. The clustering coefficient was 0.030, and the average path distance was 4.76. The average characteristics of the randomly generated networks had a markedly lower clustering coefficient (0.0005), a smaller range of degree (0 to 10) and a larger average path distance between farms (14.49), compared to the observed network.

Of the study population, 963 (59%) had a non-zero score for out-degree (off-farm movement connections), whereas 1,120 (69%) had non-zero in-degree (inward movement connections). A small difference between movement connections on and off farms was shown, with a range of 0-17 out-degree, and between 0-15 in-degree, with a mean for both of 1.5 (median = 1) (Figure 5.3). Farms with an in-degree of zero would be assumed to be breeder farms that bred their own replacements for that period, whereas a farm with zero

out-degree might be a finisher farm, as movement connections to abattoirs were not included in this network.

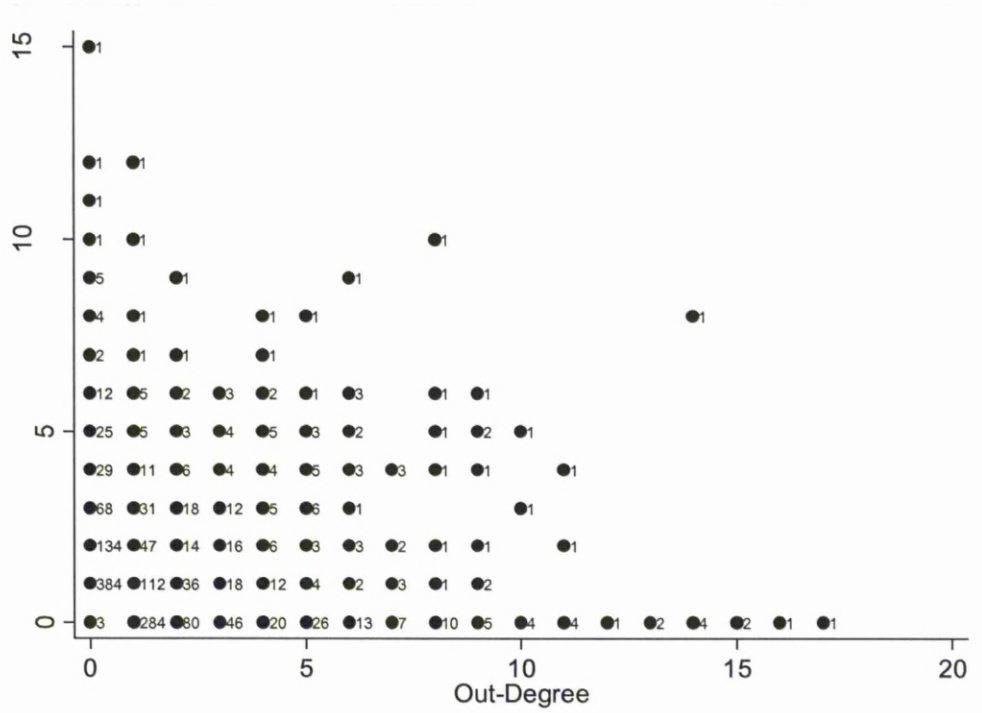

Figure 5.3: Scatterplot of in-degree and out-degree for pig movement connections between UK Quality Assurance Scheme registered pig farms (n=1,633). The labels signify the number of pig farms at each point.

For the observed network, the network centralization index was very close to zero for both out-degree and in-degree (0.00095 and 0.00083 respectively) indicating the absence of highly connected nodes in the network. Furthermore, the 10 farms with the highest out-degree accounted for 6% of the total outgoing movement connections, whereas the top 10 in-degree farms accounted for 4% of the total incoming movement connections. Only one node (a farm in the Yorkshire and Humber region belonging to a large company) had high in-degree and out-degree (14 and 8, respectively).

Only 393 farms (24%) had non-zero betweenness, and 149 (9%) had a betweenness above the mean of 35.2 (median = 0, standard deviation = 193.3). Only a small number of farms had high betweenness and high degree (Figures 5.4 and 5.5). Four farms had high values of centrality that might indicate that they were more important 'network hubs' than other nodes in the network: three farms had betweenness and in-degree scores that were in the top ten of all the farms, whereas one node was in the top 20 for out-degree and betweenness. These four farms were all from large companies and three were located in the East England region (one farm had unspecified location data).

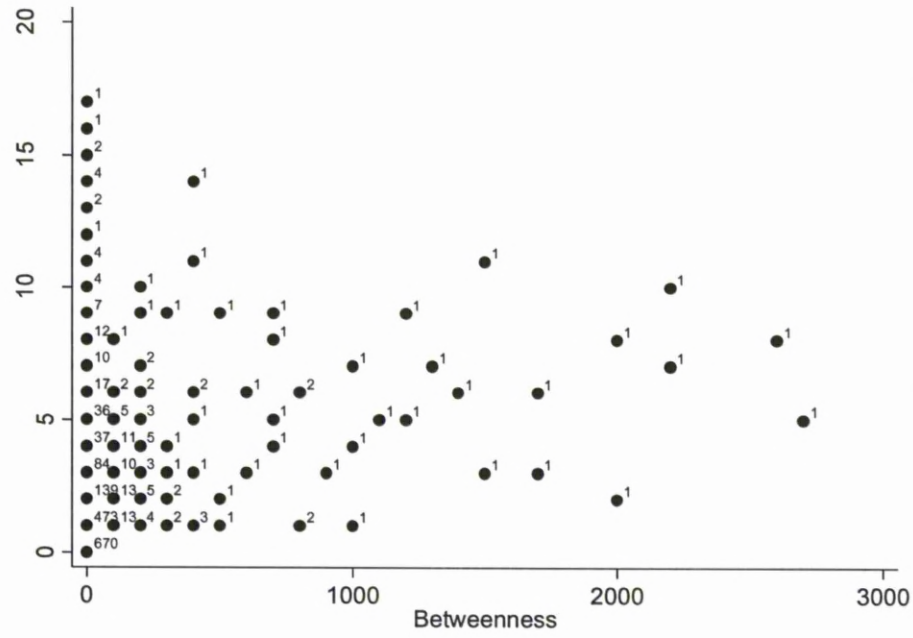

Figure 5.4: Scatterplot of out-degree and betweenness for pig movement connections between UK Quality Assurance Scheme registered pig farms (n=1,633). Betweenness has been rounded up to the nearest 100. The labels signify the number of pig farms at each point.

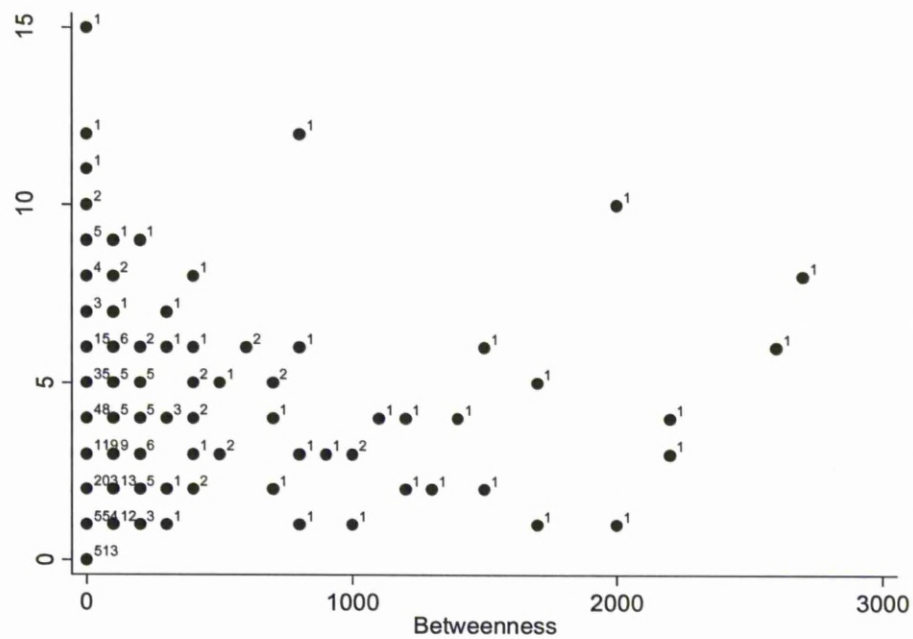

Figure 5.5: Scatterplot of in-degree and betweenness for pig movement connections between UK Quality Assurance Scheme registered pig farms (n=1,633). Betweenness has been rounded up to the nearest 100. The labels signify the number of pig farms at each point.

Farms within the QMS scheme showed almost equal tendency for internal and external connections (E-I index = -0.101), whereas both the ABP and GQA schemes had a tendency towards within-scheme movement connections (-0.362 and -0.441 respectively). The examination of the regions showed that farms in Northern Ireland and Wales had a strong tendency for external connections, whereas Scotland, Eastern England and the South West had higher proportions of internal connections (Table 5.1).

Table 5.1: External-Internal (E-I) index of pig movement connections within and between regions for Quality Assured UK pig farms.

Region	E-I
Scotland	-0.461
Northern Ireland	0.479
North East	0.248
North West	0.102
Yorkshire & Humber	-0.044
East England	-0.342
East Midlands	0.094
West Midlands	-0.099
Wales	0.857
South East	-0.023
South West	-0.348

Abattoir analysis

In total, 1,424 farms (593 ABP, 222 QMS and 609 GQA) recorded the use of at least one abattoir. A further 30 abattoir connections were present in the ABP scheme dataset but were omitted from the analysis as the abattoirs used were not identifiable. The range of abattoirs used by each farm was different for each scheme, with GQA farms listing the most (mean 1.8, range 1-6), with less listed under QMS (1.3, 1-5) and ABP (1.3, 1-4) farms (permutation T-test comparison between GQA v ABP+QMS $P<0.001$). The overall mean usage was 1.6 abattoirs linked to each farm within a yearly assessment period. The mean average distance to an abattoir was 91km (median 70km) and 1.8 hours travel time (Figures 5.6 and 5.7), although no travel time duration data were included from QMS farms. Scottish farms had the longest travel distances and journey durations (Scottish farms v other regions, $P<0.001$), with Northern Irish farms having the least ($P<0.001$).

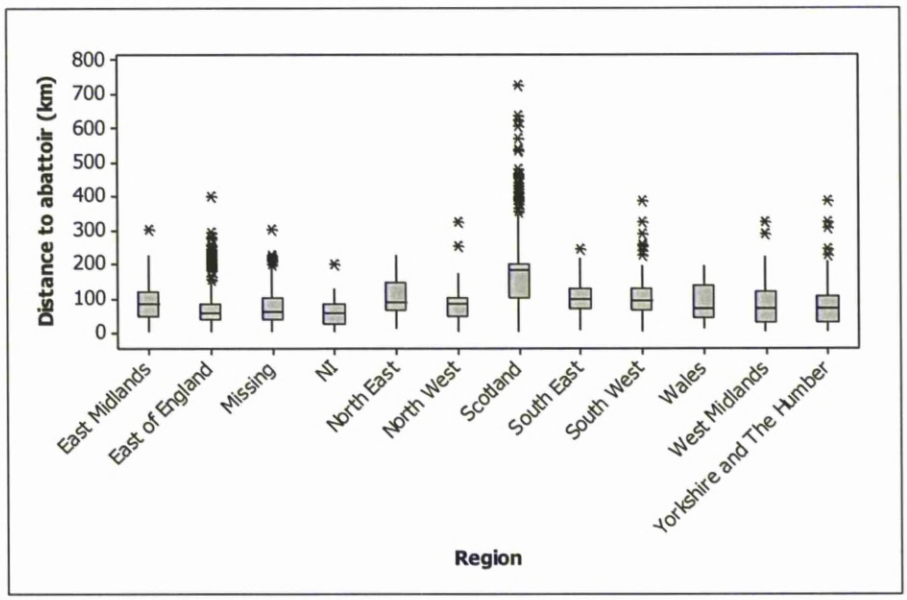

Figure 5.6: Average distance in kilometres from Quality Assured pig farms to the nearest abattoir, for farms in each geographical region. For each region, the upper and lower extent of the box represents the 75th and 25th percentile respectively; the horizontal line intermediate of these is the median. The vertical line represents 25th percentile - 1.5*(75th-25th percentile) and 75th percentile + 1.5*(75th-25th percentile), and * indicates outliers. NI = Northern Ireland.

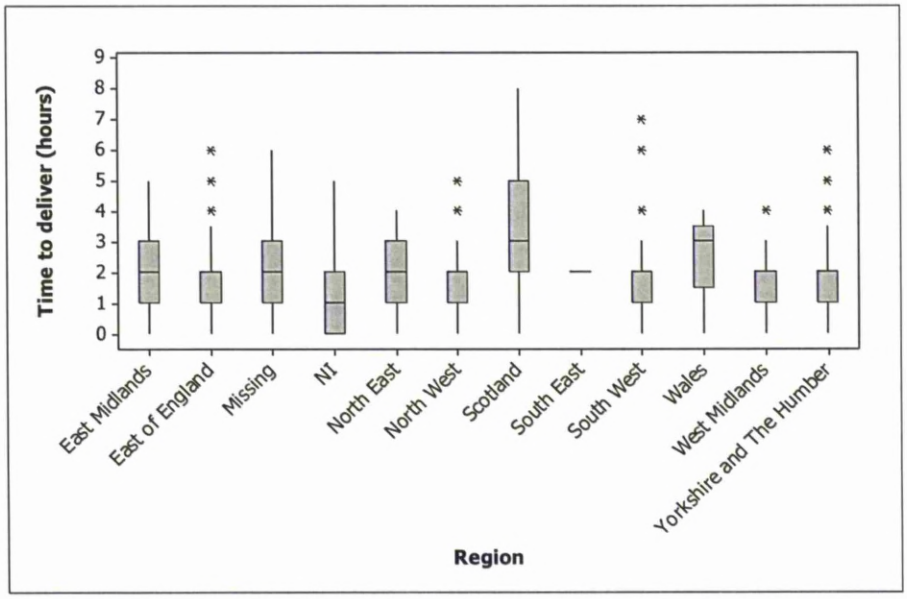

Figure 5.7: Average travelling time in hours from Quality Assured pig farms to the nearest abattoir for farms in each geographical region. For each region, the upper and lower extent of the box represents the 75th and 25th percentile respectively; the horizontal line intermediate of these is the median. The vertical line represents 25th percentile - 1.5*(75th-25th percentile) and 75th percentile + 1.5*(75th-25th percentile), and * indicates outliers. NI = Northern Ireland.

QA farms were linked to a total population of 89 unique abattoirs and each abattoir was linked to between 1 and 224 farms (mean = 27, median = 3). These abattoirs were unevenly spread within the regions (Figure 5.8), with only small numbers of abattoirs in South East England (3) and Northern Ireland (3), and none in Wales.

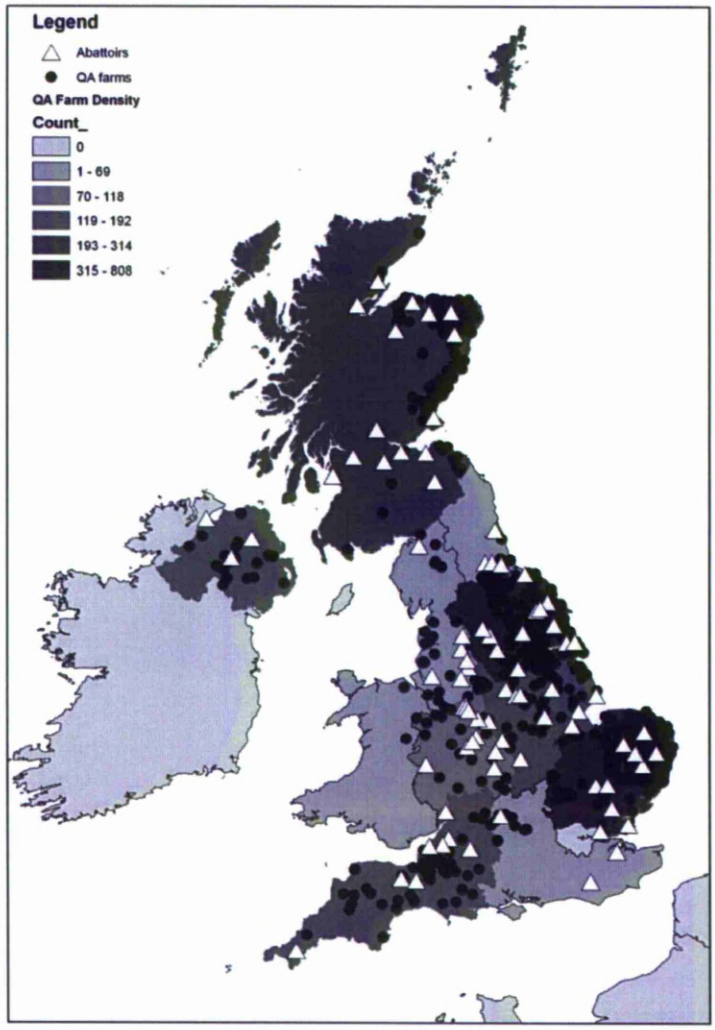

Figure 5.8: Location of 1,266 Quality Assurance (QA) scheme farms, with available map references, that moved pigs to abattoirs and the location of the 87 abattoirs listed by the QA schemes, with available map references. The data are plotted onto a background of QA pig farm density by region.

Abattoirs in Scotland only serviced Scottish farms, and Northern Irish farms only used Northern Irish abattoirs, whereas East Midland abattoirs serviced every region apart from Northern Ireland (Figure 5.9). Farms frequently used abattoirs within the same region, with an average of 56% of connections between farms and abattoirs in the same region, ranging

from 8-100% depending on region (excluding Wales, where there was no abattoir). The largest percentage of trips were short and less than half of the mean distance (28% were 40km or less). However, the majority of abattoir connections for farms in Wales, North East and South East England were with abattoirs in another geographically close region (North West/ West Midlands, North West, and South West respectively).

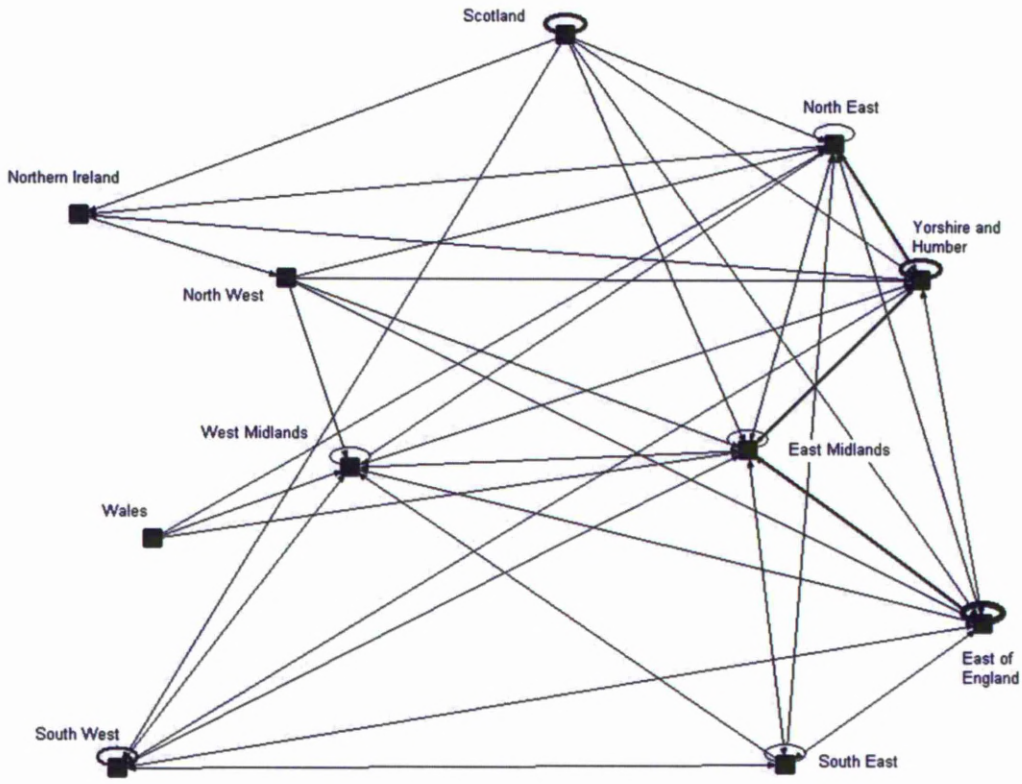

Figure 5.9: Diagram, arranged by region, of pig movement connections from farms to abattoirs within and between each region. Farms with unspecified regions have been omitted. The width of the line indicates the number of connections.

Haulier info

There were 1,445 farms from the ABP and GQA schemes (QMS did not record haulier use) that reported the use of 252 unique hauliers. These farms used between 1 and 7 hauliers (mean 1.4, median 1.0), with farms from the GQA scheme (mean 1.6, median 1.0) using more on average than ABP (mean 1.2, median 1.0) (two-sample permutation T-test $P<0.001$). Each unique haulier identifier reference was linked to between 1 and 133 farms (mean 8.0, median 1.5) from 1 to 6 regions. Most hauliers (67%) operated within a single region, with a mean of 1.6 regions.

Company size network comparisons

In the dataset, there were 7 large, 27 medium and 458 small companies, covering 1,359 farms, with 274 (17%) farms not recording company/ farm name information. Most farms

from large companies were located in East of England, accounting for 72% of the total number of farms in the region (Table 5.2). The majority of farms also belonged to large companies in two other regions, Scotland (70%) and North East England (52%). However, Northern Ireland, and Wales contained no farms from large companies.

Table 5.2: Proportion of Quality Assurance scheme pig farms belonging to large, medium and small companies, by geographical region.

	No. of farms by size of company (% of total in region)		
Region	Small	Medium	Large
Missing	39 (22.8%)	61 (35.7%)	71 (41.5%)
East Midlands	39 (44.3%)	29 (33.0%)	20 (22.7%)
East of England	83 (20.7%)	29 (7.2%)	289 (72.1%)
Northern Ireland	26 (74.3%)	9 (25.7%)	0 (0.0%)
North East	12 (44.4%)	1 (3.7%)	14 (51.9%)
North West	15 (57.7%)	5 (19.2%)	6 (23.1%)
Scotland	32 (20.5%)	15 (9.6%)	109 (69.9%)
South East	14 (56.0%)	2 (8.0%)	9 (36.0%)
South West	67 (77.9%)	1 (1.2%)	18 (20.9%)
Wales	1 (50.0%)	1 (50.0%)	0 (0.0%)
West Midlands	29 (69.0%)	10 (23.8%)	3 (7.1%)
Yorkshire and The Humber	165 (55.0%)	73 (24.3%)	62 (20.7%)

Farms that were part of large and medium companies were most likely to have movement connections to farms within the same company (90% and 76% respectively; Table 5.3), whereas farms in small companies infrequently moved pigs within the company and mainly moved pigs to other small or large companies. A comparison of network parameters between company sizes showed that small companies had a significantly smaller out-degree and betweenness, than medium and large companies, but there was no significant difference between in-degree (Table 5.4).

Table 5.3: Pig movement connections between categorised sizes of pig farm companies (percentage of total connections in brackets).

		Company size of receiving farm				
Company size of sending farm	Same company	Small	Medium	Large	Missing	Total
Large	861 (89.8)	25 (2.6)	4 (0.4)	24 (2.5)	45 (4.7)	959
Medium	260 (75.6)	29 (8.4)	22 (6.4)	20 (5.8)	13 (3.8)	344
Small	35 (5.3)	278 (41.7)	26 (3.9)	287 (43.1)	40 (6.0)	666
Missing	-	94 (20.8)	24 (5.3)	250 (55.3)	84 (18.6)	452

Table 5.4: Comparison of network parameters between small and medium or large pig companies (farms with missing company data were omitted from the networks).

Company Size	Statistic	Number of farms	Mean	Range	Permutation T-test *P*-value
Small	In-degree	481	1.5	0-11	0.915
Medium/ Large		775	1.5	0-12	
Small	Out-degree	481	1.3	0-14	0.048
Medium/ Large		775	1.6	0-17	
Small	Betweenness	481	24.0	0-1879	0.023
Medium/ Large		775	49.9	0-2502	

The size of company was also associated with the average number of hauliers and abattoirs connected to each farm. Small companies used an average of 0.8 hauliers and 0.8 abattoirs, whereas medium companies used a mean of 1.0 haulier and 1.3 abattoirs, and large companies used 1.3 hauliers and 1.3 abattoirs. However, the larger sizes of company had a higher probability that farms within the same company were using the same abattoir or haulier. Analysis of the number of unique abattoirs and hauliers, divided by the total number of farms, within a company, showed that large companies had a ratio of 0.1 unique abattoirs per farm and 0.1 unique hauliers, which was lower in comparison with medium (0.2,0.2) and small companies (0.6,0.7). The results indicated that large companies were more likely to be using fewer hauliers and abattoirs per farm, probably integrated into the company, with each haulier and abattoir having more farms connected to them.

5.2.4 Discussion

This study provides the first description of the connections between UK pig farms: through a farm-to-farm network; examination of the use of abattoirs and hauliers recorded at a yearly QA assessment; and the differences detected between regions and QA schemes. By analysing the structure of pig movement connections, using social network analysis and descriptive analysis, the outputs help to explain the potential transmission dynamics of *Salmonella* between pig farms. However, as the records related to unique movement connections within a yearly period and did not record the frequency of movements, the specific risks to pigs caused by the movement of a batch of pigs to an abattoir or to a farm, or from the contamination of a haulier environment, cannot be assessed. It should also be noted that the analysis did not collect data on any other types of *Salmonella* transmission routes (e.g. wildlife), but the movement of pigs is widely recognised as the biggest risk of disease introduction (Stärk et al., 2002).

The network analysis of the farm-to-farm movement connections showed a near absence of any farms with high in- and out-degree, and most farms showed both low in- and out-degree. These findings are notable and indicative of a network where no key nodes exist that link many others, and could act as hubs for transmitting diseases. The four farms identified with

relatively high in- and out-degree, or high betweenness and out-degree, were all located in areas of high pig herd density and were all owned by large pig companies, which may explain why these farms were playing a more central role in farm connections than others. Network parameters are useful in predicting the risk of becoming infected and so these four farms may be at relatively greater risk of receiving and transporting infected pigs. The pigs on these farms may be at particular risk of *Salmonella* infection, as a previous study had shown that herds receiving stock from more than three supplier herds had three-times higher odds to test seropositive to *Salmonella* antibodies than herds which bred their own replacement stock or used a maximum of three supplier herds (Lo Fo Wong et al., 2004).

The farm-to-farm network was more clustered and with a shorter average distance, than the random datasets, indicative of a small world network (Watts and Strogatz, 1998). If the network had been less clustered but with a high heterogeneity of connections, then controls could be targeted on just those with high in- and out-degree, but in a small world network, with multiple pathways between many pairs of farms, these controls are not as effective (Albert, Jeong and Barabasi, 2000; Newman, 2000). A reduction in degree by all farms (by farms moving pigs to fewer individual farms) may be effective in limiting the spread of infectious organisms and would reduce the betweenness score of the farms within the network. Reducing the centrality values of the farms would help isolate the effect of high prevalence farms e.g. a high prevalence farm would transport infected pigs to fewer farms, which would subsequently transport their infected pigs to fewer farms. High prevalence farms with relatively high centrality values could be subject to targeted surveillance and control measures, to reduce the effect of these high risk nodes within the network.

Within a small world network, a disease spreads quickly but the total size of an outbreak of disease would be smaller than from a random network (Christley et al., 2005). Hence, according to the transmission routes measured here, an outbreak of a novel *Salmonella* strain in the pig farm network may be more likely to spread predominantly within a specific company or a region e.g. an outbreak of disease on farms in Northern Ireland and Wales would be more likely to spread outside of the region due to the movement of pigs and so heightened surveillance could be extended to the regions with which the farms are most likely to trade pigs. Targeting control to a specific group has been shown to be useful in pneumonia control in people, where caregivers in an in-patient institution were identified as important routes of transmission and specific control strategies were formulated (Meyers et al., 2003).

The majority of pig farms in both Scotland and North East and Eastern England belonged to large companies, and this finding may help explain the lower *Salmonella* seroprevalence in these areas, when compared with Yorkshire and Humber which has a high seroprevalence (Smith, Clough and Cook, 2010; Smith et al., 2011). In integrated companies, biosecurity

measures are usually enforced throughout the company and the risk of mixing pigs of differing health status is reduced. Small company farms were more likely to use their own transport rather than haulier companies and not to send animals to slaughter (i.e. breeder farms). Furthermore, farms in large companies had connections to more abattoirs/hauliers but at a company level these were fewer unique abattoirs/ hauliers than a similar number of farms in small companies i.e. each farm within a company has links to the small number of abattoirs integrated within that company. Large integrated companies using the same abattoirs and hauliers may help to ensure that these facilities are only used by herds of a similar health and biosecurity status which reduces the threat of indirect spread between pigs from different farms.

The larger company size categories had greater numbers of connections within the company than to other companies. However, although small companies have a lower betweenness and out-degree, the results show that they trade mainly with other small companies and large companies, and so, may play an important disease transmission role in bridging between large companies in the network. However, this analysis of company data may be affected by errors in the listing of company in the datasets, where the farm name rather than company name may have been provided meaning that large or medium company farms may have been listed as individual farms.

The low *Salmonella* seroprevalence previously detected in Scotland may be related to the predominance of farms belonging to large companies and the large number of internal pig movements within the region, so that farms trade with similarly low prevalence farms. However, the East of England region was also found to have these characteristics but was not found to have a low seroprevalence, which may indicate that other factors are associated with *Salmonella* infection but low numbers of external movements may help maintain a prevalence level.

The distribution of abattoirs covered all the main geographical areas of pig production in the UK, with many being in central England, where they could be accessed by a large number of farms within a short travelling time. Farms frequently had connections with abattoirs within the same region, or a neighbouring region. Interestingly, there were many abattoirs located in Scotland, even in areas of low pig farm density, but Scottish farms also sent pigs over long distances, as far as South West England.

A previous study has shown that a pig may become colonised by *Salmonella* after only two hours of exposure to a highly contaminated source, which may indicate that pigs may be at risk of infection during the average transport time identified in this study, if the haulier vehicle environment was contaminated (Hurd et al., 2001). The stress caused by transit can also cause infected pigs to shed larger numbers of bacteria in their faeces, which could

contaminate the hides of other pigs and cause a risk to human health if the pigs are being transported for slaughter (Thiry et al., 1987; Moberg and Mench, 2000). However, the effect of the distance of transport on pig health has not been clearly shown, with only a minimal association demonstrated with *Yersinia* faecal prevalence, and no association with VTEC O157, *Salmonella* or *Campylobacter,* in a UK abattoir study (Milnes et al., 2009). Two Spanish studies have shown that the effect of transport duration may be complicated, as in one study, very short transport (15 minutes) caused a more intense stress response than pigs transported for three hours, as the lengthier duration may have allowed the pigs to adapt to the transport conditions (Perez et al., 2002). In the second study, weaned piglets transported for either one or eight hours (the maximum distance in our study) showed a mixed set of blood chemistry results linked to stress and meat condition, so that it was unclear whether short or long transport times were more detrimental to pigs (Averos et al., 2009).

The main reason for a farmer's selection of an abattoir is believed to be the price paid per pig (VLA personal communication, 2010). Use of a nearby abattoir would mean a smaller cost to the farmer in relation to transport costs, and this may impact upon a farmer's profit per pig. However, farms linked to a company may need to send pigs to a more distant abattoir, due to contractual arrangements between the company and the abattoir. Decisions on the length of transport in the UK may be further affected by transport regulations that state that journeys over 65km need transporter authorisation, and haulier drivers require a scheduled break after 4 ½ hours of driving (Defra, 2010a). The stopping of hauliers was not thought to be a disease transmission risk to other livestock premises en-route, as animal hauliers are only permitted to stop at hard standings at service stations, lorry parks or official lay-bys (Defra, 2010b).

A surprising number of abattoirs were registered as being used by each farm during the study period, with some farms using up to six abattoirs. No data were recorded on the number of pigs sent to these abattoirs and it is expected that a number of the unique abattoirs listed might have been used infrequently. This may happen for the slaughter of a relatively small number of pigs, when a farm had produced more pigs than required under contract with a main abattoir, and thus the extra pigs may be sent to another abattoir with sufficient capacity in its schedule. Infrequent movements of pigs to an abattoir may also represent the culling of sows and boars at specialist abattoirs designed to handle large pigs.

The results in this study pertaining to abattoirs may have been biased by missing data in the dataset. Of the 697 farms that did record a pig movement connection but did not provide a reference for any abattoirs utilised, 100 (14%) had records from abattoir surveillance of *Salmonella* for that year (Zoonoses National Control Plan, BPEX, 2010b) indicating that these farms were sending pigs to slaughter. It was unknown whether similar amounts of

missing data were present in the entire dataset. If similar amounts of missing data were present in the farm-to-farm network then it was probable that this missing data would decrease the accuracy of the calculation of the measures of centrality in a predictable manner and it has been suggested that centrality measures are quite robust at small amounts (~10%) of missing data (Borgatti, Carley and Krackhardt, 2006). Hence, the missing data in this study, if assumed to be approximately 14%, may have had some (but perhaps limited) impact on the veracity of the results.

The information on the use of hauliers by QA farms showed that hauliers mainly collected pigs from farms within a single region. The spread of farms served by each haulier company indicates the potential for disease transmission between farms that do not share pig movements between them but could use the same haulier within a short time period. This highlights the need to ensure the adequate cleaning and disinfection of the transporters between pickups. The relative geographical clustering of the use of each haulier may also support the hypothesis that regional differences in *Salmonella* prevalence may be due to farms within a high prevalence region being at a higher risk of infection from contaminated vehicles and thus perpetuating the prevalence of that region. All vehicles carrying livestock are required to be cleaned and disinfected prior to departure but these vehicles can be difficult to completely clean (Defra, 2009), although no data on this was collected from this study. Significantly more hauliers were linked to GQA farms than ABP, which may represent a bias in the way this information was collected by the different schemes.

Due to the source of the datasets used in this study, the results may not apply to the entire UK pig population. The QA records may suffer from self-reporting and recall bias (Freeman, Romney and Freeman, 1987), caused by the farmer only remembering the most important or the most regular connections. The dataset may also have omitted connections to non-QA registered herds, and so the connectivity of farms may have been underestimated. The dataset also did not provide information on the strength of the connections, such as the number of movements per year or the numbers of animals moved. As the QMS dataset did not record which feeder units were supplied, this may have underestimated the connections between many Scottish farms. However, movements of Scottish pigs to supply farms registered under the other schemes would have been recorded. This difference in data collection may explain why the farms in QMS had a different E-I index from the other schemes. For this reason, the results of analysis of comparisons of QMS or Scottish farms to others should be treated with caution.

The QA dataset may have been biased towards a particular type and size of herd that would benefit more from QA status. Although no appropriate data on non-QA registered farm were available, it has been estimated that non-QA registered farms are smaller and less conventional (BPEx personal communication, 2010). For example, a network on the QA

dataset may not contain the large number of small holdings and semi-professional farms that may play a role of indirectly connecting professional farms or by providing an alternative reservoir of disease. The dataset also lacked information on the movements of pigs to and from markets. It is estimated that between 4-5% of pig movements by professional pig farms are to one of the 365 livestock markets in the UK (VLA personal communication, 2011), and these markets are also used by small holdings, and would most likely have high betweenness properties within the network. Previous research has shown that markets can play an important role in the speed of transmission between farms in a network, although this study examined only cattle and sheep farms (Ortiz-Pelaez et al., 2006).

The pig movement connections explored in this study demonstrate a structured organisation, with particular heterogeneities of movements between farms belonging to companies of different sizes and between the different regions. The results also showed the grouping of farms using particular hauliers and abattoirs, and the range of hauliers and abattoirs used by each farm, which indicated that many farms were connected by these indirect means. Knowledge of these structures may assist with targeting *Salmonella* surveillance and control strategies within the UK, with more emphasis on monitoring and reducing the prevalence on farms with high centrality values, as well as attempting to reduce the number of connections between all farms. The information on the differences of connections between regions and between farm company sizes may also inform these strategies and assist in identifying high risk areas in an outbreak situation.

5.3 Additional Pig Movement Network Analysis

5.3.1 Aim

A) To determine whether other data sources could be used to validate the recording of farm-to-farm connections in the Quality Assurance (QA) scheme datasets. By validating the recording of movements in the QA schemes, we might determine the quality and coverage of the dataset.

B) To analyse the farm-to-farm movement connections recorded by the QA schemes for consistency over time, to review whether the movements for each farm remained stable and to quantify the amount of any change. The consistency of the dataset would inform whether results from the dataset are applicable to other periods of time.

C) To test the association of the farm network characteristics to *Salmonella* seroprevalence results, recorded by the Zoonoses National Control Plan (ZNCP), by adding the outputs of the network analysis to 1) a base risk factor model and 2) to the risk factor model, containing the significant farm characteristics, designed in Chapter 3.

5.3.2 Method

A) To verify the completeness of the recording of pig movements in the QA datasets, the records were compared against movement data collected independently. The Animal Movement Licensing System (AMLS) contains information from England and Wales on commercial movements of deer, goats, pigs, and sheep. It was implemented in mid-2004 by Animal Health on behalf of local health authorities, to follow on from the legislation requiring registration of movements initiated in 1997. The AMLS system records movements to and from Scotland but not movements within Scotland. However, the Scottish Animal Movement System (SAMS) was added to the AMLS data to cover all Scottish pig movements, within, into, or out of Scotland. SAMS is operated by the devolved Scottish government to record movements of sheep, pig and goats and the recording of animal movements in SAMS began in 2002. As the QA data recorded network connections between farm holdings, rather than individual movements, the AMLS/ SAMS data were used to compare the unique connections between farms.

A dataset was extracted from AMLS and SAMS of all pig movements, from English, Scottish and Welsh agricultural holdings to other holdings, from 1st January 2004 to 31st December 2008. The movements were summarised, in MS Access, to a list of unique movement connections between farms for the four year period. A database query was used to match QA records from the dataset collected in Chapter 3 and the pig movement data, using the

CPH (county parish holding identifier), to verify if a corresponding connection between farms was present in both datasets.

B) Holdings that recorded farm movements, or abattoirs/ haulier usage, from more than one assessment period in the QA dataset, were analysed to determine the number of unique movement/ usages that were consistent over all the recorded assessments.

An analysis in MS Access was completed individually for each table of movement data recorded by the QA schemes (Assured British Pigs (ABP), Genesis (GQA) and Quality Meat Scotland (QMS)), as some tables only recorded a single assessment period for each farm. Summaries of each table were generated to identify unique holdings with multiple assessment periods and then comparing whether the farm, haulier or abattoir name or identifier was consistent across the assessments. Where QA farms were linked to multiple farms, hauliers or abattoirs, the data were analysed to assess whether the combination of either farms, abattoirs or hauliers was consistent or whether just individual connections were consistent.

C) The QA farms that had recorded farm-to-farm movements in the QA dataset were linked via QA scheme identifier to *Salmonella* serology results and other risk factor data, gathered for Chapter 3. Farms that could not be linked to any sample results were dropped from the analysis. The five main network parameters generated for each farm holding within Chapter 5 (in-degree, out-degree, betweenness and External-Internal (E-I) index for region and scheme) were added to a linear regression model, with holding identifier added as a random effect. The outcome of the model was the log-transformed meat juice ELISA ratio results, used to determine *Salmonella* seroprevalence, as explained in Chapter 3.

The network variables were added to the model independently, and then all five network parameters were added to a multivariable model that contained the significant farm management risk factors identified previously. Model diagnostics were used to determine how the network variables may have affected the fit of the model.

5.3.3 Results

A) Validation of recorded QA movement connections

The AMLS dataset included 59,964 unique movements from farm-to-farm, within the four year time period (2004-2008) used for the QA datasets. SAMS recorded 3,875 unique movement connections, with 123 unique movements being duplicated as recorded in both datasets. From this total of 63,716 movement records, 11,391 (17.9%) linked to QA identifiers by County Parish Holding (CPH) number for both the sending and receiving farms.

In the QA dataset there were 2,421 unique movements, of which 1,592 had one or more CPH reference for both the sending and receiving holding (a total of 1,739 unique CPH combinations). Of the records in the QA schemes, the holdings could be attributed to 4,231 CPHs, and of these 2,405 (56.8%) were found to have movements (in or out) recorded in AMLS/SAMS during the study period, whereas 1,401 had recorded movements in the QA dataset. When the QA and AMLS/SAMS datasets were combined by CPH of sending and receiving holding, 915 (52.6%) of QA movements were detected in the corresponding dataset.

B) Consistency of QA movements over time

A number of tables in the QA dataset only recorded data from a single assessment period for the holdings. These included feeder farms supplied by ABP farms; hauliers used by ABP farms; weaner farms supplying QMS farms; and abattoirs used by QMS farms. The QMS dataset did not record information on haulier use or feeder farms supplied.

The remaining ABP tables contained only four farms with multiple assessment records. Only one farm had multiple assessment records on abattoir usage, which recorded the use of two abattoirs. One abattoir was consistently used, whereas the other was not. Three farms recorded two assessment records of the sources of weaners, with two farms consistently recording the use of the same single weaner farm, whereas the other farm showed inconsistent weaner farm use.

For the use of abattoirs by GQA farms, 322 holdings had multiple assessment records (697 assessments). Of these holdings, 138 (42.9%) had consistent abattoir use, whereas 184 (57.1%) showed differences between the records. The differences between assessment periods were further broken down as 337 abattoirs were used only once by the 184 inconsistent farms, and 246 abattoirs were used more than once. This corresponds to 92 farms that used an abattoir only once (inconsistent uses) and 72 farms that used a specific abattoir on more than one occasion (consistent uses), indicating only 20 farms had totally inconsistent data.

For the use of livestock hauliers by GQA farms, 405 holdings had multiple assessment records (900 assessments). Of these holdings, 195 (48.1%) had consistent haulier use and 210 (51.9%) had inconsistent movements. The haulier use by the inconsistent farms showed that 367 hauliers were used only once by a farm and 260 were used by a farm more than once. This corresponds to 197 farms that had hauliers used only once (inconsistent uses) and 176 farms that used a haulier more than once (consistent uses), indicating only 21 farms had totally inconsistent data.

Of the 320 GQA farms with multiple assessment records (698 assessments) for farm-to-farm movements, 49 (15.3%) were consistent in the recorded farm movements and 271 (84.7%) had non-identical records. Of the farms with inconsistent records, there were a total of 1,144 connections to farms that were only traded with once and 181 multiple moves to a farm used by a GQA holding. This corresponds to 101 of the 271 farms having at least one consistent movement record between the assessment periods and 170 farms that recorded only inconsistent pig movements.

C) Effect of adding network parameters to risk factor model

A total of 259 farm holdings were able to connect to *Salmonella* seroprevalence results and questionnaire data, from a total of 1,633 (15.9%) holdings that recorded farm-to-farm connections in the QA database. The holding linked to a total of 51,612 samples (mean = 199, range 3-1,094) over a four year period. However, when the farm identifiers were linked to the data for significant farm characteristics identified by the risk factor model, 21 farms contained missing questionnaire data and were dropped from the multivariable analysis.

When the five network parameters were entered independently into the mixed linear model, three variables, betweenness, in-degree and E-I index for region were significant at the 0.05 *P*-value significance cut-off (Table 5.5). When all five parameters were added to the list of significant farm characteristics in the final risk factor model (Table 5.6), none of the network variables were found to be significantly associated with *Salmonella* seroprevalence and the model fit was not improved, as indicated by the Akaike Information Criterion. The correlation between the network parameters and the other fixed variables were compared by estimated sampling correlations, but none of the network parameters were strongly correlated with any other (>0.7) within the multivariable model, although some linkage was detected between region and E-I region (0.27).

Table 5.5: Univariable risk factor model results for five network parameters individually applied to a linear regression model as predictors of logged meat juice ELISA ratio. The unique Quality Assurance farm identifier was added as a random effect. (259 farms, 51,612 samples).

Variable	Odds Ratio	95% confidence intervals	*P*-value
Betweenness	1.002	1.000-1.003	0.008
In-degree	1.093	1.010-1.183	0.027
Out-degree	1.050	0.995-1.109	0.078
E-I region	1.419	1.205-1.671	<0.001
E-I scheme	1.160	0.972-1.385	0.100

Table 5.6: Results for five network parameters added to the final risk factor model for logged meat juice ELISA ratio. The unique Quality Assurance farm identifier was added as a random effect (238 farms, 49,488 samples).

Variable	Odds Ratio	95% confidence intervals	*P*-value
Betweenness	1.001	1.000-1.002	0.204
In-degree	1.004	0.939-1.074	0.900
Out-degree	1.023	0.981-1.069	0.286
E-I region	1.090	0.928-1.280	0.292
E-I scheme	0.967	0.821-1.138	0.686

5.3.4 Discussion

A) The recording of pig movements between farm holdings in the QA schemes was shown to be different from that recorded in AMLS/SAMS, with just over half of the movements corresponding to those recorded in AMLS/SAMS. The missing movement connections may indicate that the identifiers of sending and receiving farms were poorly recorded in the QA scheme and could not be linked to the correct farm in AMLS/SAMS. Errors may have been compounded as the farm data were connected by linking each QA scheme identifier to a CPH, and either identifier may have been incorrectly recorded. More than one QA member identifier can be linked to each CPH, as units may have changed scheme providers, and a single CPH can cover a group of linked holdings under the same owner. This may have caused problems in linking these identifiers. However, the only other identifying information recorded in the QA dataset, was postcode, which was completed for a small population of members and also has the problem that more than one farm could be recorded under a single postcode, especially in farm dense areas of Britain (Yorkshire and Humber and East England). Another reason for the large disparity between the datasets could be that the recording of movements by AMLS/SAMS was not complete and farmers may not have submitted all of their pig movements to the scheme.

Another finding of this analysis was that AMLS/SAMS recorded almost five times more unique movements between those QA-registered farms that contained CPH details. The time periods of the two datasets do not allow direct comparison as the QA data recorded movement connections at a yearly assessment between 2004 and 2008, whereas the extracted AMLS/SAMS data covered all unique movements over the four years to support the requirements to try and match up these movement connections in the two sources. However, the number of AMLS/SAMS movement connections was larger than would be expected.

The movement data recorded in the QA schemes may have omitted movements as the schemes only recorded specific movement classes (weaner farm suppliers, feeder farms supplied), and so the movement of gilts and boars between farms would not have been

recorded. The schemes also predominantly recorded movements between farms from the same QA scheme and this may represent a real bias in the selection of supplying herds or could be due to problems with recording the identifiers of other scheme identifiers. The QA data may also have suffered from self-reporting and recall bias (Freeman, Romney and Freeman., 1987), caused by the farmer only remembering the largest or the most regular connections, and so smaller infrequent movements were never recorded.

The validation of the QA farm-to-farm movement connections has shown some agreement between the datasets, but also a large number of unmatched movements. These discrepancies may have been an artefact of the purpose for the recording of movements in the two datasets and that data recording errors that may be present. The lack of data on the actual movement of pigs on specific dates has meant that there is little scope for further exploration of the reasons for missing movements. As both datasets did not appear to represent a complete dataset, it was deemed that it was reasonable to use the QA dataset for analysis but that any analysis would need to take into account the potential biases in the dataset.

B) The comparison of the recording of the movement connections was limited by the number of tables that recorded multiple assessment periods and the number of farms within these tables that recorded their use for more than one assessment. However, these data showed that the use of hauliers and abattoirs was consistent on approximately half of the holdings, whereas farm-to-farm movements showed a much larger amount of inconsistency, with 53% of the farms displaying only inconsistent connections. Very few farms recorded totally inconsistent (5-6%) haulier or abattoir usage, which highlights the strength of consistency. This finding provides evidence that over the four year period, data were recorded in a standardised manner. The analysis suggested that farmers infrequently make large changes to their use of haulier and abattoirs, although over half of the records showed that the use of at least one abattoir had changed. It was proposed that farmers may utilise one or two regular abattoirs, with which they are contracted to supply pigs, whereas additional abattoirs are irregularly used for special occasions, e.g. for the slaughter of sows or boars at abattoirs that have specialist equipment for pigs of that size, or an abattoir selected to slaughter pigs that were surplus to the requirements of their main abattoir contract.

The higher variability of farm-to-farm connections may indicate that farmers may be more likely to change the feeder farms that they supply or the weaner farms that supply them, between annual assessment periods. Frequent changing of farm-to-farm connections may have implications for disease transmission as this would increase the number of farms infected by a single farm, whereas standard movement connections would allow epidemiologists to utilise previous knowledge to target disease control and intervention strategies.

The data used for this analysis predominantly came from GQA farms, as there were very few recordings of multiple assessment periods in the ABP and QMS datasets. The removal of the majority of Scottish farms from this analysis may have implications for the consistency of connections, as farms in Scotland may have been influenced by geographical isolation and a subsequent limited choice of different connections within a reasonable distance.

C) The univariable model results showed that three network characteristics were significantly associated with *Salmonella* seroprevalence results. The significant association with the E-I index for region was not unexpected as the geographical region of the farm holding was previously shown to be associated with *Salmonella* seroprevalence (Chapters 2 and 3). Regions with low prevalence may maintain this status by buying livestock from similarly low prevalence farms within that region or the regional differences may represent similar herd management and veterinary advice provided to neighbours and farms within the same region. Higher E-I scores indicate more external movement connections and the association with *Salmonella* could mean that external movements are longer in duration and distance than internal movements, which may cause increased stress to the pigs and longer exposure to a potentially contaminated haulier environment. However, research has shown that the length of transport may not necessarily be important to stress as very short transport times (15 minutes) caused a more intense stress response than pigs transported for three hours (Perez et al., 2002; Averos et al., 2009).

In-degree and betweenness characteristics were also found to be significant in the univariable model, which highlights that farms that have a large number of incoming movements have higher *Salmonella* seroprevalence, as suggested by previous research (Lo Fo Wong et al., 2004) and receiving pigs from farms with similarly large numbers of sources may exacerbate this risk. Farms with high in-degree characteristics that submitted serology samples would be likely to be specialist finisher farms, rather than breeder-finisher farms, which would have a lower requirement for incoming pigs movements. Previous studies of the British serological *Salmonella* surveillance data have shown a lower seroprevalence is present in breeder-finisher herds when compared to finishers (Sanderson, 2005; Clough et al., 2007).

Once the network variables were entered into the multivariable model, none were found to be significant and the model fit was not improved. This would indicate that the network variable associations were not as important as the variables already included in the model and they did not improve on the variables on region and number of pig deliveries in explaining how farm movement connections may be associated with *Salmonella* seroprevalence on farms. The correlation results showed little connection between the network parameters and the other fixed variables, although it was unsurprising to find some

linkage between region and E-I region. It may be that in the multivariable model, the underlying risk factors within the network parameters are explained by a combination of the existing risk factors. It is important to note that the number of farms in the combined model was almost half of the size of the original risk factor model and this smaller population may not be a true representation of all of the original study population. The difference in the population has affected the model characteristics of the original risk factors (i.e. some risk factors are no longer statistically significant) and so the relevance of this comparison has been hampered by the missing data in the network data for all of the original 566 study farms.

It should be noted that the use of multivariable analysis on network parameters is problematic as the network parameters are different ways of expressing the same group of farm connections and so are not independent of each other, thus conflicting with a standard model assumption. The analysis may also have been affected because the farms linked to *Salmonella* serology results were only those farms that send pigs to slaughter and so it was not possible to evaluate the effect of network parameters on specialist breeder farms.

5.4 References

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6. General discussion

This thesis has explored sources of information that allowed for a wide-ranging analysis of pig *Salmonella* in the UK. The literature review (Chapter 1) identified that although there were a number of studies of pig *Salmonella*, there was still the need to expand this work to a comprehensive set of risk analysis questions on a large study population. The literature reviews also proposed that the *Salmonella* serosurveillance might be a useful way of determining an outcome for such an analysis, as it covered a large population. Chapter 2 covered the search for explanatory variable data and reviewed the data collected by the Quality Assurance schemes, which regularly collected data from commercial pig farms at quarterly visits. This followed on to descriptive analysis, map presentations, risk factor analysis and K-function spatial analysis, to evaluate the usefulness of the data. The data gaps and problems identified with the data led to the creation of a questionnaire and a subsequent risk factor analysis in Chapter 3. The risk factor analysis identified a number of significantly associated factors linked to *Salmonella* seroprevalence, which ranged from feed types, prominence of health conditions, farm management, vehicular deliveries, but also temporal, meteorological and spatial variables.

The examination of spatio-temporal factors continued in Chapter 4, where the temporal patterns of *Salmonella* seroprevalence were assessed and the incorporation of a geostatistical approach to an epidemiological model showed how the variables identified in the risk model could account for the spatial heterogeneity that had been identified, even when the spatial risk factor variables had been removed from the model. Similarly, a network analysis and analysis of pig movement data was completed in Chapter 5, to further explore the relevant variables uncovered in the risk factor model. This study provided a far more comprehensive method of examining pig movements than could be covered by an epidemiological model and the work showed an absence of farms with network characteristics that would indicate that they were particularly important and central to the pig movement connections. There were also differences in pig movements and abattoir use between regions and in different pig company size categories.

The findings of these analyses are all particularly relevant in the context of supplying evidence and advice for future *Salmonella* control and surveillance activities, such as those that will be required by each European Union Member State for the upcoming national control plans to reduce *Salmonella* prevalence In Europe (European Union Zoonoses Regulation (EC) No. 2160/2003). Also, recently there has been increased focus on *Salmonella* since the rapid rise in cases of tetra-resistant monophasic *Salmonella* Typhimurium. This strain is a concern for human health and it was the causal organism in three recent German outbreaks in 2010. Pigs may be an important reservoir for human infection by this strain as the outbreaks were all individually linked to the consumption of pig

meat, and, from UK pig surveillance, the strain was found to be the 2nd most common serovar (21.8%) in 2010 behind classic *S.* Typhimurium (52.1%) (EFSA, 2012). These developments stress the importance for evidence-based decisions on *Salmonella* control in pigs.

The epidemiological risk factor analysis was a statistically robust and powerful examination of pig *Salmonella*. The study used a large study population and a very large dataset of possible explanatory variables, which ensured that the assessment was comprehensive and representative of commercial pig farms. The results have uncovered interesting implications for *Salmonella* control. One area of *Salmonella* control that could be encouraged would be the movement to feeds that are homemixed and have a higher percentage of barley, as these factors have been shown to be associated with a positive gut environment that encourages helpful bacteria and restricts the colonisation by *Salmonella*. However, it would be hard to encourage the use of these controls without financial incentives, as they both have implications for decreased feed conversion efficiency and may decrease pig growth performance and thus the farmer's profit. Although most *Salmonella* infection is subclinical, it is believed that infection may still have implications for pig performance. However, the evidence for weakened performance may not be enough to convince farmers to spend money to control *Salmonella* on their farm. Unless feed control could be shown to assist other pathogens or could be shown to have another effect that negated this cost, then this control would be hard to enact.

The study highlighted that more needs to be done in controlling *Salmonella* on non-conventional farm enterprises. Evidence from other studies suggests that *Salmonella* controls are harder to carry out on farms with outdoors production. More needs to be done to protect outdoor pigs from wildlife and environmental sources of *Salmonella*, possibly by adding bird nets. To this end, the use of feed troughs with 'roofs' that restrict access to rodents and to large birds, and also protect the feed from becoming wet, may be particularly useful for outdoor herds. Other ideas that could be tested could be the use of bird scarers and the rotavation of land to stop newly introduced pigs being infected by an environment contaminated by *Salmonella* left by the previous group of pigs.

For all types of farms, the results suggested that biosecurity controls for vehicular collections and deliveries may not be effective and that an increased number of vehicular visits were associated with *Salmonella* prevalence. Controls could cover improved management of the pig herd so that collections/ deliveries could be grouped and the number of visits could be reduced. When visits are necessary then vehicles should not come into the vicinity of the pig buildings and pigs should be loaded and unloaded at the farm boundary. The cleaning and disinfection of the vehicle wheels may also help to reduce *Salmonella* contamination, although this would need to follow a rigorous format so that organic matter was adequately

removed before the use of an effective disinfectant at the correct concentration. Research into the effectiveness of specific disinfectants may also inform the procedures. These controls can be time consuming to complete and may involve the difficult restructuring of the farm layout, and so may not be easy interventions to complete on a number of farms.

One area of control that farmers may show interest in applying would be in taking a more holistic view of pathogen control, as the control of other organisms that have a direct consequence to pig health and the farmer's business, will have a knock-on effect on *Salmonella* prevalence. By reducing the prevalence of respiratory and wasting diseases, possibly by improving ventilation and temperature control, or the use of treatments and vaccines, may keep the pig's immune system robust and the pig less prone to infection from *Salmonella*. Work is currently underway at VLA (now called AHVLA) to look at what practical interventions could be used to control both pig *Salmonella* and other health conditions, and which might offer the most cost-benefit to the farmer.

It has been shown that spatial clustering of pig *Salmonella* infection is significant within areas of the UK but that the epidemiological risk factors described may account for any global spatial heterogeneity. The use of a number of spatial analysis techniques were explored and the benefit of using more than one, allowed for a more thorough assessment of spatial heterogeneity. The variogram analysis was particularly useful, as this allowed for all types of outcome measures to be used without the need to aggregate the data to a binary outcome at the farm level. The use of modelling techniques in the variogram also allows for the benefits of adding and testing covariates. However, the K-function and variogram analyses suffered from a reduction in confidence in the results from areas of low numbers of spatial points. The Scan statistic and the adaptive bandwidth smoothing 'hot spot' analyses used methods that allowed for the analysis of data without restricting the confidence in areas of poorer data.

The temporal trends that were identified were included as part of the risk factor model and should be taken account of when looking at the effectiveness of control plans or accounted for when examining surveillance trends e.g. a short-term reduction in seroprevalence may be related to season factors, such as routine farm management and meteorological weather conditions, rather than an actual decrease in prevalence caused by an intervention.

Regional differences in farm management were detected, such as the differences in the use of outdoor production or use of homemixing of feed. When combined with the pig movement findings, results showed how the clustering of high or low prevalence within a region might be maintained by regions that predominantly traded within their own region. The large range and distance of pig movements throughout the UK might suggest that farmers were likely to select trading farms by company instructions or by pig price. A greater motivation to select farms by *Salmonella* status should be encouraged and driven by the industry. If farmers

were to purchase pigs from low prevalence farms, and to reduce the total number of farms from which they bring in pigs, then this would improve the chances of maintaining farms at a low prevalence, once on-farm interventions have been enacted. Similar challenges to the way hauliers are used may also be beneficial i.e. with a reduction in the total number of hauliers used.

The network results also have implications for outbreak control, such as after the introduction of a novel type of *Salmonella* or an outbreak of Foot and Mouth Disease. Movement restrictions and heightened surveillance could be targeted within regions related to the index farm, depending on their regions External-Internal score, rather than applying global controls. The network findings also showed that although farms in larger companies had a high percentage of movements within their own company, the use of control within a single company network may not be successful due to their connections to small companies, which provide an indirect means of connecting the large companies. There were no farms identified that would be of particular importance in a disease outbreak situation (i.e. no farms acting as key transport hubs for pig movements) and so it would be inefficient to use control strategies targeted at specific farms.

The QA scheme data were a significant resource that were extracted and evaluated through this study. A large number of pig farms were included in the schemes, and although not all of the data collected were suitable for analysis, the large dataset provided great statistical power and high confidence in the results. The analyses have also highlighted improvements that could be applied that would greatly improve the data collected by the QA schemes. The scope and quality of data collected at QAS audits could be improved to collect more epidemiologically useful information on biosecurity and infection risk, and initial conversations with the QAS have shown a willingness to incorporate some of these ideas. The introduction of greater data validation would make a large impact, as the study was hampered by: 1) errors in the recording of farm identifiers, which could be improved by using pick lists of current members or by using input masks to ensure that the identifiers can only be data entered in the standard format, and 2) missing values in the dataset, potentially caused by data not being collected by auditors at every visit. In areas of small amounts of missing data, imputation methods could be used to ensure that records are not dropped from a model, by 'imputing' values. Various methods have been suggested and a number have been shown to be useful in the epidemiological context (Schafer, 1997; Shieh, 2003; den Uijl, Swart and van Shaik, 2012). The standardisation of the questions collected at audit by the three main schemes would also improve the data useful for epidemiological analysis. Improvements on these fronts would greatly improve the scale of the dataset that could be used for statistical modelling, and subsequently the statistical power and representativeness of the analysis, whereas otherwise these records would be dropped from the analysis.

Another area where data quality was important was in the recording of farm locations. Although the farms were generally linked to spatial locations that were fairly accurate, improvements could be made. Farm coordinates were usually gathered using either the CPH identifier that was linked to departmental records on farm locations or the postcode provided was used. Both of these identifiers can be problematic in providing the location of the pigs, as the postcode is often the location of the farmer's home or farm office rather than the actual location of the pigs, which could be distinct from that location. In areas of high pig farm density, multiple farms could provide the same postcode and so these coordinates had to be randomly 'agitated' to another close location to allow them to be categorised as separate locations in the spatial analysis. The CPH identifier has been a routine problem when identifying unique farms as multiple pig units can be covered by the same CPH if they are all owned by the same company, even though these may be quite distant from each other. The collation of exact GPS coordinates for each pig building would be a far more accurate way of determining spatial locations, although, for this study it would have been unrealistic to achieve that for such a large dataset. However, the collection of the geo-location of pig buildings could be an aim of the QA schemes, as this information could be useful for a number of studies and would allow the unique referencing of pig building rather than individual farms.

The ZAP/ ZNCP scheme has provided Governmental policy-makers, the pig industry and individual farmers with a way of monitoring pig *Salmonella* infection. The studies in this thesis have also shown how useful the data were for statistical analysis. However, the serological nature of the tests does limit its use as an outcome, as the individual serovars most important to human health cannot be distinguished and the ELISA result does not accurately predict whether a pig is currently infected or not. The use and frequency of this method of serological testing has been questioned in a number of areas (PVS, 2008), with some pushing for further changes to the protocol or a switch to bacteriological testing. A common response from farmers and vets contacted through this study was that the scheme's results fluctuated greatly with no apparent reason and that this meant that the monitoring of prevalence and consequence of a high prevalence in a scoring period was difficult to interpret. Changes to prevalence may have been caused by meteorological events or temporal associations, or the appearance of a new strain of *Salmonella*, which are beyond the control of the farmer. These factors could be taken account of when providing a farm's ZNCP score, so that an adjusted score is provided. However, the scheme provides a cost-effective route for the collection of *Salmonella* surveillance data, due the cheap cost of serological testing and it has the added benefit of detecting subclinically infected pigs. After the end of the work completed for this thesis (July 2012), the seroprevalence survey was suspended. The removal of this scheme and the lack of a replacement will affect the ability of the UK industry to monitor and set baselines for *Salmonella* prevalence. However, with the increasing importance of monophasic strains, it would be important for any new scheme to

utilise microbiological culturing. This would differentiate between the spread of novel strains between farms, causing outbreaks of high prevalence, and high prevalence of 'home' strains maintained on farms, to aid the selection of interventions.

The work completed here could be advanced by using an intervention study to test whether any of the suggested control measures show a significant effect in reducing prevalence, or whether it is the expertise in applying the control measures that is key to controlling *Salmonella*. It would also be interesting to see how interventions could be effective on individual farms and also on a network basis. If the QAS datasets were improved sufficiently, then a second epidemiological *Salmonella* analysis would be recommended to see if the same set of risk factors were selected from a population of all the QAS members. A representative population of non-member pig farm holdings could also be included to fill this data gap, to see whether findings are applicable to these holdings. It would also be useful to reproduce the study using on-farm faecal sampling and bacteriological tests to check that the use of serological abattoir-collected samples had not biased the study findings. This study could also examine whether the risk factors detected were applicable to *S.* Typhimurium or other serovars. If a new bacteriological study used a longitudinal study design then this would allow factors to be detected that influence new *Salmonella* infection in pigs.

The results also had implications for further examination of pig movements. Scottish farms were found to send pigs over a greater transport distance on average, but had on average a significantly lower seroprevalence. As transport durations would not be long enough for a pig to seroconvert it would be important to test whether longer transportation has an effect on bacteriological *Salmonella* results and especially to see whether any increase in hide contamination is detected. This may show that Scottish farms, which have a low seroprevalence related to the farm condition, pose a risk to human health due to greater *Salmonella* shedding and infection in transit caused by longer journeys to abattoirs.

This work has shown that the use of a broad set of skills and techniques is valuable. Although epidemiological modelling can provide an excellent basis for understanding the factors that influence infection and prevalence, the incorporation of spatial and network analyses in this study helped extract the most out of these data and to create a broader understanding of the disease. Although not described here, close working with risk analysts and statistical modellers has also allowed for disease spread to be modelled, control strategies to be simulated and for data gaps and areas of critical importance to be identified. These types of close collaboration with staff with a range of technical expertise, along with a team of industry, laboratory and veterinary colleagues who 'ground truth' the work and provide expert opinion on farms, abattoirs, laboratory testing or disease aetiology, all make a study far more effective and helpful to the policy makers.

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8. Bibliography

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Presentations at international scientific meetings

Smith R.P., Clough H.E., Cook A.J.C.	Analysis of Meat Juice ELISA results and questionnaire data to investigate farm-level risk factors for *Salmonella* infection in UK pigs **(winner of best speaker award)**	01/10/09	Proceedings of the Safepork 2009 conference, Quebec, Canada.

Presentations at industry/ veterinary meetings

Smith R.P., Sanchez M.	An Analysis of Quality Assurance and Zoonoses Action Plan Data	13-14/11/08	Proceedings of the Pig Veterinary Society Autumn Meeting, Breadsall Priory, Derbyshire

Presentations at VLA/ University of Liverpool meetings

Clough H., Smith R.P.	ZAP role of routine surveillance data in understanding geography and timing of Salmonella on UK pig farms	27/11/07	Presentation to CERA & pig salmonella project staff
Smith R.P.	An Analysis of Quality Assurance and Zoonoses Action Plan Data	06/11/08	RVC-VLA seminar day, VLA Weybridge

Smith, R.P., Christley, R., Clough, H.E	Analysis of Meat Juice ELISA results and questionnaire data to investigate farm-level risk factors for *Salmonella* infection in UK pigs	26/03/10	UoL poster day

Other related papers/ reports/ talks

Smith, R.P., Sánchez-Vázquez, M.J., Cook, A.J.C., Edwards, S.E. (2011) Abattoir-based study investigating the association between gross pathological lesions and serological tests for *Salmonella* infection in pigs. Veterinary Record, 168 (9): 240.

Sánchez-Vázquez, M.J., Smith, R.P., Gunn, G.J., Lewis, F., Strachan, W.D., Edwards, S.A. (2010) The Identification of Risk Factors for the Presence of Enzootic Pneumonia-Like Lesions and Pleurisy in Slaughtered Finishing Pigs Utilizing Existing British Pig Industry data. Pig Journal, 63: 25-33.

Other related presentations at industry/ veterinary meetings

Smith R.P., Sanchez M.	Pilot study of clinical sign surveillance on pig farms	25-26/05/10	Pig Veterinary Society Spring Meeting, Dunston hall, Norfolk

Chapter 2

Appendix A: Review of QAS data

Scheme	ABP	QMS	GQA
Transport			
Own transport	[redacted]	yes/no	[redacted]
Feed			
Home mix feed used	yes/no	yes/no	
Zootech feed used	yes/no	[redacted]	yes/no
Dairy liquid co-products fed	yes/no		yes/no
Non-dairy liquid co-products fed	yes/no		yes/no
Dry co-products fed	yes/no		yes/no
Antibiotic	weaner, grower, finisher (all yes/no)		yes/no
Antibiotic Growth Promoter	weaner, grower, finisher (all yes/no)		weaner, grower, finisher (all yes/no)
Probiotic	weaner, grower, finisher (all yes/no)		weaner, grower, finisher (all yes/no)
Feed Enzyme	weaner, grower, finisher (all yes/no)		weaner, grower, finisher (all yes/no)
Pellets	sow, weaner, grower, finisher (all yes/no)		boars, dry sows, maiden sows, service sows, farrowers, weaner, grower, finisher
Meal	sow, weaner, grower, finisher (all yes/no)		boars, dry sows, maiden sows, service sows, farrowers, weaner, grower, finisher
Compound feeds	[redacted]	yes/no	[redacted]
Wet	sow, weaner, grower, finisher (all yes/no)	[redacted]	boars, dry sows, maiden sows, service sows, farrowers,

		[redacted]	weaner, grower, finisher
liquid feed	[redacted]	yes/no	[redacted]
Weaner fed	feed rate? To appetite, restricted, B? (A/B/R)	[redacted]	To appetite, restricted, (A/R)
Grower fed	feed rate? To appetite, restricted, B? (A/B/R)		To appetite, restricted, (A/R)
Finisher fed	feed rate? To appetite, restricted, B? (A/B/R)		To appetite, restricted, (A/R)
Boars fed	[redacted]		To appetite, restricted, (A/R)
Dry sows fed			To appetite, restricted, (A/R)
Maiden sows fed			To appetite, restricted, (A/R)
Service sows fed			To appetite, restricted, (A/R)
Farrowers fed			To appetite, restricted, (A/R)
Feed type sources		free text field holding types of feed and sources	[redacted]
<u>Stock movement</u>			
What Hauliers do you use	3 fields of numeric Ids	[redacted]	7 numeric fields
Source of weaners	6 fields of numeric IDs	up to 8 fields of member numeric IDs	7 fields with numeric assurance refs + name and postcode
grower supplier distance	[redacted]	[redacted]	7 numeric fields
grower supplier travelling time			7 numeric fields
When		date of weaner supply	
Do you supply other Feeding units - which	6 fields of numeric Ids, N = Not known	[redacted]	7 fields with numeric assurance refs + name and postcode
Distance to each feed herd	6 numeric fields		7 numeric fields

Time to each feed herd	6 numeric fields	[redacted]	7 numeric fields
Do you supply abattoirs - which	6 fields of numeric IDs	free text field listing abattoirs used and types of pigs moved	7 numeric fields
Distance to each abattoir	6 numeric fields	[redacted]	7 numeric fields
Time to each abattoir	6 numeric fields		7 numeric fields
average age (days)	[redacted]		7 numeric fields
average weight (kg)			7 numeric fields
<u>Site info (multiple for each member)</u>			
CPH	full cph	full cph	
address	text	[redacted]	
County	text		
Primary site	yes/no		
Slap marks	slap mark ID, type (primo/ registered/ unregistered), site number		
<u>Stock & veterinary</u>			
Number of maiden gilts	numeric	[redacted]	numeric
No. of in pig gilts	numeric		numeric
No. of in pig sows	numeric		numeric
Suckling sows	numeric		numeric

Other sows	numeric		numeric
Total no. of sows	[redacted]	numeric	[redacted]
Boars	numeric	[redacted]	numeric
Feeders<30kg	numeric		numeric
Feeders>30kg	numeric		numeric
Breeding unit	[redacted]	Yes/no	[redacted]
Feeding unit		Yes/no	
Weaning unit		Yes/no	
No. of finished pigs		numeric	
Herd size		numeric	
Teeth clipped	none, some, all	[redacted]	none, some, all
Tails docked	none, some, all		none, some, all
Ears notched	none, some, all		none, some, all
Ear tattooed	none, some, all		none, some, all
<u>Housing systems</u>			
Breeding	indoor or outdoor (both questions yes/no)	[redacted]	indoor or outdoor (both questions yes/no)
Housing type weaners	[redacted]	solid floor, full slats, part slats, straw (all yes/no)	[redacted]
Growing	indoor or outdoor (both questions yes/no)	[redacted]	indoor or outdoor (both questions yes/no)
Housing type growers	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)	solid floor, full slats, part slats, straw (all yes/no)	[redacted]

Finishing	indoor or outdoor (both questions yes/no)	[redacted]	indoor or outdoor (both questions yes/no)
Housing type finishers	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)	solid floor, full slats, part slats, straw (all yes/no)	[redacted]
Housing type boars	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)	solid floor, full slats, part slats, straw (all yes/no)	
Housing type maiden	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)	[redacted]	
Housing type dry sows	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)		
Housing type wean to serve	full slats, part slats, no slats or bedding, no slats but bedding, outdoors (multiple choice)		
Is farrowing carried out on unit	yes or no		
Housing type farrow	full slats, part slats, no slats or bedding, no slats but bedding (multiple choice)		
Housing type sows	[redacted]	solid floor, full slats, part slats, straw (all yes/no)	

<u>Pen level details</u>

Total number of pigs per pen/ building	[redacted]	boars/ dry/ maiden/ service/weaners/ growers/finishers
Total area (metres) per pen/building		boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers
full slats/ part slat/ none		boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers
bedding - none, woodshavings, straw, sawdust		boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers

ventilation	[redacted]	natural, forced, auto - boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers
Pen Condition score		scored 1-4, boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers
Pig Condition score		scored 1-4, boars/ dry/ maiden/ service/Farrowers/Weaners/growers/finishers
Piglet protection		Yes/no
Farrowing crate length		length (metres)
Farrowing housing		crate, loose, outdoors
Average weight of pigs (kg)		Weaners/growers/finishers

Chapter 3

Appendix A: Pig farm biosecurity survey questionnaire

Veterinary Laboratories Agency

Assurance Scheme Membership number – please give full reference (**letters** and numbers): _____

Date form completed: ___/____/___

Please be aware that the information given below will be kept in confidence

Pig Class Definitions

Weaner = 3-10 weeks old or approximately 8-30 kg.
Grower = 11-14 weeks old or approximately 30-50kg.
Rearer = weaners & growers.
Finisher = 15+ weeks old or approximately 50-110kg.

1. Business details

1.1 Current enterprise type (*tick all that apply*)?

☐ Organic ☐ Conventional ☐ Freedom foods

1.2 Have pigs been reared on contract within the last 12 months?: ☐ Yes ☐ No

if yes, from which companies……

1.3 Production system (*tick all that apply*)? ☐ All-in/ All-out on shed basis

☐ All-in/ All-out on whole farm basis ☐ Continuous

1.4 Number of pig places?

Pig category	Maximum number of pig places
Weaner	
Grower	
Finisher	
Adults	

1.5 Other animals currently present on premises:-

Species	Animals present on farm in last 12 mths (*tick applicable box*)	Number of animals currently present
Cattle	☐ Present ☐ Absent	
Sheep	☐ Present ☐ Absent	
Goats	☐ Present ☐ Absent	
Poultry	☐ Present ☐ Absent	
Horses	☐ Present ☐ Absent	
Other1, specify……		
Other2, specify……		

2. Pig unit details

2.1 Are separate groups of pigs mixed together at the following stages?:-

When weaner group made ☐ Yes ☐ No
At end of production/ slaughter ☐ Yes ☐ No
At any other time ☐ Yes ☐ No

2.2 Are sick pigs isolated? ☐ Yes,always ☐ Yes,sometimes ☐ Yes,rarely ☐ Never

If yes,

2.2a Are sick pigs isolated in - ☐ Separate pen ☐ Separate building

2.2b Are sick pigs mixed in with healthy ones on recovery?

☐ Always ☐ Sometimes ☐ Rarely ☐ Never

2.3 Feed used on farm

Feed Type	Weaner	Grower	Finisher	Sows
Acidified feed	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
If yes, when used	☐ Always ☐ Sometimes ☐ Rarely	☐ Always ☐ Sometimes ☐ Rarely	☐ Always ☐ Sometimes ☐ Rarely	☐ Always ☐ Sometimes ☐ Rarely
Acidified water	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
If yes, when used	☐ Always ☐ Sometimes ☐ Rarely	☐ **Always** ☐ Sometimes ☐ Rarely	☐ Always ☐ Sometimes ☐ Rarely	☐ Always ☐ Sometimes ☐ Rarely
Fermented liquid feed	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Unfermented liquid feed	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Home mix	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Bought-in concentrate	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Wheat	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Approx % of wheat in total diet				
Barley	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Approx % of barley in total diet				
Feed additive/ growth promoter/ prebiotic/ probiotic	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
If yes, which types				

2.4 How many deliveries of pigs have been received in the last 12 months? *(please enter the name & short address of source company or farm)*

.....

***2.5** How is water supplied for finishers* (tick all that apply)*?:-*

☐ *Mains* ☐ *Borehole* ☐ *Other, specify*.....

2.6 What type of drinker is used in the finisher houses *(tick all that apply)*?:-

☐ Nipple ☐ Bowl
☐ Trough ☐ Mixed with feed ☐ Other, specify.....

2.7 How often is drinking system in finisher housing cleaned?

☐ Between batches ☐ Every other batch ☐ Quarterly
☐ Biannually ☐ Annually

3. Biosecurity

3.1 When are pig finisher houses cleaned? (*tick one box*):–

☐ Between batches ☐ Every other batch ☐ Quarterly ☐ Biannually
☐ Annually ☐ Not applicable (go to question 3.4)

3.2 How are the pig finisher houses cleaned? (*tick all that apply*)

☐ Scraped ☐ Brushed ☐ Washed with hose
☐ Pressure washed ☐ Steam cleaned

3.3 When are pig finisher houses disinfected? (*tick one box*):–

☐ Between batches ☐ Every other batch ☐ Quarterly
☐ Biannually ☐ Annually ☐ Never

3.4 Frequency of visits:-

•Delivery of feed = ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Vet = ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Pig delivery = ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Live Pig collection = ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Dead stock collection= ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Vermin controller = ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Other 1, specify.....
= ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never
•Other 2, specify.....
= ☐ >11/year ☐ 6-11/ year ☐ 2-5/year ☐ 1/year ☐ Never

3.5a Do pig delivery lorries unload at the perimeter gate?:–

☐ Always ☐ Sometimes ☐ Rarely ☐ Never

3.5b Do feed delivery lorries unload at the perimeter gate?:–

☐ Always ☐ Sometimes ☐ Rarely ☐ Never

3.6a Do you have facilities in place for vehicles to disinfect their wheels? ☐ Yes ☐ No

3.6b How frequently do drivers use them? (*please tick one box*)?
☐ Always ☐ Sometimes ☐ Rarely ☐ Never

3.7a How frequently are boot dips used on entry to pig buildings? – ☐ Always
☐ Sometimes ☐ Rarely ☐ Never

3.7b Are boot dips present (*tick all that apply*)? ☐ Yes, outside each pig building
☐ Yes, at entrance of site ☐ None

3.7c Are the boot dips changed:-
☐ Daily ☐ Weekly ☐ Monthly ☐ Less than monthly ☐ N/A

3.7d Are boot dips protected from rain? ☐ Yes ☐ No

4. Herd health

4.1 Health status (*tick all that have been diagnosed/ reported in the pig herd by your vet over the last year*):-

☐ Enzootic Pneumonia (EP) ☐ Actinobacillus PleuroPneumonia (APP)
☐ Postweaning Multisystemic Wasting Syndrome (PMWS) ☐ Glasser's
☐ Porcine Reproductive & Respiratory Syndrome (PRRS) ☐ Streptococcus suis
☐ Coccidiosis ☐ Swine dysentery ☐ Enteric parasite
☐ Porcine Dermatitis & Nephropathy Syndrome (PDNS) ☐ Clinical Salmonellosis

Ailment list: Scour, wasting, respiratory disease, nervous disease, skin disease, lameness, tail biting, sudden death, other type (please specify).

4.2 Please rank the top three ailments from the list above that have caused the most pig deaths over the last 12 months and the estimated percentage of herd effected:-

1st (Most deaths)_____
2nd _____
3rd _____

4.3 Please rank the top three ailments from the list above that have been treated with antibiotics over the last 12 months and the estimated percentage of herd treated:-

1st (Most treated)_____
2nd _____
3rd _____

Many thanks for your help

Appendix B: Pig farm biosecurity questionnaire assessment form

Assurance Scheme
Membership number: _____ Date form completed: ___/____/___

1. How long did it take to complete the questionnaire?.....Minutes

2. Did you have to consult your records to answer any questions? ☐ Yes ☐ No

If so, for which question numbers?.....

How are your records stored? ☐ Paper ☐ Computer

How long did it take to consult your records?.....Minutes

3. Are there any particular questions you had problems with?.....

4. How could this questionnaire be improved?

.....

.....

Appendix C: Enrolment letter to private veterinary surgeons

Our Ref: OZ0323

To: Private Veterinary Surgeons working for Pig Quality Assurance Companies

Dear Sir/ Madam

Assistance with data collection
I am contacting you to seek your assistance in a research project on the epidemiology of *Salmonella* in pigs. The Veterinary Laboratories Agency (VLA) is leading a Defra-funded project "An integrated risk-based approach to the control of *Salmonella* in pigs" which we are delivering with support from the British Pig Executive (BPEX) and Zoonoses Action Plan (ZAP) *Salmonella* Monitoring Scheme. Our aims are to improve our understanding of the epidemiology of *Salmonella* in pigs, by making the most of available data, to enable science-based advice to be given on control. Our previous research has been disseminated to private vets and to industry, and you might have seen our presentation at the "Serious about *Salmonella*" meeting at Stoneleigh, funded by FSA.

We would like you to help all assured farmers that submit samples to the ZAP scheme, i.e. farms with finishers, complete an additional questions form (please find four copies attached) at your next quarterly visit. The form has been piloted to ensure that the questions are easy to answer and will only take 10-15 minutes to complete. The form can then be faxed to me (01932 349983) or posted to the address below using the provided reply-paid envelopes. We do appreciate that volunteering to complete the supplementary form is an imposition upon the time of both the farmer and yourselves, however, as in other projects, we rely on individuals making a modest personal contribution to the project in the interests of all.

We will utilise routine data collected by assurance schemes along with this supplementary information and sample level data from the ZAP scheme (rather than using the ZAP score) to carry out a detailed and thorough investigation. We have researched the topic extensively to ensure that every question we ask has not been collected in a useful format elsewhere and is essential information for our analysis. The analysis will tackle issues highlighted by earlier research, such as the spatial clustering of high prevalence *Salmonella* farms, and also inform a risk assessment and cost/benefit analysis of interventions. We are hoping that with your invaluable help we can gain maximum participation with this study, which will allow us to provide results with real statistical power and significance.

We will send a summary of the project findings to the participating farmers and yourselves, and our aim is that the project analysis will create new knowledge that will help farmers/ vets monitor their *Salmonella* status, choose interventions and predict their effect and cost.

We hope you feel that you can help us with this study. If you have any queries or require more forms and envelopes, please do not hesitate to contact me (01932 357803).

Yours sincerely

Richard Smith
CERA
Veterinary Laboratories Agency - Weybridge
Woodham Lane
New Haw
Addlestone
Surrey KT15 3NB

Appendix D: Enrolment supporting letter from ABP

Secretariat:
Unit 4B,
Highway Farm,
Horsley Road, Downside,
Cobham,
Surrey
KT11 3JZ
Direct Line & Fax: 01932 589800

May 2007

Dear ABPigs Registered Veterinary Surgeon,

Assured British Pigs Veterinary Reporting – QVR

On behalf of the ABPigs Board I would like to thank you for your ongoing co-operation with the Assured British Pigs standards and in particular with regard to the completion and return of the Quarterly Veterinary Report (QVR).

The Board has received feedback indicating that the re-drafted form introduced in 2005 has addressed criticisms which had been levelled at the Scheme both by farmers and some members of the veterinary profession and notes that veterinarians are now able to spend more time assessing and advising on health and welfare matters rather than completing paperwork. The completion of these reports is as you know a vital component of the integrity of the scheme. It is therefore essential that the information recorded is accurate and complete. May I therefore take this opportunity of reminding all veterinarians that:-

- It is important to confirm whether any additional location(s)/sites have been visited on each quarterly visit and note this on the QVR - or if the additional sites have not been assessed then please could you advise the CB of the reasons why this was not undertaken. It is essential that this is done accurately because we need to be aware exactly which sites we are assuring as there have been incidences of unscrupulous operators only showing inspectors some of their sites and then fraudulently using their assurance number to cover additional sites. This check on the QVR form allows us to regularly keep a check on which sites are assured.
- It is important to confirm stock numbers – these details should be completed in full but are often left blank or only partially completed.
- The source of any incoming weaners should be recorded in full - often a surname only is given with no address and no / incorrect assurance registration number. ABPigs needs information to be recorded to facilitate the verification of the assured status of all incoming weaners. This should be readily available in the farm's movement records.

Defra Research Project on the epidemiology of Salmonella in pigs

Enclosed with this letter is a letter and additional questions form prepared by VLA asking for your help with a Defra funded project "An integrated risk-based approach to the control of *Salmonella* in pigs". ABPigs is already collaborating with this project and is mindful of the need to reduce the burden of form filling at all stages of production.

Appendix E: Enrolment supporting letter from GQA

GENESIS QUALITY ASSURANCE LIMITED

RYKNIELD HOUSE – ALREWAS – BURTON-ON-TRENT – STAFFORDSHIRE – DE13 7AB
Tel: +44 (0) 1283 79 2100 Fax: +44 (0) 1283 791500 E-mail: info@genesisqa.com Website: www.genesisqa.com

30 May 2007

Dear Sir or Madam

Re: Veterinary Laboratories Agency Project
"An Integrated risk-based approach to the control of *Salmonella* in pigs"

Approximately three years ago assurance schemes were asked to support the work of the ZAP Salmonella Scheme by introducing it as a requirement of the scheme. After considerable debate this was incorporated primarily on the basis of food safety.

Whilst intentions on all sides were good the penalties for producers that do not meet the targets are severe – i.e. loss of assured status and it has become somewhat of a thorn in the side of producers, their veterinary surgeons/advisers and scheme operators. Not least because there appears to be a lack of scientifically proven measures that producers can use in their efforts to reduce the prevalence of *Salmonella* when all the basic measures have been properly implemented and as I am sure you are aware this seriously affects the morale of producers.

Genesis QA is therefore supporting this project and whilst we appreciate that veterinary surgeons already contribute significant time towards assurance scheme requirements, particularly in the completion Quarterly Veterinary Visit Reports, we would be grateful if you would endeavour to assist Richard and his team by completing the questionnaire for any Genesis QA members within your practice.

Your assistance in this matter would be greatly appreciated however if you have any concerns or queries and wish to discuss this matter further please do not hesitate to contact either Richard or myself.

Yours sincerely

Jane Johnson
General Manager

Appendix F – Farmer enrolment letter

7 April 2008

Our Ref: OZ0323

To: Quality Assured Pig Farmers

Dear Sir/ Madam

Salmonella in pigs: Assistance with data collection

At the Veterinary Laboratories Agency, we understand that this is a very difficult time for pig producers. As a member of a quality assurance scheme, you will know that *Salmonella* levels are another problem that you have to face. We are currently studying *Salmonella* in pigs in order to provide the scientific evidence for *Salmonella* control on farms. Results from our previous research have been provided to private vets and the pig industry, and you may have seen our presentation at the "Serious about *Salmonella*" meeting at Stoneleigh, funded by FSA.

Our current Defra-funded project "An integrated risk-based approach to the control of *Salmonella* in pigs" is supported by the British Pig Executive (BPEX) and the Zoonoses Action Plan (ZAP) *Salmonella* Monitoring Scheme. As part of this project, we would like to invite all assured farmers that submit samples to the ZAP scheme, i.e. farms with finishers, to complete the attached biosecurity form. This information will be used to carry out a detailed and thorough investigation into *Salmonella* prevalence on pig farms. The form will only take 10-15 minutes to complete and can either be faxed to me (01932 349983) or posted to the address below using the reply-paid envelope provided.

We appreciate that completing this form will take time and so we will make an inconvenience payment of £20 on receipt of your completed form. This could be either by direct payment or by Marks & Spencer voucher, an invoice form is attached for you to complete. Please try and ensure that the biosecurity form and payment form are returned to me within two weeks of receipt.

We hope you feel that you can help us with this study. If you have any queries or require more biosecurity forms, please do not hesitate to contact me (01932 357803).

Yours sincerely,

Richard Smith
CERA
Veterinary Laboratories Agency - Weybridge
Woodham Lane
New Haw
Addlestone
Surrey KT15 3NB

Appendix G – Pig farm company enrolment letter

2 April 2008

Our Ref: OZ0323

To: Quality Assured Pig Farmers

Dear Sir/ Madam

Salmonella in pigs: Assistance with data collection

At the Veterinary Laboratories Agency, we understand that this is a very difficult time for pig producers. As a member of a quality assurance scheme, you will know that *Salmonella* levels are another problem that you have to face. We are currently studying *Salmonella* in pigs in order to provide the scientific evidence for *Salmonella* control on farms. Results from our previous research have been provided to private vets and the pig industry, and you may have seen our presentation at the "Serious about *Salmonella*" meeting at Stoneleigh, funded by FSA.

Our current Defra-funded project "An integrated risk-based approach to the control of *Salmonella* in pigs" is supported by the British Pig Executive (BPEX) and the Zoonoses Action Plan (ZAP) *Salmonella* Monitoring Scheme. As part of this project, we would like to invite your assured farmers that submit samples to the ZAP scheme, i.e. farms with finishers, to complete the attached biosecurity form. Please see the attached list for the farms that we believe may submit samples to the ZAP scheme that we are interested in. This information will be used to carry out a detailed and thorough investigation into *Salmonella* prevalence on pig farms. The form will only take 10-15 minutes to complete and can either be faxed to me (01932 349983) or posted to the address below using the reply-paid envelope provided.

We appreciate that completing this form will take time and so we will make an inconvenience payment of £20 to you or direct to the farmer, on receipt of your completed form. This could be either by direct payment or by Marks & Spencer voucher, an invoice form is attached for you or the individual farmer to complete. Please try and ensure that the biosecurity form and payment form are returned to me within two weeks of receipt.

We hope you feel that you can help us with this study. If you have any queries or require more biosecurity forms, please do not hesitate to contact me (01932 357803).

Yours sincerely,

Richard Smith
CERA
Veterinary Laboratories Agency - Weybridge
Woodham Lane
New Haw
Addlestone
Surrey KT15 3NB

Appendix H: Univariable model results

Variable	Count of holdings	% of all holdings	% samples positive	Coef.	*P*-value
QA scheme					
ABP	305	53.9	22.0	Baseline	
GQA	171	30.2	29.3	0.458	<0.001
QMS	90	15.9	7.1	-0.658	<0.001
NUTS Region of farm					
East Midlands	33	5.8	14.5	-1.055	<0.001
East and South East England	129	22.8	24.9	-0.427	<0.001
Northern Ireland	43	7.6	13.1	-0.974	<0.001
North East	6	1.1	18.5	-0.560	0.112
North West	17	3.0	17.7	-0.699	0.002
Scotland	93	16.4	7.1	-1.336	<0.001
South West	63	11.1	12.8	-1.052	<0.001
West Midlands & Wales	39	6.9	17.5	-0.755	<0.001
Yorkshire and The Humber	139	24.6	32.5	Baseline	
NUTS Region of farm (recoded)					
Other	469	82.9	23.9	Baseline	
Scotland	93	16.4	7.1	-0.824	<0.001
Season that sample was collected from					
Spring	n/a		20.6	Baseline	
Summer	n/a		21.4	-0.169	<0.001
Autumn	n/a		24.1	-0.133	<0.001
Winter	n/a		22.2	-0.099	<0.001
Organic					
TRUE	9	1.6	14.8	-0.134	0.680
FALSE	557	98.4	22.2	Baseline	
Conventional					
TRUE	515	91.0	21.3	-0.741	<0.001
FALSE	51	9.0	35.1	Baseline	
Freedom foods					
TRUE	86	15.2	33.0	0.583	<0.001
FALSE	480	84.8	20.9	Baseline	
Reared On Contract					
No	292	51.6	25.4	0.385	<0.001
Yes	254	44.9	20.8	Baseline	
Number of Rearer Companies					
Missing	15	2.7	24.9	Baseline	
Multiple Companies	5	0.9	38.3	0.656	0.194
Single company	228	40.3	25.2	-0.163	0.536
Single Individual	5	0.9	27.5	-0.402	0.423
All-in/All-out on shed basis					
TRUE	155	27.4	23.1	0.075	0.421
FALSE	411	72.6	21.6	Baseline	
All-in/All-out on shed basis					
TRUE	190	33.6	23.0	0.115	0.200
FALSE	376	66.4	21.9	Baseline	
Continuous production					
TRUE	219	38.7	21.4	-0.160	0.062
FALSE	347	61.3	22.6	Baseline	
Cattle Present On Farm					

Absent	373	65.9	22.9	Baseline	
Present	193	34.1	20.4	-0.282	0.001
Sheep Present On Farm					
Absent	419	74.0	22.3	Baseline	
Present	147	26.0	21.6	-0.241	0.011
Goats Present On Farm					
Absent	555	98.1	22.2	Baseline	
Not Known	1	0.2	2.4	-0.944	0.327
Present	10	1.8	19.8	-0.195	0.526
Poultry Present On Farm					
Absent	449	79.3	21.9	Baseline	
Not Known	1	0.2	2.4	-0.953	0.323
Present	116	20.5	23.2	-0.063	0.545
Equine present on farm					
Not Known	1	0.2	2.4	-0.926	0.337
Absent	450	79.5	21.5	Baseline	
Present	115	20.3	24.6	0.076	0.478
Cats present on farm					
Absent	554	97.9	21.5	Baseline	
Present	12	2.1	35.9	0.636	0.025
Dogs present on farm					
Absent	549	97.0	21.4	Baseline	
Present	17	3.0	36.0	0.264	0.287
Camelids present on farm					
Absent	564	99.6	22.2	Baseline	
Present	2	0.4	7.8	-0.808	0.235
Other birds present on farm					
Absent	560	98.9	22.0	Baseline	
Present	6	1.1	28.7	0.046	0.908
Pigs Mixed At Weaner Group Stage					
No/NA	142	25.1	24.4	Baseline	
Yes	390	68.9	21.0	-0.368	<0.001
Pigs Mixed At Production/ Slaughter					
NA	1	0.2	6.7	-0.274	0.782
No	202	35.7	21.0	Baseline	
Yes	327	57.8	22.6	-0.104	0.246
Pigs Mixed At Other Time					
No/NA	376	66.4	20.8	Baseline	
Yes	114	20.1	23.5	0.209	0.046
Pigs Not Mixed in Any Of The Above					
No	494	87.3	21.5	Baseline	
Yes	72	12.7	27.1	0.477	<0.001
Are Sick Pigs Isolated					
Always	316	55.8	21.4	Baseline	
Sometimes	217	38.3	22.8	0.042	0.637
Rarely	21	3.7	24.9	-0.061	0.793
Never	9	1.6	22.5	0.626	0.074
Where Are Pigs Isolated					
Both	20	3.5	24.3	Baseline	
Separate Building	193	34.1	23.8	0.239	0.297
Separate Pen	340	60.1	20.8	0.162	0.471
Are Sick Pigs Mixed On Recovery					

Always	33	5.8	20.9	Baseline	
Sometimes	147	26.0	26.6	0.049	0.794
Rarely	142	25.1	20.2	-0.081	0.668
Never	231	40.8	20.8	-0.120	0.512
Any Homemix					
no	334	59.0	26.0	Baseline	
yes	144	25.4	13.9	-0.548	<0.001
Any Wet feeding					
no	448	79.2	23.7	Baseline	
yes	65	11.5	14.8	-0.434	<0.001
Any Compound feeding					
no	67	11.8	14.2	Baseline	
yes	446	78.8	23.9	0.500	<0.001
Weaners Fed Acidified Feed					
NA	192	33.9	24.7	0.148	0.122
No	263	46.5	22.0	Baseline	
Yes	111	19.6	18.6	-0.030	0.791
Weaners Acidified Feed - When Used					
NA	455	80.4	23.0	0.126	0.264
Missing	8	1.4	13.7	0.029	0.936
Always	94	16.6	19.1	Baseline	
Sometimes	9	1.6	19.0	0.405	0.231
Weaners Fed Acidified Water					
NA	192	33.9	24.7	0.150	0.099
No	351	62.0	21.3	Baseline	
Yes	23	4.1	18.2	-0.113	0.589
Weaners Acidified Water - When Used					
NA	528	93.3	22.3	0.325	0.290
Missing	17	3.0	18.4	0.307	0.425
Always	10	1.8	15.7	Baseline	
Sometimes	10	1.8	25.4	0.602	0.167
Rarely	1	0.2	2.5	-0.830	0.415
Weaners Fed Fermented Liquid Feed					
NA	192	33.9	24.7	0.142	0.113
No	366	64.7	21.4	Baseline	
Yes	8	1.4	6.5	-0.646	0.061
Weaners Fed Unfermented Liquid Feed					
NA	192	33.9	24.7	0.151	0.098
No	345	61.0	21.0	Baseline	
Yes	29	5.1	21.7	-0.079	0.681
Weaners Fed Home Mix					
NA	192	33.9	24.7		
No	285	50.4	24.2	Baseline	
Yes	89	15.7	13.5	-0.623	<0.001
Weaners Fed Bought-in Concentrate					
NA	192	33.9	24.7		
No	128	22.6	23.0	Baseline	
Yes	246	43.5	19.7	-0.182	0.031
Weaners Fed Bought-in Compound Feed					
NA	192	33.9	24.7	0.158	0.079
No	370	65.4	21.0	Baseline	
Yes	4	0.7	26.8	0.122	0.801

Weaners Fed Wheat					
NA	192	33.9	24.7	0.102	0.328
No	187	33.0	23.0	Baseline	
Yes	187	33.0	19.5	-0.108	0.289
Weaners Fed Barley					
NA	192	33.9	24.7		
No	190	33.6	24.8	Baseline	
Yes	184	32.5	17.8	-0.272	0.002
Weaners Fed Feed Additives/ Prebiotics etc					
NA	192	33.9	24.7	0.191	0.052
No	231	40.8	19.4	Baseline	
Yes	143	25.3	23.7	0.090	0.390
Growers Fed Acidified Feed					
NA	160	28.3	25.7	0.216	0.029
No	304	53.7	21.5	Baseline	
Yes	102	18.0	20.1	0.046	0.686
Growers Acidified Feed - When Used					
NA	464	82.0	22.5	0.115	0.325
Missing	3	0.5	5.1	0.418	0.474
Always	86	15.2	19.0	Baseline	
Sometimes	11	1.9	28.8	0.496	0.110
Rarely	2	0.4	33.7	dropped	
Growers Fed Acidified Water					
NA	160	28.3	25.7	0.205	0.030
No	397	70.1	21.2	Baseline	
Yes	9	1.6	19.4	0.046	0.886
Growers Acidified Water - When Used					
NA	557	98.4	22.1	0.053	0.903
Always	5	0.9	15.5	Baseline	
Sometimes	3	0.5	28.2	0.500	0.479
Rarely	1	0.2	2.5	dropped	
Growers Fed Fermented Liquid Feed					
NA	160	28.3	25.7	0.181	0.055
No	392	69.3	21.7	Baseline	
Yes	14	2.5	9.5	-0.625	0.016
Growers Fed Unfermented Liquid Feed					
NA	160	28.3	25.7	0.163	0.086
No	372	65.7	22.0	Baseline	
Yes	34	6.0	15.6	-0.461	0.007
Growers Fed Home Mix					
NA	160	28.3	25.7		
No	280	49.5	25.0	Baseline	
Yes	126	22.3	14.7	-0.572	<0.001
Growers Fed Bought-in Concentrate					
NA	160	28.3	25.7	0.284	0.008
No	183	32.3	20.4	Baseline	
Yes	223	39.4	22.0	0.150	0.128
Growers Fed Bought-in Compound Feed					
NA	160	28.3	25.7	0.208	0.027
No	402	71.0	21.0	Baseline	
Yes	4	0.7	33.9	0.423	0.379
Growers Fed Wheat					

NA	160	28.3	25.7		
No	180	31.8	24.2	Baseline	
Yes	226	39.9	19.3	-0.302	<0.001
Growers Fed Barley					
NA	160	28.3	25.7		
No	185	32.7	25.8	Baseline	
Yes	221	39.0	17.8	-0.404	<0.001
Growers Fed Feed Additives/ Prebiotics etc					
NA	160	28.3	25.7	0.186	0.061
No	294	51.9	21.8	Baseline	
Yes	112	19.8	19.3	-0.066	0.546
Finishers Fed Acidified Feed					
NA	40	7.1	26.5	0.315	0.068
No	425	75.1	21.1	Baseline	
Yes	101	17.8	25.1	0.214	0.051
Finishers Acidified Feed - When Used					
NA	465	82.2	21.4	-0.077	0.519
Missing	3	0.5	4.0	0.328	0.577
Always	82	14.5	22.4	Baseline	
Sometimes	13	2.3	34.6	0.633	0.029
Rarely	3	0.5	38.5	0.568	0.316
Finishers Fed Acidified Water					
NA	40	7.1	26.5	0.280	0.104
No	519	91.7	21.7	Baseline	
Yes	7	1.2	29.8	0.419	0.251
Finishers Acidified Water - When Used					
NA	559	98.8	21.9	-0.457	0.413
Not Known	1	0.2	33.3	0.424	0.702
Always	3	0.5	24.5	Baseline	
Sometimes	1	0.2	29.9	0.243	0.827
Rarely	2	0.4	34.5	-0.531	0.547
Finishers Fed Fermented Liquid Feed					
NA	40	7.1	26.5		
No	501	88.5	22.7	Baseline	
Yes	25	4.4	11.6	-0.591	0.003
Finishers Fed Unfermented Liquid Feed					
NA	40	7.1	26.5	0.231	0.178
No	466	82.3	23.2	Baseline	
Yes	60	10.6	16.2	-0.356	0.007
Finishers Fed Home Mix					
NA	40	7.1	26.5		
No	385	68.0	25.2	Baseline	
Yes	141	24.9	16.1	-0.539	<0.001
Finishers Fed Bought-in Concentrate					
NA	40	7.1	26.5	0.250	0.162
No	230	40.6	22.4	Baseline	
Yes	296	52.3	21.3	-0.043	0.622
Finishers Fed Bought-in Compound Feed					
NA	40	7.1	26.5	0.283	0.099
No	519	91.7	21.7	Baseline	
Yes	7	1.2	34.5	0.645	0.080
Finishers Fed Wheat					

NA	40	7.1	26.5	0.218	0.218
No	271	47.9	23.4	Baseline	
Yes	255	45.1	20.8	-0.112	0.198
Finishers Fed Barley					
NA	40	7.1	26.5		
No	269	47.5	25.7	Baseline	
Yes	257	45.4	19.2	-0.284	0.001
Finishers Fed Feed Additives/ Prebiotics etc					
NA	40	7.1	26.5	0.300	0.084
No	424	74.9	21.7	Baseline	
Yes	102	18.0	22.9	0.130	0.231
Sows Fed Acidified Feed					
NA	276	48.8	25.9	0.403	<0.001
No	261	46.1	19.3	Baseline	
Yes	29	5.1	19.1	-0.011	0.952
Sows Acidified Feed - When Used					
NA	537	94.9	22.3	0.337	0.127
Always	20	3.5	13.9	Baseline	
Sometimes	7	1.2	25.2	0.335	0.430
Rarely	2	0.4	27.7	0.603	0.397
Sows Fed Acidified Water					
NA	276	48.8	25.9	0.401	<0.001
No	285	50.4	19.3	Baseline	
Yes	5	0.9	13.6	-0.224	0.603
Sows Acidified Water - When Used					
NA	561	99.1	22.1	0.063	0.928
Missing	1	0.2	3.7	-1.390	0.244
Sometimes	2	0.4	3.1	Baseline	
Rarely	2	0.4	23.6	-0.160	0.870
Sows Fed Fermented Liquid Feed					
NA	276	48.8	25.9		
No	282	49.8	19.7	Baseline	
Yes	8	1.4	8.2	-0.899	0.009
Sows Fed Unfermented Liquid Feed					
NA	276	48.8	25.9	0.385	<0.001
No	266	47.0	19.8	Baseline	
Yes	24	4.2	15.1	-0.225	0.264
Sows Fed Home Mix					
NA	276	48.8	25.9		
No	187	33.0	23.5	Baseline	
Yes	103	18.2	13.1	-0.660	<0.001
Sows Fed Bought-in Concentrate					
NA	276	48.8	25.9	0.449	<0.001
No	160	28.3	18.3	Baseline	
Yes	130	23.0	20.7	0.099	0.385
Sows Fed Bought-in Compound Feed					
NA	276	48.8	25.9	0.407	<0.001
No	288	50.9	19.0	Baseline	
Yes	2	0.4	37.8	0.304	0.649
Sows Fed Wheat					
NA	276	48.8	25.9	0.396	<0.001
No	126	22.3	20.7	Baseline	

Yes	164	29.0	18.3	-0.014	0.900
Sows Fed Barley					
NA	276	48.8	25.9		
No	134	23.7	23.5	Baseline	
Yes	156	27.6	15.9	-0.440	<0.001
Sows Fed Feed Additives/ Prebiotics etc					
NA	276	48.8	25.9	0.391	<0.001
No	272	48.1	19.4	Baseline	
Yes	18	3.2	17.6	-0.218	0.348
Finisher Water Supply - Mains					
No	188	33.2	18.6	Baseline	
Yes	366	64.7	23.6	0.218	0.014
Finisher Water Supply - Borehole					
No	376	66.4	23.2	Baseline	
Yes	178	31.4	19.1	-0.223	0.013
Finisher Water Supply - Other types					
Running water	1	0.2	27.3		
Still water	32	5.7	16.4		
Unspecified	4	0.7	17.9		
Drinker type In Finisher Houses - Mixed with feed					
FALSE	485	85.7	22.4	Baseline	
TRUE	62	11.0	19.7	-0.235	0.072
Drinker type In Finisher Houses - Nipple					
FALSE	170	30.0	18.5	Baseline	
TRUE	377	66.6	23.1	0.249	0.006
Finisher Houses Scraped Clean					
FALSE	423	74.7	22.3	Baseline	
TRUE	143	25.3	21.2	-0.078	0.429
Finisher Houses Brushed Clean					
FALSE	489	86.4	22.0	Baseline	
TRUE	77	13.6	22.8	0.039	0.751
Finisher Houses Washed With Hose					
FALSE	551	97.3	22.2	Baseline	
TRUE	15	2.7	19.7	0.115	0.670
Finisher Houses Pressure Washed					
FALSE	173	30.6	22.0	Baseline	
TRUE	393	69.4	22.1	0.034	0.706
Finisher Houses Steam Cleaned					
FALSE	508	89.8	21.9		
TRUE	58	10.2	23.3	0.145	0.292
Do Pig Deliveries Unload At Gate					
NA	31	5.5	16.5	-0.302	0.118
Always	179	31.6	21.9	Baseline	
Sometimes	20	3.5	39.4	0.580	0.012
Rarely	15	2.7	28.0	0.177	0.531
Never	313	55.3	21.2	-0.023	0.799
Do Feed Deliveries Unload At Gate					
NA	3	0.5	42.2	0.833	0.138
Always	149	26.3	18.9	Baseline	
Sometimes	26	4.6	28.6	0.197	0.356
Rarely	14	2.5	20.5	-0.158	0.571
Never	369	65.2	23.2	0.160	0.098

Are Facilities to Disinfect Wheels Present					
NA	2	0.4	40.0	1.052	0.122
No	294	51.9	22.3	Baseline	
Yes	268	47.3	21.4	-0.042	0.619
How Frequently Do Drivers Use Them					
NA	296	52.3	22.5	0.064	0.638
Always	69	12.2	24.9	Baseline	
Sometimes	98	17.3	24.8	0.225	0.156
Rarely	85	15.0	13.0	-0.332	0.042
Never	16	2.8	24.5	0.575	0.032
How Frequently Are Boot Dips Used					
NA	55	9.7	20.8	-0.079	0.590
Always	231	40.8	22.4	Baseline	
Sometimes	183	32.3	22.5	0.068	0.491
Rarely	62	11.0	21.1	0.179	0.216
Never	29	5.1	20.8	0.006	0.976
Where Are Boot Dips Present					
All buildings	164	29.0	23.3	Baseline	
Entrance	266	47.0	21.5	-0.034	0.730
Entrance & All buildings	48	8.5	25.2	0.113	0.484
Entrance & Some buildings	1	0.2	2.9	-1.417	0.142
Some buildings	5	0.9	20.5	-0.030	0.946
None	74	13.1	20.1	-0.149	0.279
How Frequently Are Boot Dips Changed					
Not Applicable	74	13.1	20.1	-0.027	0.931
Daily	12	2.1	13.2	Baseline	
Weekly	266	47.0	22.8	0.064	0.824
Less Than Monthly	40	7.1	22.1	-0.002	0.995
Monthly	167	29.5	22.5	0.206	0.484
Are Boot Dips Protected From The Rain					
NA	74	13.1	20.1	-0.167	0.181
No	379	67.0	23.2	Baseline	
Yes	106	18.7	20.2	-0.101	0.359
Enzoonotic Pneumonia Diagnosed in the last 12 months					
TRUE	264	46.6	22.7	0.187	0.026
FALSE	302	53.4	21.1	Baseline	
Actinobacillus Diagnosed in the last 12 months					
TRUE	68	12.0	21.0	0.001	0.991
FALSE	498	88.0	22.4	Baseline	
PMWS Diagnosed in the last 12 months					
TRUE	316	55.8	24.5	0.365	<0.001
FALSE	250	44.2	17.5	Baseline	
PRRS Diagnosed in the last 12 months					
TRUE	130	23.0	25.7	0.435	<0.001
FALSE	436	77.0	20.3	Baseline	
Glassers Diagnosed in the last 12 months					
TRUE	93	16.4	26.2	0.316	0.005
FALSE	473	83.6	20.9	Baseline	
Streptococcus Diagnosed in the last 12 months					
TRUE	140	24.7	20.1	-0.036	0.712
FALSE	426	75.3	22.8	Baseline	
Coccidiosis Diagnosed in the last 12 months					

TRUE	57	10.1	21.3	-0.023	0.868
FALSE	509	89.9	22.2	Baseline	
Swine Dysentery Diagnosed in the last 12 months					
TRUE	31	5.5	15.6	-0.364	0.049
FALSE	535	94.5	22.4	Baseline	
Enteric Parasites Diagnosed in the last 12 months					
TRUE	31	5.5	25.4	0.025	0.891
FALSE	535	94.5	21.8	Baseline	
PDNS Diagnosed in the last 12 months					
TRUE	200	35.3	24.1	0.157	0.072
FALSE	366	64.7	20.5	Baseline	
Clinical Salmonellosis Diagnosed in the last 12 months					
TRUE	38	6.7	34.1	0.562	0.001
FALSE	528	93.3	21.1	Baseline	
No Health Status Recorded					
TRUE	121	21.4	17.3	-0.318	0.002
FALSE	445	78.6	22.8	Baseline	
Primary Cause of Deaths In The last year					
Farrowing	6	1.1	5.4	Baseline	
Internal Physical	10	1.8	16.8	0.498	0.366
Lameness	23	4.1	23.3	0.858	0.095
Missing	5	0.9	14.8	0.429	0.502
Nervous disease	41	7.2	19.1	0.871	0.076
Respiratory disease	80	14.1	25.1	1.050	0.028
Scour	52	9.2	16.3	0.602	0.214
Skin Disease	1	0.2	0.0	-0.802	0.441
sudden death	128	22.6	16.2	0.497	0.294
Vice	12	2.1	13.8	0.471	0.381
Wasting	186	32.9	26.5	1.111	0.019
Primary cause of deaths - respiratory or wasting					
no	278	49.1	16.8	Baseline	
yes	266	47.0	26.0	0.510	<0.001
Primary Cause Of Antibiotic Treatment In last year					
Farrowing	6	1.1	12.9	Baseline	
internal physical	1	0.2	31.2	0.860	0.410
Lameness	60	10.6	23.1	0.401	0.360
Missing	3	0.5	20.7	0.604	0.383
Nervous disease	47	8.3	15.8	0.386	0.386
None	22	3.9	18.5	0.262	0.577
Respiratory disease	169	29.9	23.7	0.555	0.193
Scour	120	21.2	18.8	0.291	0.497
skin Disease	3	0.5	3.3	-0.314	0.652
Sudden death	3	0.5	9.2	-0.675	0.330
Vice	9	1.6	11.1	0.061	0.909
Wasting	53	9.4	26.5	0.665	0.131
Finishers Housed On Fully Slatted Floor					
No	386	68.2	21.5	Baseline	
Yes	138	24.4	21.5	-0.085	0.371
Finishers Housed On Partly Slatted Floor					
No	415	73.3	22.3	Baseline	
Yes	109	19.3	19.5	-0.179	0.081
Finishers Housed On Bedding					

No	198	35.0	19.3	Baseline	
Yes	326	57.6	23.3	0.109	0.210
Finishers Housed On Solid Floor					
No	275	48.6	17.2	Baseline	
Yes	249	44.0	26.5	0.462	<0.001
All Stages Of Production Indoors					
No	502	88.7	21.5	Baseline	
Yes	40	7.1	27.5	0.254	0.115
Number of feed deliveries a year					
2-5	6	1.1	16.7	Baseline	
6-11	28	4.9	18.1	-0.810	0.096
never	8	1.4	13.0	-0.591	0.292
over 11	517	91.3	22.4	-0.220	0.625
Vet visits a year					
1	6	1.1	12.4	Baseline	
2-5	458	80.9	21.5	-0.043	0.914
6-11	75	13.3	24.6	0.151	0.713
over 11	20	3.5	29.3	0.264	0.556
Pig deliveries a year					
0-5	343	60.6	18.1	Baseline	
6-11	83	14.7	28.8	0.702	<0.001
over 11	132	23.3	26.0	0.483	<0.001
Live pig collections a year					
0	21	3.7	14.3	Baseline	
1-5	21	3.7	31.0	0.814	0.001
6-11	74	13.1	22.9	0.626	0.009
over 11	442	78.1	21.9	0.570	0.007
Dead stock collections a year					
0-5	114	20.1	16.7	Baseline	
>6	442	78.1	23.6	0.380	<0.001
Number of vermin controller visits					
0	237	41.9	21.5	Baseline	
>0	295	52.1	23.2	0.193	0.026
Any other visitors					
no	460	81.3	23.2	Baseline	
yes	106	18.7	16.8	-0.331	0.002
Number of auditor visits a year					
0	564	99.6	22.1	Baseline	
1	2	0.4	23.8	-0.044	0.948
Number of farm inspector visits a year					
0	543	95.9	22.3	Baseline	
1	16	2.8	21.0	-0.049	0.843
2-5	7	1.2	12.8	-0.480	0.192
Number of other deliveries					
0-11	564	99.6	22.3	Baseline	
over 11	2	0.4	2.4	-1.512	0.026
Number of herdman/other staff visits a year					
0-5	509	89.9	22.5	Baseline	
>6	50	8.8	17.2	-0.246	0.110
missing	7	1.2	9.6	-0.604	0.103
Number of machine loan visits a year					
0	565	99.8	22.1	Baseline	

2-5	1	0.2	0.0	-1.131	0.243
Number of pig specialist visits a year					
0-5	562	99.3	22.2	Baseline	
>6	4	0.7	5.1	-0.920	0.057
Number of visits of general public a year					
0	561	99.1	22.2	Baseline	
1	2	0.4	12.3	-0.436	0.522
2-5	1	0.2	31.3	0.609	0.530
missing	2	0.4	21.7	0.231	0.735
Number of school/ student visits a year					
0	563	99.5	22.1	Baseline	
2-5	1	0.2	0.0	-1.694	0.079
6-11	1	0.2	7.3	-0.680	0.480
over 11	1	0.2	39.1	1.166	0.229
Slurry/ bedding collections a year					
0	565	99.8	22.0	Baseline	
2-5	1	0.2	47.8	1.109	0.249
Tradesman visits a year					
0	556	98.2	22.1	Baseline	
1	1	0.2	0.8	-1.310	0.175
2-5	6	1.1	22.8	-0.216	0.584
6-11	1	0.2	1.6	-1.012	0.294
over 11	2	0.4	29.2	0.483	0.478
Continuous variables					
No. Weaners	280	49.5	-	0.000	0.333
No. Growers	270	47.7	-	0.000	0.179
No. Finishers	469	82.9	-	0.000	0.184
No. Adult pigs	249	44.0	-	0.000	0.720
No. Combined Weaners & Growers	18	3.2	-	0.000	0.343
No. Combined Growers & Finishers	31	5.5	-	0.000	0.889
Total No. Of Pigs (questionnaire data)	565	99.8	-	0.000	0.263
No. of sows (QAS data)	438	77.4	-	0.000	0.745
Log sows (QAS data)	438	77.4	-	-0.036	0.030
No. of finishers (QAS data)	438	77.4	-	0.000	0.901
Log. Finishers (QAS data)	438	77.4	-	0.023	0.398
Total number of pigs (QAS data)	438	77.4	-	0.000	0.920
Log. Total (QAS data)	438	77.4	-	0.022	0.474
Number of cattle Currently Present	164	29.0	-	-0.001	0.019
Number of sheep Currently Present	122	21.6	-	0.000	0.228
Number of goats Currently Present	7	1.2	-	-0.008	0.868
Number of poultry Currently Present	103	18.2	-	0.000	0.601
Number of equine Currently Present	97	17.1	-	-0.003	0.731
Number of cats Currently Present	9	1.6	-	0.053	0.442
Number of dogs Currently Present	14	2.5	-	0.060	0.604
Number of camelids Currently Present	1	0.2	-	-0.351	0.275
Number of other Birds Currently Present	5	0.9	-	0.000	0.991
Weaners - Percentage Of Wheat	152	26.9	-	-0.004	0.063
Weaners - Percentage Of Barley	151	26.7	-	-0.014	<0.001
Growers - Percentage Of Wheat	200	35.3	-	-0.005	0.020
Growers - Percentage Of Barley	200	35.3	-	-0.019	<0.001
Finishers - Percentage Of Wheat	218	38.5	-	-0.002	0.287
Finishers - Percentage Of Barley	223	39.4	-	-0.014	<0.001

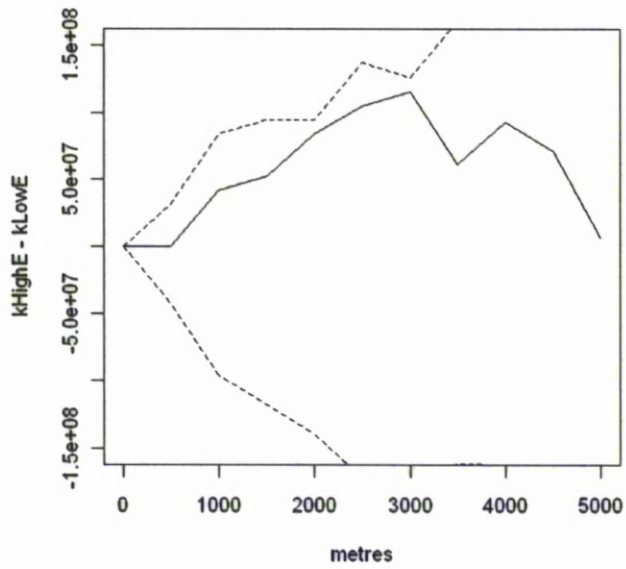
Sows - Percentage Of Wheat		140	24.7	-	-0.006	0.012
Sows - Percentage Of Barley		131	23.1	-	-0.016	<0.001
Number Of Pig Deliveries in last 12 months		401	70.8	-	0.000	0.170
Number Of Pig Farms Within 3 km Radius		554	97.9	-	0.085	<0.001
Number Of Pig Farms Within 10 km Radius		554	97.9	-	0.021	<0.001
How FrequentlyDrinking System Cleaned/Year		533	94.2	-	0.004	0.930
How Frequently Finisher Houses Cleaned/Year		511	90.3	-	-0.033	0.642
How Frequently Finisher Houses Disinfected/Year		514	90.8	-	0.074	0.147
Monthly maximum temperature actual for farm's region (oC)		505	89.2	-	0.001	0.385
Monthly maximum temperature anomaly for farm's region (oC)		505	89.2	-	0.023	<0.001
Monthly minimum temperature actual for farm's region (oC)		505	89.2	-	0.003	0.013
Monthly minimum temperature anomaly for farm's region (oC)		505	89.2	-	0.032	<0.001
Monthly mean temperature actual for farm's region (oC)		505	89.2	-	0.001	0.136
Monthly mean temperature anomaly for farm's region (oC)		505	89.2	-	0.031	<0.001
Monthly rainfall actual for farm's region (mm)		505	89.2	-	0.001	<0.001
Monthly rainfall anomaly for farm's region (mm)		505	89.2	-	<0.001	0.004
Monthly sunshine actual for farm's region (hours)		505	89.2	-	<0.001	<0.001
Monthly sunshine anomaly for farm's region (hours)		505	89.2	-	0.001	<0.001
Quarterly temporal cycle						
	Cos	566	100.0	-	-0.051	<0.001
	Sin	566	100.0	-	-0.038	<0.001
Yearly temporal cycle						
	Cos	566	100.0	-	-0.070	<0.001
	Sin	566	100.0	-	0.060	<0.001

NA = not applicable.

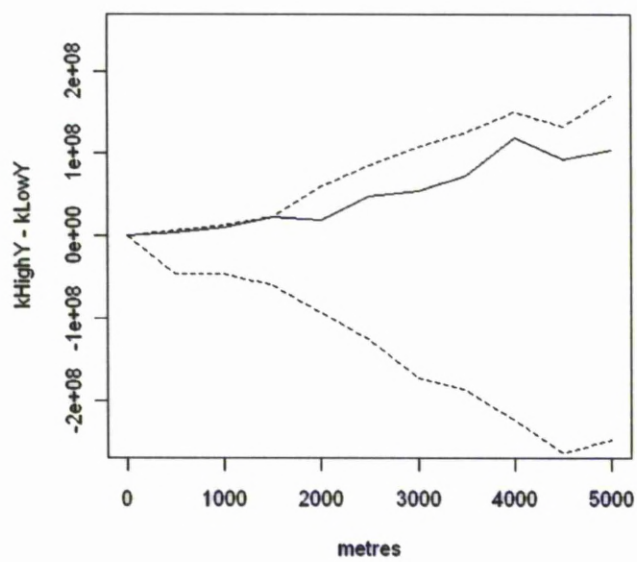
Chapter 4

Appendix A: Regional K-function plots

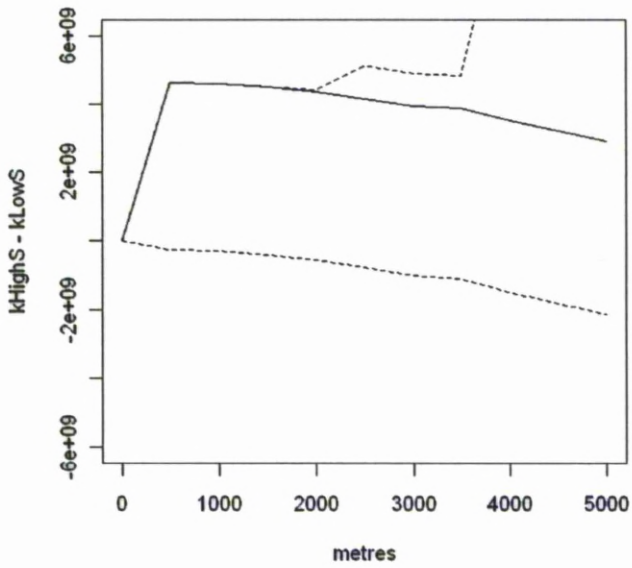
East England



Yorkshire and the Humber



Scotland


kHighS - kLowS
6e+09
2e+09
-2e+09
-6e+09
0
1000
2000
3000
4000
5000
metres