Control of *Salmonella* infection in pigs at the farm level in Great Britain

A thesis submitted for the Degree of Doctor of Philosophy

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by

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Declaration

I, Alasdair James Charles Cook, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed

Date: 04 April 2014

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Many colleagues from AHVLA and elsewhere contributed to the research studies and it is impossible to name them all. However, special thanks go to Rob Davies, Ian McLaren and the team in the Department of Bacteriology. Within the former Centre for Epidemiology and Risk Assessment, Sophie Pascoe, Sandy Miller, Eli Marier, Zoe Chapman, Renee Sheehan, Richard Smith, Julia O'Connor, Charlie Byrne, Mark Arnold, Alberto Vidal-Diez, Emma Snary and Andy Hill deserve my gratitude. Throughout this period, I was also fortunate to have the support of Derek Armstrong and the British Pig Executive for the research studies, which I very much appreciated. The work on farms and in abattoirs would have been impossible without the kind co-operation of the many farmers and managers involved.

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Statement of Contribution

The research reported in this thesis would not have been possible without important contributions from others. I have benefitted throughout from advice and feedback from my supervisor, Prof Simon Cousens. The further contributions from others are described with reference to each Chapter below:

Chapter 1

This is entirely my own work.

Chapter 2

The laboratory tests performed as part of this research and reported in chapters 3, 4 and 6-8 were conducted by colleagues at the Animal Health and Veterinary Laboratories Agency (AHVLA) Weybridge (formerly VLA Weybridge) and AHVLA Bury St. Edmunds. I am especially indebted to Rob Davies, Ian McLaren, Sue Bedford and Mark Breslin in Weybridge and to Peter Heath in Bury St. Edmunds for their expertise and assistance.

Chapter 3

The data for this analysis were derived from a UK abattoir survey of *Salmonella* in pigs which was conducted as part of a European Union (EU) baseline survey. I was a member of the European Food Safety Agency (EFSA) working group that recommended the survey design to the EU and of a second EFSA working group established to analyse and report on the survey results. I was the project leader responsible for the implementation of the survey in UK, which was managed on a day to day basis by Elizabeth Marier (CERA-VLA). Samples were collected by staff from the Meat Hygiene Service and laboratory tests were conducted by staff in VLA's National Reference Laboratory for *Salmonella* under the leadership of Dr. Rob Davies and colleagues (see Chapter 2 above). I conducted the Bayesian analysis with supervision from Mark Arnold (CERA-VLA). We applied a model based on Branscum et al (2005 (1) using their computer code downloaded from a public domain website. Alberto Vidal-Diez (CERA-VLA) provided additional assistance in the use of WinBugs (http://www.mrc-bsu.cam.ac.uk/bugs/winbugs)

and BetaBuster (http://www.epi.ucdavis.edu/diagnostictests/betabuster.html) software. Funding was provided by Defra and FSA.

Chapter 4

This survey of GB finisher farms was funded by Defra. I was responsible for the design of the project and was the project leader during its implementation. Recruitment was assisted by the British Pig Executive (BPEx) and I am especially grateful to Derek Armstrong MRCVS for his support. Sophie Pascoe, Alexander Miller and Elizabeth Marier (CERA-VLA) managed the project and the project database. Samples were collected from participating farms by their own private veterinary surgeons, paid for(1) by the project. Meat juice samples were either collected by Meat Hygiene Service staff, or on a limited number of occasions, by the private veterinary surgeon. Dr Rob Davies and colleagues (see Chapter 2 above) undertook the microbiological testing at the National Salmonella Reference Laboratory in Weybridge and Peter Heath and colleagues conducted the MJ ELISA test at AHVLA Bury St. Edmunds.

Chapter 5

This work was funded by Defra. Data were provided by BPEx and Assured British Pigs (ABP); I am again very grateful to Derek Armstrong and also to Marcus Woods of ABP. The combined dataset described in this chapter was created with assistance from the data management team at CERA-VLA and I am especially grateful to Charles Byrne for his help. I was responsible for the data analysis that is reported here.

Chapters 6-8

These Chapters report the outcome of a longitudinal intervention study, which was funded by Defra. I was responsible for the design of the study and I was the project leader during its implementation. I was ably assisted by Zoe Chapman, Elizabeth Marier and Renee Sheehan as project managers. The database was created and managed by colleagues in CERA-VLA, including Alexander Miller and Charles Byrne. Many colleagues from VLA Weybridge and from the VLA's Regional Laboratories assisted in abattoir sample collection. Dr Rob Davies and colleagues undertook the microbiological testing at the National Salmonella

Reference Laboratory in Weybridge and Peter Heath and colleagues conducted the MJ ELISA test at AHVLA Bury St. Edmunds (see Chapter 2 above). BPEx once again provided considerable support for the project. Professor Martin Woodward kindly provided feedback on the text in Chapter 6.

Chapter 9

This was entirely my own work.

1. Branscum AJ, Gardner IA, Johnson WO. Estimation of Diagnostic-Test Sensitivity and Specificity through Bayesian Modeling. Preventive Veterinary Medicine. 2005;68(2-4):145-163.

List of Publications and Presentations

The work described in Chapters 2, 6, 7 and 8 was funded by Defra in a project entitled “Epidemiological studies of *Salmonella* in pigs and control by intervention – OZ0316”. The project final report can be accessed at:

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=9747&FromSearch=Y&Publisher=1&SearchText=oz0316&SortString=ProjectCode&SortOrder=Asc&Paging=10

The work described in Chapter 4 was funded by Defra in a project entitled “Use of routine data to investigate risk factors for Salmonella infection in pigs – FZ2014”

The project final report can be accessed at:

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=9747&FromSearch=Y&Publisher=1&SearchText=fz2014&SortString=ProjectCode&SortOrder=Asc&Paging=10

Peer-Reviewed Papers Associated with this thesis

The following papers made use of information derived from data collected in the research reported in this thesis.

Author	Title		Journal/publication
Ivanek R., Snary E., Cook, A.J.C, Grohn Y.T.	A mathematical model for the transmission of Salmonella typhimurium within a Grower-Finisher pig herd in Great Britain.	2004	Journal of food protection 67 (11), 2403-2409.
Arnold M.E., Cook, A.J.C, Davies, R.H.	A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of salmonella in pigs.	2005	Journal of the Royal Society Interface 2, 365-372/
Hill A., Snary E., Arnold M.E., Alban L., Cook, A.J.C	Dynamics of salmonella transmission on a British pig grower-finisher farm: a stochastic model.	2007	Epidemiology and Infection, Volume 136, 320-333

Clough, H.E., Fenton S.E.; French;N.P. Miller, A.J. & Cook, A.J.C.	Evidence from the UK Zoonoses Action Plan in favour of localised anomalies of *Salmonella* infection on UK pig farms.	2009	Preventive Veterinary Medicine 89 67-74
Arnold, M.E. & Cook, A.J.C.	Estimation of Sample Sizes for Pooled Faecal Sampling for Detection of *Salmonella* in Pigs.	2009	Epidemiology & Infection 137: 1734-1741
Fraser, R.W., Williams, N.T., Powell, L.F. & Cook, A.J.C.	Reducing Campylobacter and Salmonella Infection: Two Studies of the Economic Cost and Attitude to Adoption of On-farm Biosecurity Measures.	2010	Zoonoses & Public Health 57 E109-E105 DOI: **10.1111/j.1863-2378.2009.01295.x**
Van Dam, Y., Frewer, L.J., Marier, E., Armstrong, D. & Cook, A.J.C.	Barriers to adoption of measures to control Salmonella in pigs in the UK: A stakeholder analysis.	2010	The Pig Journal 63, 50-58
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	2010	Zoonoses & Public Health 57 39-48 DOI: 10.1111/j.1863-2378.2010.01362.x
Snary, E.L.; Munday, D.K.; Arnold, M.E. & Cook, A.J.C.	Zoonoses Action Plan *Salmonella* Monitoring Programme: An Investigation of the Sampling Protocol.	2010	Journal of Food Protection 73, 488-494

Scientific Conferences and external seminars

Author	Title	Date	Venue
Cook, A.J.C, Davies, R.H, Miller, A.J., Gopal, R. Byrne, C. Heath, P.J. Cousens, S. & Pascoe, S.J.S.	Monitoring salmonella infections on pig farms in GB by meat juice ELISA and culture of pen faecal samples	Nov 03	10th ISVEE Conference
Cook, A.J.C, Davies, R.H, Evans, S.	Salmonella in pigs- towards control	May 01	Presentation to PigVetSoc
Cook, A.J.C.	Contribution to a joint VLA-IAH meeting	May 03	VLA Weybridge
Cook, A.J.C.	Salmonella in pigs-data sources and control programmes in UK	May 03	Safood chain meeting, Switzerland
Cook, A.J.C., Davies,R.H, Miller, A.J. , Gopal, R., Byrne, C. Heath, P.J. & Cousens, S.	A randomised controlled trial to reduce salmonella infection in finisher pigs	Oct 03	5th International Symposium on the Epidemiology and control of zoonotic pathogens in pork.
Cook, A.J.C	Measuring the impact of salmonella control in finishing pigs- lessons from a pilot study	Nov 03	Presentation to PigVetSoc Pig Journal **53** 157-163

Cook, A.J.C, Davies, R.H, Miller, A.J. , Gopal, R. Byrne, C. Heath, P.J. & Cousens, S.	A pilot randomised trial of an enhanced hygiene and biosecurity programme to control salmonella infection in a group of pig finisher units in Great Britain.	Nov. 2003	10th International Society for Veterinary Epidemiology and Economics Conference, Vina del Mar, Chile.
Cook, A.J.C	ZAP Update	Jan 04	NPA-TASC, National pig association meeting
Cook, A.J.C	Strategies to control Salmonella in pigs	Feb 04	LSHTM seminar
Cook, A.J.C	Salmonellas in animals- recent trends and future threats	Feb 04	South, West and Mid Wales Zoonoses Liaison Group Annual Meeting VLA, Carmarthen
Cook, A.J.C, Miller, A.J. & McLeod, A.	Identifying the costs of a hygiene and biosecurity intervention to control salmonella infection on pig farms in UK- a pilot study	Mar 04	SVEPM conference
Cook, A.J.C	Zapping salmonella in British pigs	Jul 04	World meat inspection and hygiene conference, Cambridge
Davies R.H, Cook, A.J.C	The UK ZAP salmonella scheme for pig meat production	Sep 04	HPA conference, Warwick
Evans, S. Cook, A.J.C., Twomey, D.F., Milnes, A.	Ongoing and future salmonella surveillance and research	Nov 04	Food Borne pathogens in cattle, sheep and pigs in Great Britain – Conference at VLA
O'Connor, J. Snow, L. & Cook, A.J.C	An on farm trial investigating the effect of organic acids in weaner pigs diets on salmonella from UK finisher pig farms.	Mar 05	AVTRW Conference

Cook, A.J.C	Zoonoses action plan- the development of UK food protection policy	Jun 05	HPA Zoonoses conference, Liverpool
Cook, A.J.C, Miller, A.J. Snow, L. & Davies, R.H	Epidemiological studies of salmonella in pigs	Jun 05	MedVetNet 1st General scientific meeting Winchester
Hill, A.,Snary, E., Davies, R.H & Cook, A.J.C.	A transmission model for salmonella in grower-finisher pigs	Sep 05	6th International Symposium on the Epidemiology and control of foodborne pathogens in pork.
Cook, A.J.C	Risk factors for a positive meat juice ELISA result- An analysis of routine data from Britain.	Sep 05	6th International Symposium on the Epidemiology and control of foodborne pathogens in pork.
Cook, A.J.C	Optimization of Pooled Faecal Samples For the isolation of Salmonella from Finisher pigs in GB	Sep 05	6th International Symposium on the Epidemiology and control of foodbourne pathogens in pork.
Cook, A.J.C	HPA-VLA collaboration in Epidemiology	Dec 05	HPA-VLA Liason Meeting
Snary, E., Hill, A. & Cook, A.J.C.	An integrated risk based approach to the control of salmonella in the UK	Sept 06	COST 920 Conference. Future Challenges to Foodborne Zoonosis.
Cook, A.J.C, Arnold, M.E. & Davies, R.H	An estimation of the sensitivity of pooled faecal samples for the detection of salmonella spp in pigs	Oct 06	CRL Epidemiology workshop

Cook, A.J.C, Williamson, S. Miller, A.J. O'Connor, J Twomey, D.F., Marier, E; Snow, L, Davies, R.H, & Featherstone, C.	Investigation the ZAP Level 2 or 3 farm- case studies	Mar 07	Pig Vet Society conference The Pig Journal **58** pp 190-203
Twomey D.F., Cook A.J.C., Miller A.J., Armstrong J.D., Gayford P.J.R. & Howell, M.V.	The Zoonoses Action Plan for the Control of Salmonella in Finishing Pigs.	2007	Government Veterinary Journal **17,** 28-31

Meetings with industry and communication

Author	Title	Date	Venue/publication
Cook, A.J.C	Summary results of the observational study at Site 1	Feb 02	farmers meeting Regional Veterinary Laboratory at Bury St Edmunds
Cook, A.J.C	Presentation at the National pig-poultry fair	May 02	Stoneleigh
Cook, A.J.C	Salmonella in UK pigs	Oct 02	Frank Wright LTD/BASF conference
Anon	Mentioned in Farmers Weekly	Nov 02	Farmers Weekly pg 41
Cook, A.J.C. & Miller, A.J.	Letter in Veterinary Record	Nov 02	Veterinary Record pg 643
Cook, A.J.C.	Intervention group feedback	Dec 02	Farmers meeting Bury St Edmunds regional laboratory
Cook, A.J.C. & Miller, A.J.	Salmonella: a pilot intervention study	Feb 03	Farmers meeting Bury St Edmunds
Anon	Mentioned in Farmers Weekly	Feb 03	Farmers Weekly p45
Anon	Mentioned in Farmers Guardian	Feb 03	Farmers Guardian pg 89
Cook, A.J.C	Salmonella and pigs tests and control	Jul 03	Darlington Pig discussion group
Cook, A.J.C	Salmonella and pigs, an update	Feb 04	Stotfold pig discussion group

Cook, A.J.C. & Papadopoulou, C.	Effects of different management options on health and productivity for replacement gilts	Nov 04	Joint conference- SFT/MLC/HGCA Nutrition and management for improving pig health and productivity.
Cook, A.J.C.	How producers can discover the secrets of reducing salmonella in batch-finished pigs	Mar 05	The Pig World magazine
Marier, E; Miller, A.J. & Cook, A.J.C.	Recent VLA work on pig salmonella	May 05	MLC's ZAP update brochure
Anon	Research to find effective controls for salmonella in pigs	May 05	MLC TechTalk newsletter
Cook, A.J.C.	More producers needed for pig salmonella study	Jun 05	The Pig World magazine
Cook, A.J.C.	Monitoring control of salmonella infection in pigs- a GB perspective.	Nov 05	Italian national Swine Industry Meeting in Soave.

Internal VLA and DEFRA

Author	Title	Date	Venue/publication
Cook, A.J.C.	Salmonella in pigs- an update	May 01	To VLA pig group
Cook, A.J.C.	Epidemiological studies of salmonella in pigs and control by intervention	Feb 02	DEFRA FES science review
Miller, A.J. & Cook, A.J.C.	Epidemiology of salmonella on pigs farms National study	Sept 03	VLA, CERA seminar
Cook, A.J.C. and Miller, A.J.	Project workshop- Interventions to control salmonella in finishing pigs	Nov 03	DEFRA London

Cook, A.J.C.	Bullet points for FES science highlights OZ0316- salmonella in pigs study		FES Programme Science Highlights reports
Cook, A.J.C.	ZAP update	Jan 04	FES programme meeting
Cook, A.J.C.	ZAP3 support visits and research activities at VLA	May 04 - Feb 07	ZAP Salmonella programme management steering group meetings.
Snow, L. O'Connor, J. & Cook, A.J.C.	An on farm trial investigating the effect of organic acids in weaner pigs' diets on salmonella prevalence in finisher pigs	Jul 05	Final report
Cook, A.J.C.	The VLA's involvement in ZAP and views on changes	Jan 07	ZAP review, DEFRA Page Street.
Miller, A.J. & Cook, A.J.C.	Introduction to projects OZ0316 and OZ0323	Feb 07	FES meeting, VLA

Abbreviations

^{o}C	Degrees centigrade
95% CI	95% Confidence Interval
ABPigs	Assured British Pigs
AHVLA	Animal Health and Veterinary Laboratories Agency
Approx.	Approximately
BPEx	British Pig Executive
BPW	Buffered Peptone Water
BQAP	British Quality-Assured Pork
C&D	Cleaning and Disinfection
CC	Caecal Content
CERA-VLA	Centre for Epidemiology & Risk Analysis, Veterinary Laboratories Agency
Ci	Confidence Interval
CovDp	Covariance Dependence
CPH	County-Parish-Holding number
Cri	Credible Interval
CS	Carcass Swab
deff	Design Effect
Defra	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Agency
e.g.	For example
ELISA	Enzyme-linked immunosorbent assay
ES	Effect Size
EU	European Union
EU MS	European Union Member State
FSA	Food Standards Agency
GB	Great Britain
GEE	Generalised Estimating Equations
h	Hours
HBS	Hygiene and Biosecurity Score
IMS	Immunomagnetic separation

IRR	Incidence Rate Ratio
LN	Lymph Node
LPS	Lipopolysaccharide
MCMC	Markov Chain Monte Carlo Simulation
MHS	Mean Hygiene Score
MJ	Meat Juice
MJE	Meat Juice ELISA
MJ10	Meat Juice ELISA test cut-off is s:p ratio of 0.10
MJ25	Meat Juice ELISA test cut-off is s:p ratio of 0.25
MLC	Meat and Livestock Commission
MLVA	Multi-locus variable number tandem repeat analysis
MLST	Multi-locus sequence typing
MSRV	Modified Semi Solid Rappaport Vassiliadis medium
NPA	National Pig Association
OD	Optical Density
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCV2	Porcine circovirus type 2
PDNS	Porcine Dermatitis and Nephropathy Syndrome
PFGE	Pulsed-Field Gel Electrophoresis
PMWS	Porcine multi-systemic wasting syndrome
PRRS	Porcine Reproductive and Respiratory Syndrome
PVS	Private Veterinary Surgeon
RV	Rappaport – Vassiliadis media
QA	Quality Assurance
QMS	Quality Meats Scotland
Secc	Sensitivity of caecal content culture
Secs	Sensitivity of carcass swab culture
Seln	Sensitivity of lymph node culture
Semj	Sensitivity of MJ ELISA
SOP	Standard Operating Procedure
Spmj	Specificity of MJ ELISA
s:p	Sample:Positive ratio
USA	United States of America

UK	United Kingdom
VLA	Veterinary Laboratories Agency
VNTR	Variable Number Tandem Repeat
ZAP	Zoonoses Action Plan
ZNCP	Zoonoses National Control Plan

Abstract

Salmonella is an important zoonotic pathogen and 10,000 cases of human salmonellosis are reported annually in the UK. The most commonly implicated serovars are *S*. Enteritidis and *S*. Typhimurium. Since a quarter of British pigs carry *Salmonella* in their gut at slaughter, there is an urgent requirement for improved control strategies that could benefit human health. A literature review showed that hygiene, biosecurity and feed exposures were important risk factors for *Salmonella* infection in pigs, which originates from environmental contamination or introducing infected pigs into the herd. The aim of this research was to design and test an intervention to control *Salmonella* in pigs. The following objectives were achieved:

1. An evaluation of tests for *Salmonella* in pigs: isolation by culture and the meat juice (MJ) ELISA, to inform test selection for the intervention study.
2. A national farm-level survey to estimate the variation in *Salmonella* prevalence between farms and to investigate risk factors associated with infection.
3. An analysis of a merged MJ ELISA dataset with a quality assurance dataset to provide additional information on risk factors.
4. A randomised controlled trial of an enhanced hygiene and biosecurity protocol intended to control *Salmonella* infection in finisher pigs.

The intervention was tested on 48 farms. The primary outcome was the pen incidence rate of *Salmonella* infection, measured by culture of pooled pen floor faecal samples. No important change in incidence between intervention and comparison groups was seen. Analysis by reported behaviour showed that improved attention to between-batch cleaning and disinfection was beneficial. The prevalence of infected pens shortly after re-stocking had an overwhelming effect on incidence whilst improved hygiene during production had relatively little effect. Therefore, enhanced hygiene and biosecurity may yield benefits in *Salmonella* control, but these may be overwhelmed by the introduction of infection at re-stocking or through residual environmental contamination.

Contents

List of Tables

List of figures

Chapter 1: Introduction and Literature review

There are estimated to be nearly 94 million cases of nontyphoidal *Salmonella* gastroenteritis in the world every year, with up to 300,000 deaths and more than 80% of these cases are foodborne (1). These cases are an important component of the overall burden of diarrhoea in older children, adolescents and adults (2).

Salmonella bacteria have a wide host range and may be found in the intestinal tract of all animals. Although some serovars are host-adapted, many are capable of infecting a wide range of animals, including man. Infected animals excrete *Salmonella* in their faeces, leading to environmental contamination and new infections occur mainly following ingestion of contaminated materials, including food. Infection may be asymptomatic or may lead to a spectrum of clinical signs varying from mild enteritis to septicaemia and death. Different serovars vary in their pathogenicity and this may be mitigated or exacerbated by host factors including intercurrent infection or disease, age and immune status. The control of *Salmonella* infection in animals can make a significant contribution to the alleviation of the global burden of human salmonellosis.

In 2012, there were approximately 9,000 laboratory confirmed cases of human salmonellosis in the United Kingdom (UK) (3). However, a study of infectious intestinal disease in England concluded that only one third of cases in the community were actually reported (4), so the total number of cases is approximately 50,000 (3). Whilst the incidence of salmonellosis has declined, further reductions are necessary if deaths from foodborne infection are to be avoided (5). Across Europe, there are some 100,000 reported cases (6). Although most of these cases are sporadic and the source of the infection is unknown, outbreaks occur regularly and are often foodborne. *Salmonella* Enteritidis is particularly associated with poultry and *S.* Typhimurium has been associated with a wide range of foods including beef, dairy products, pork, lamb, poultry and non-animal sources including herbs and spices (7). The predominant serovar found in pigs varies across the European Union (EU) and the prevalence amongst EU member states (MS) also varies from negligible in Sweden to high in

countries including UK and Spain (8, 9). A quantitative microbial risk assessment (QMRA) predicted that the contribution of pigs to human disease varied amongst groups of EU MS and could be as much as a third of *S*. Typhimurium cases, although the results of these models are subject to very wide confidence intervals (10). In the UK, the cost of a human case of salmonellosis due to *S.* Typhimurium has been estimated as nearly £1100 (11) and therefore, there is also a potential economic motive for policy-makers to promote the control of infection in pigs. Under EU legislation, livestock producers are required to act to safeguard human health. The EU considers that *S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Virchow and *S*. Hadar are the most important serovars with respect to human health (6). Interestingly, *S*. Derby, which is the second most frequent isolate from pigs, is not associated with a substantial human morbidity (9). Following EU baseline surveys (8, 9), there is an expectation that the EU will set targets for the prevalence of *Salmonella* infection in pigs that MSs will be required to achieve, although to date none have been agreed.

In pigs in Great Britain (GB), *Salmonella* infection is seldom associated with clinical salmonellosis, although a wide range of clinical syndromes from enteritis to septicaemia have been reported. Exceptions to this generality include the pig-specific serovar *S*. Choleraesuis and the recently emerging monophasic variant of *S.* Typhimurium 1,4,[5],12:i:- (12). The former is rarely isolated in UK; *S*. Choleraesuis var Kunzendorf has only been isolated once since 2008 (12) whereas monophasic variants of *S*. Typhimurium have become increasingly frequent in UK and Europe (12, 13) and are more frequently associated with clinical disease in weaned pigs. While most infected pigs are asymptomatic, they nevertheless are able to transmit infection to other pigs or other hosts, including humans. The potential contribution of *Salmonella* originating from pigs to human disease was highlighted by a serious outbreak of *S.* Infantis in Denmark in 1994 affecting more than 500 people that was attributed to pigs (14). This stimulated the Danish Pig Industry to invest in a monitoring and control programme (15).

A survey conducted in 1999 – 2000 found that 23% of finisher pigs slaughtered in UK abattoirs carried *Salmonella* in their caecal contents (16) and later surveys found no important change in prevalence (9, 17). The most frequent serovar was

S. Typhimurium, which is the second most frequent cause of human salmonellosis. In 2002, the UK pig industry launched the Zoonoses Action Plan *Salmonella* Monitoring Programme (ZAP). The prevalence of pigs that are positive at slaughter in a meat juice enzyme-linked immunosorbent assay (MJ ELISA) was used to classify participating farms with respect to their *Salmonella* status (18) (7). The aim of ZAP was to reduce the prevalence of *Salmonella* infection in pigs in the UK to protect public health. A similar programme was instigated in Denmark in 1995 and is believed to have contributed to a reduction in human cases of salmonellosis over the following 5 years (19, 20). The ZAP programme was replaced by the Zoonosis National Control programme (ZNCP) in 2008. Monitoring by MJ ELISA continued and the classification system was amended, with those farms that achieved a prevalence of below 10% receiving a "Platinum Pig" award. However, MJ ELISA testing was suspended in July 2012 and ZNCP now focuses on provision of advice on the control of *Salmonella*.

Clearly, the success of such programmes depends upon both the efficacy of the monitoring programme and the availability of acceptable measures to control *Salmonella*. The purpose of the research described in this thesis was to develop and test a farm level intervention to reduce the incidence of *Salmonella* infection within UK finisher pig herds.

Microbiology

The evolution of *Salmonella* bacteria has been traced back over more than 120 million years, prior to the emergence of the first warm-blooded creatures on earth. Ancestral forms are believed to have existed as microparasites of the intestines of cold-blooded reptiles. When new, warm-blooded hosts became available, other related members of the family Enterobacteriaceae including *Escherichia coli* evolved to occupy this ecological niche (21). Some *Salmonella* continued to colonise the intestine of reptiles. However, others became adapted to warm-blooded hosts through the acquisition of genes that mediate invasion of epithelial cells.

Eberth was the first to identify a member of the *Salmonella* genus in 1880, in the tissues of a patient who died from typhoid and Gaffky subsequently isolated *S.*Typhi in 1884. Just two years later, *S.* Choleraesuis was isolated from pigs by Salmon and Smith, who incorrectly believed that it was the cause of Classical Swine Fever (22). Theobald Smith later observed that *Salmonella* could be differentiated from other Enterobacteriaceae by their inability to ferment lactose. In common with other members of the Enterobacteriaceae*, Salmonella* are straight, Gram-negative, non-sporing rods that are often motile, due to their peritrichous flagellae. They are easily cultivable on ordinary laboratory media and grow in aerobic or anaerobic conditions. Optimum growth occurs at 37^{o}C although a wide temperature range (7^{o}C – 48^{o}C) is tolerated. The International Journal of Systematic Bacteriology publishes accepted names for bacterial species. A genomic species is one in which all isolates demonstrate at least 70% DNA relatedness. Under this definition, two species of *Salmonella* are recognised – *Salmonella enterica* and *S. bongori*.

Six sub-species of *S. enterica* are recognised:

1. *S. enterica* subsp *enterica*
2. *S. enterica* subsp *salamae*
3. *S. enterica* subsp *arizonae*
4. *S. enterica* subsp *diarizonae*
5. *S. enterica* subsp *houtenae*
6. *S. enterica* subsp *indica*

Biochemical characteristics are sufficient to identify species and sub-species but further differentiation depends upon serological typing, which is used to identify isolates as serovars. Only serovars of *S. enterica* subsp *enterica* receive names – for example, *S. enterica* subsp *enterica* serovar Typhimurium (22). Full systematic names such as *Salmonella enterica* subsp *enterica* serovar Typhimurium will be expressed as *Salmonella* Typhimurium or *S.* Typhimurium in this thesis, as is common practice in the scientific literature. There are at least 2449 different serovars of *Salmonella* of which at least 1443 are *S. enterica* subsp *enterica* (23).

Salmonella present three classes of antigens that are used for typing (24):

1. “O” antigens – somatic heat stable lipopolysaccharides that have a structural role in the cell wall;
2. “H” antigens – heat labile proteins of the peritrichous flagellae;
3. “Vi” antigens – surface polysaccharides that inhibit agglutination by homologous “O” anti-sera. These antigens are only found in *S.* Typhi, *S.* Paratyphi and *S.* Dublin.

The somatic O antigens comprise a polysaccharide core that is common to all Enterobacteriaceae and a lipopolysaccharide side chain that confers specificity. The O antigens are detected by agglutination tests, in which specific antisera are mixed with suspensions of the organism. Cross-reactions with other enterobacteria are common but can be avoided by absorption of the antisera.

The H antigens of *Salmonella* are normally biphasic – they may exist in one of two distinct antigenic forms. The switch between these forms occurs at a characteristic frequency and can be induced in the laboratory. Each flagellum is composed of proteins known as flagellins. *Salmonella* in phase1 express flagellin of the *fli*C gene whilst in phase2, the flagellin of the *flj*B gene is expressed, mediated by a repressor of the phase1 gene and aided by the *flj*A gene. A small number of serovars are monophasic, existing only in phase1 or phase2. As mentioned previously, these include the emergent monophasic strain of *S*. Typhimurium (13). There are even rare triphasic serovars (25). These antigens are also identified by agglutination tests. In order to fully define a serovar, both phases must be identified. Other classification systems, for example based on the H antigen, have also been proposed (26, 27).

Order was brought to the classification of *Salmonella* by the development of the Kauffmann-White scheme in the 1920s. It has continued to develop and remains in use today. The antigenic formula for each serovar is expressed in terms of the O serogroup, O antigens and Phase1 and Phase2 H antigens. O serogroups are now numbered from 01 – 067, although there are only 46 serogroups, as some bacteria that were initially thought to be *Salmonella* were later re-classified. Previously, some serogroups were designated by capital letters and many people

continue to use this designation. More than 95% of *S. enterica enterica* serovars belong to six O serogroups and for most purposes, serotyping can be conducted using 12 O, 18 H and Vi antisera (28).

The ability of *Salmonella* to colonise warm-blooded hosts is determined by the orchestration of numerous pathogenicity associated genes often located in close proximity on the *Salmonella* genome. The *Salmonella* pathogenicity islands (SPI's) encode the Type III protein secretion systems (T3SSs) which enables *Salmonella* to adhere, invade, replicate and survive in the host cell. The T3SS has been likened to "molecular syringes" that inject bacterial effector proteins into the host cell (29). Following multiplication in the lumen of the intestine, *Salmonella* may use fimbriae and flagellae to adhere to the epithelial surface of the intestine and then penetrate the host cell, forming a *Salmonella*-containing vacuole (SCV). The *Salmonella* can survive and replicate inside the SCV until they infect a neighbouring cell. The T3SS-components are vital to this process in pigs and induce membrane ruffling that favours bacterial uptake into these vacuoles. A distinct T3SS-2 system plays a key role in intracellular survival through its effect on the membrane of the intracellular vacuole and by altering the host immune response. Other SPIs influence adhesion, which varies by host species and thus are involved in conferring host specificity upon serovars and strains (29). These adaptations enable *Salmonella* to avoid competition with other bacteria (22). The proteins encoded by T3SSs also influence the clinical outcome, e.g. by disrupting the gut epithelium and activity of neutrophils engaged in the host immune response and by influencing the ability of the strain to migrate beyond the intestine and cause systemic disease (29). As Stevens *et al*. (2007) conclude "the outcome of infection … is not predetermined but reflects the interaction of multiple host and pathogen processes" (29). For example, the anatomy of the gastrointestinal tract in pigs features characteristically more substantial accumulations of lymphoid tissue than seen on other species. In the spiral colon, these Peyers patches may present with necrosis, forming "button ulcers" that must be distinguished from a similar pathology due to Classical Swine Fever virus. Recently, the role of a large number of genes in the intestinal colonisation of cattle, pigs and chickens in comparison to mice has shown that whilst many genes are necessary in all species, a few are host-specific. This

knowledge may presage development of new approaches to control, including vaccination (30).

For epidemiological purposes, including outbreak investigations, discrimination at a level below the serovar is often necessary. Approaches including phage typing, antimicrobial sensitivity testing, pulsed-field gel electrophoresis (PFGE) (14) or multi-locus variable number tandem repeat (MLVA or VNTR) analysis have frequently been used. In a recent review, 17 different methods were described which are finding increasing favour (31). Amongst these, MALDI-Tof mass spectrometry applied to whole killed organisms has been found to be quick and cheap but insufficiently discriminatory for subspecies typing. Multi-locus sequence typing (MLST), which “compares the sequence of 7 housekeeping genes” has been used to redefine *Salmonella* groups that are equivalent to serovars and is highly reproducible but also lacks the capacity to differentiate within serovars (31).

Some serovars are understood to have a wide host range, e.g. *S.* Typhimurium, whilst others are generally host-specific e.g. *S.* Typhi. However, even within a serovar with a wide host range, there may be some variants which are host-adapted to a greater extent than others (32). For the purposes of this review, consideration will be limited to the level of serovar; this simplification is justified since on farms, even the serovar may be unknown and farm level interventions cannot be variant-specific.

Literature review

A systematic search was conducted to identify the scientific literature relating to the epidemiology of *Salmonella* infection in pigs and its control. The review was restricted to articles in English and published since 1995, as this was the year that Denmark introduced a surveillance and control programme for *Salmonella* in slaughter pigs (15, 33).

Methods

A series of search terms were developed and grouped to search the literature for the following purposes:

1. To identify variables associated with *Salmonella* infection on pig farms. This knowledge can be used to develop control strategies by avoiding factors that increase the risk of infection and encouraging factors that reduce this risk.
2. To identify farm-level studies that have tested intervention strategies against *Salmonella* infection in pigs.

A report on interventions against *Salmonella* was commissioned by the British Pig Executive and published online in 2011 (34). This report was a starting point for this literature review. An earlier review (35) was also consulted although much of the information that it contained has since been superseded. Abattoir interventions and laboratory-based experimental studies, such as artificial challenge experiments, are excluded from this review.

Search terms that were identified for potential risk and protective factors are shown in table 1.1. The following databases were searched: Web of Knowledge, Scopus, Vet CD; Biosis and Cabi International.

Table 1.1: Terms and results from a literature search across 5 databases on risk factors for *Salmonella* infection in pigs and its control.

Search number	Description	Search Terms	Further Search Terms	Number of articles*
1	Animal level	Pig*, porcine, swine, hog, sow, sows, gilt, gilts, finisher*, fattener*	AND NOT pigm* AND NOT pige*	338,574
2	Outcome	Salmonel*		98.282
3	Risk factors	Feed	Feed*, diet, food, nutrition*, particle size, compound, pellet*, meal, liquid	1.8 m
4	Study type	Observational, survey, cross-sectional, epidemiologic*, risk factor		2.4 m
5	Combine 1 & 2			2,621
6	Combine 5 & 3			1,243
7	Combine 6 & 4			264
8		NOT abattoir OR slaughter* OR meat OR carcass		
9	Combine 7 & 8			83
10	Risk Factors	Management	Husbandry, management, biosecurity, hygiene*, clean*, disinfect*,	2.1 m
11		Environmental	outdoor*, indoor*, rat*, rodent*, vermin, bird*, insect*, slat*, floor*, solid floor, bed*, slurry, scrape, housing	1.2 m
12	11 OR 10 AND 9			30
13	Risk factors	Herd	Herd size; herd density, seasonal*	164,687
14	13 & 9			15
15	Intervention	Farm-level	Intervention, control*, eradicat*, eliminat* vaccin*, acid* feed, acid* water, probiotic, prebiotic, immuni*, biosecurity, hygien*, clean*, disinfect*, sanita*, NOT experiment*	7.6 m
16	15 AND 9			108

* Excluding duplicates

The full title, authors, journal and abstract for each article listed in search strings 12, 14 and 16 in table 1.1 were obtained from each database and then the lists were compared. The titles were screened and if these indicated that an article was relevant, then the abstract was read. Experimental studies, e.g. of immunological responses to oral inoculation, or review articles that did not present new data were excluded. The remaining papers were selected for this review. In addition, reference lists from the identified papers were scrutinised in case further materials could be identified.

Results

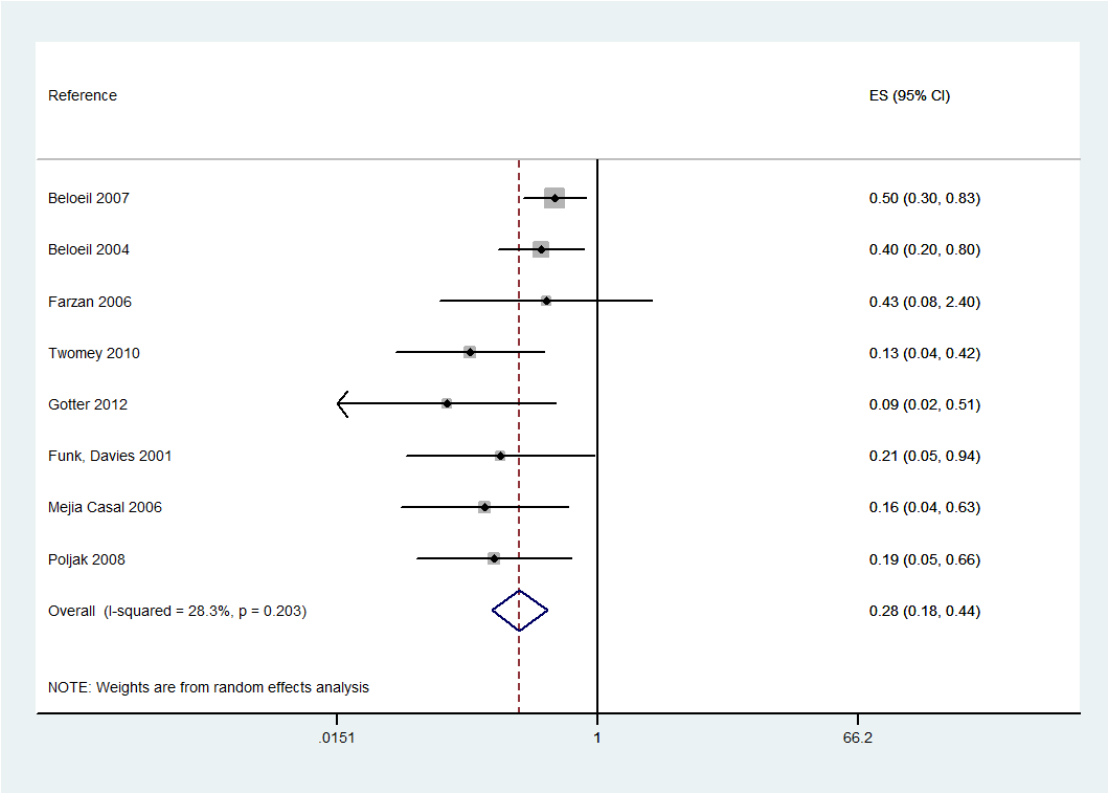
The literature search disclosed 153 papers, once duplicates were excluded. Of these, 15 were laboratory experiments or challenge studies and 11 were reviews or replicates of other papers. The full transcripts of 127 papers were evaluated for this review.

A total of 45 observational studies that consider risk factors for *Salmonella* infection in pigs were identified. Of these, 32 were cross-sectional studies, 8 were longitudinal studies, 1 was an outbreak study and 4 were review articles. Six of the 41 research articles used molecular typing methods to demonstrate shared *Salmonella* strains but did not report on any quantitative measure of association. These found common strains in isolates collected on transport and on farms (36, 37), from feed and from transport delivering feed (38) and from various environmental samples (39, 40). A further study showed that *Salmonella* could be isolated from wild birds on livestock farms, including pig farms, and speculated that these could transmit infection within and between farms (41). One of the review articles supported this suggestion although only through consideration of corroborative evidence (42) whilst a cross-sectional study in Spain did present quantitative evidence of an association (43).

Where quantitative data were presented that either provided an estimate of effect or enabled an estimate to be calculated, then a meta-analysis was undertaken using the "metan" command in Stata (44). Some quantitative papers did not present data that could be incorporated. For example, Baptista et al used a factor analysis and then used logistic regression to explore the association with each

factor. However, the paper only presented the p-value for the association, not the odds ratio (45). Other studies were essentially case reports, where no association could be estimated (38, 40, 46-53). Two papers tested for differences in prevalence of *Salmonella* infection amongst farms with varied management approaches (54, 55) but did not estimate the prevalence odds nor present data amenable to such an estimation. There were sufficient data to conduct a meta-analysis for three themes: biosecurity, feed and hygiene. The results are presented in figures 1.1, 1.2 and 1.3 respectively. Other potential risk factors for which fewer reports were available are summarised and discussed in the text.

Figure 1.1. Forest plot displaying a meta-analysis of the effect of biosecurity practices on *Salmonella* infection in pigs

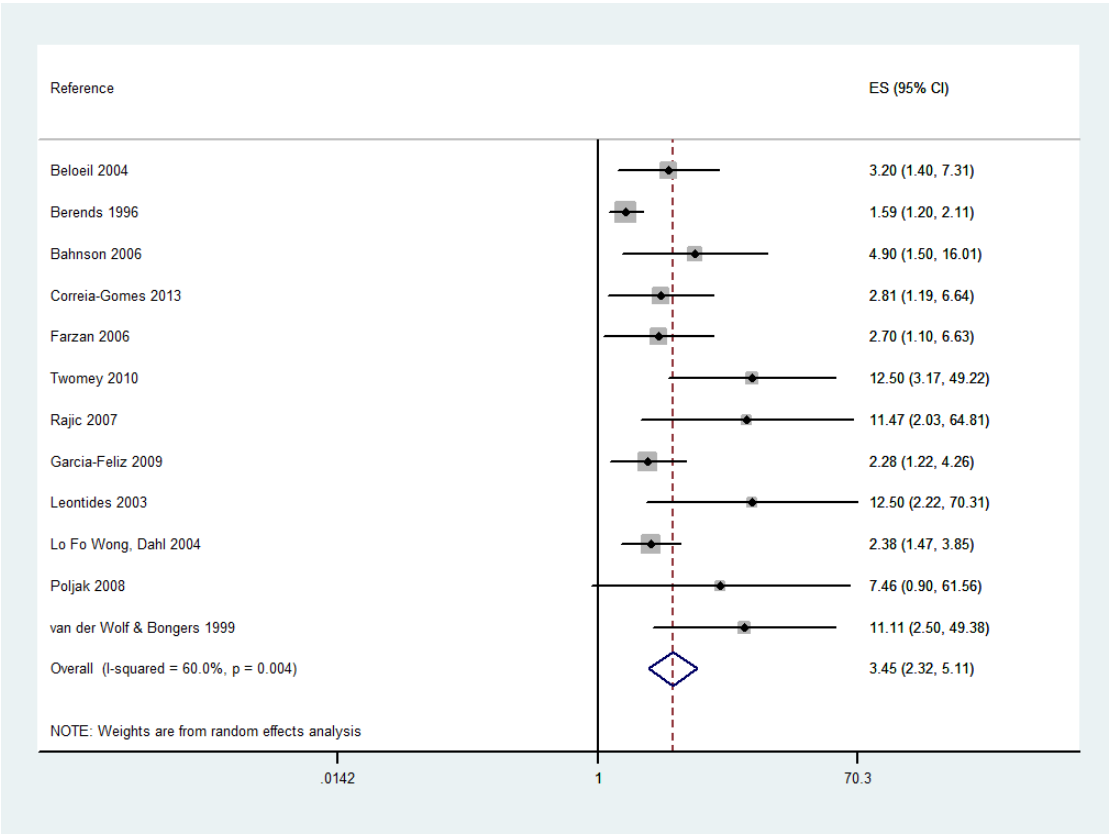


ES = Effect Size and 95% Confidence Interval

Figure 1.1 shows the results of a meta-analysis for the effect of biosecurity on *Salmonella* infection. Data from 8 studies were included (43, 56-62). The overall I^2 statistic suggests low heterogeneity amongst the studies and therefore, the overall effect size of 0.28 (95% confidence interval (ci) 0.18 – 0.44) may be accepted (44). However, the result must nevertheless be interpreted with caution.

Firstly, the studies were conducted in different countries, including GB, Canada, Germany, France, Spain and Portugal. Secondly, the measures of biosecurity varied from use of farm-specific clothing and an enclosed farm perimeter (56), use of all in/ all out batch management (58), use of wheel dips (59), visitor policies (61), presence of other livestock (43) and containment within a closed barn (62). Despite these limitations, the result of the meta-analysis provides evidence that improved biosecurity is associated with a reduced risk of *Salmonella* infection.

Figure 1.2 Forest plot displaying a meta-analysis of the effect of feed type on *Salmonella* infection in pigs

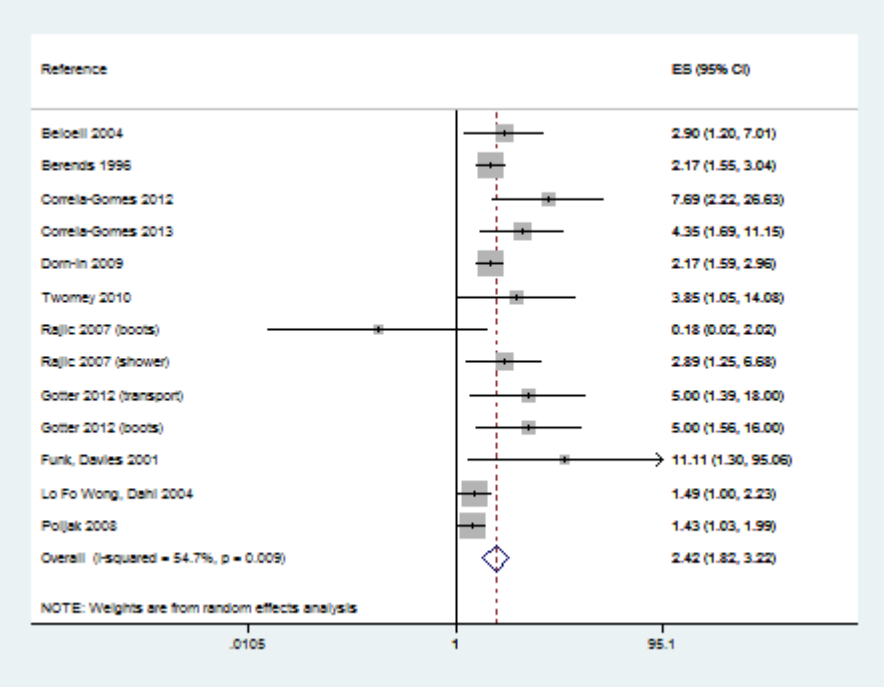


ES = Effect Size (Odds Ratio) and 95% Confidence Interval

Figure 1.2 displays the results of a meta-analysis of 12 studies that estimated the association between feed type and *Salmonella* infection. These studies considered various feed types, including liquid feed, home-milled rations, meal and commercially manufactured pelleted feed. For simplicity, the data were re-classified as pellets or other feed type. The I^2 statistic suggests very low

heterogeneity (p=0.004) amongst the studies and therefore, the overall effect size of 3.45 (95% ci 2.32 – 5.11) is plausible. The finding can be interpreted as providing strong evidence that use of commercial pelleted feed increases the risk of *Salmonella* infection by a factor of at least 2. There is consistent evidence that commercial pelleted feed is associated with an increased risk of *Salmonella* infection (48, 57, 58, 62-72) and these studies further report that use of a liquid diet is associated with a reduced risk of infection. These effects are mediated by changes in the gut pH, where commercial pelleted diets result in less acid conditions that favour growth of *Salmonella*. As discussed later, this led to the use of acidified feed as a control measure.

Figure 1.3 Forest plot displaying a meta-analysis of the effect of hygiene practices on *Salmonella* infection in pigs



ES = Effect Size (Odds Ratio) and 95% Confidence Interval

Figure 1.3 shows the results from a meta-analysis of 11 papers that reported the association between hygiene-related variables and *Salmonella* infection in pigs. Two of these papers gave estimates for two hygiene related variables (60, 73). The original data varied in whether an association was reported as a potential risk factor for failing to follow good practice (OR > 1.00) or whether adoption of a practice was a potentially protective factor (OR<1.00). In figure 1.3, all estimated

associations are given as potential risk factors – so an odds ratio above 1.00 indicates that poor hygiene increases risk. One study by Rajic et al in Canada (73) provided weak evidence that disinfection of boots might increase risk of *Salmonella* infection (OR for not disinfecting boots 0.26; 95% ci 0.07 – 2.03) although this result is not statistically significant. The I^2 statistic suggests very low heterogeneity (p=0.009) amongst the studies and therefore, the overall effect size of 2.42 (95% ci 1.82 – 3.22) may be plausible, indicating that poor hygiene may be associated with a twofold increased risk of *Salmonella* infection. However, this result should be interpreted with caution, as a wide range of different hygiene practices were considered. These included dung removal practices (57, 74), improved hygiene practices (48, 62), rodent control (63, 75), disinfection of footwear (59, 60, 73) or personal hygiene including provision of a staff toilet (61, 76). However, in all cases, the specific measure for which an estimate of association was provided was one component of hygiene and a justification for combining these data is that these may have been proxy indicators of the overall standards of farm hygiene.

The intermittent nature of *Salmonella* excretion is a major limitation for cross-sectional studies. In a longitudinal study in Denmark, *Salmonella* was isolated from more than 50% of pigs at least once but only from low numbers of pigs on any particular day of sampling (77). Similarly, an intensive longitudinal study in the USA showed that the prevalence of infection at any particular age could vary significantly between cohorts of pigs raised in the same accommodation (78). Thus, a cross-sectional snapshot may be a poor indicator of herd status with respect to *Salmonella*, which longitudinal studies have shown to be dynamic (36, 76-82). The dynamics of transmission are related to both the serovar involved and the infective dose (29, 83). Furthermore, extrapolation of results from other countries to the UK should only be done with care, since there are considerable differences in both the predominant *Salmonella* serovars that are present and in the pig husbandry systems employed (84). For example, the UK has a particular problem with *S.* Typhimurium whilst the USA is more concerned about *S.* Choleraesuis and *S*. Rissen is the main serovar isolated in the Iberian peninsula and Thailand (9, 74). Other health problems may also impact *Salmonella*. For example, in a UK case-control study of post-weaning multi-systemic wasting

syndrome (PMWS) affected herds were more likely to be infected with *Salmonella* (85, 86) and other studies have shown an association with porcine respiratory and reproductive syndrome (PRRS) virus infection or other enteric pathogens including *Lawsonia* (57). Stress has also been shown to be important and stress-related hormones such as norepinephrine have been shown to have a direct effect upon growth of *Salmonella* and faecal excretion *(87)*. The prevalence of infection in earlier work, before use of the meat juice ELISA test became widespread, was generally measured by culture of individual or pooled pig faecal samples.

Some authors report on evidence for an association with particular putative explanatory variables. A series of 3 papers from the USA concluded respectively that pig accommodation with open gutters increased the risk of *Salmonella* infection (88, 89) that infection in pigs prior to entry to a finishing barn was relatively unimportant – a finding that is contrary to reports from other authors (49) - and that slatted floors reduce risk (90). However, these papers only consider a limited range of other potentially confounding variables in the quantitative analysis.

One area of interest is the association between use of antimicrobials and *Salmonella* infection. Generally, antimicrobials are contra-indicated for salmonellosis in farmed livestock unless severe clinical disease occurs, e.g. septicaemia. However, pigs may be exposed to antimicrobials prescribed for other conditions and this has been reported to be associated with an increased risk in a number of studies (48, 58, 64, 66, 70, 91). Conversely, one study reported that the administration of antimicrobials via the water supply reduced the risk of *Salmonella* infection (68). A systematic review of the evidence for an association between antimicrobial use and *Salmonella* infection concludes that it was not possible to undertake a meta-analysis due to inadequate reporting of study design and the wide diversity of approaches (92).

A case- control study in Germany collected environmental samples from pen surfaces and areas including anterooms from farms that had either a high or a low *Salmonella* seroprevalence, as determined by a mix-ELISA test undertaken

as a part of a national monitoring programme. The samples were tested using a real-time PCR and the results showed that the odds of a positive PCR were approximately three times higher in samples from high seroprevalence herds (93). The authors suggest that this residual contamination is important for infection of pigs but do not present any additional evidence to support this assertion.

An analysis of recorded biosecurity and management practices amongst 225 farms that were visited as a result of a high seroprevalence, as determined by the ZAP scheme and 72 farms that participated in a cross-sectional survey in GB, showed that liquid feeding, fully slatted flooring and improved biosecurity practices were more frequent in the low seroprevalence group (59). Data derived from a European baseline survey with additional questionnaire information also showed an association between improved biosecurity and a lower prevalence of *Salmonella* infection in Portugal (45) as did longitudinal studies in France (56) and in Canada (94). In analogous observations, poor biosecurity was associated with an increased risk of *Salmonella* infection in Netherlands (48), France (54, 64) and the USA (61).

There was greater variation in reported associations between cleaning and disinfection practices and *Salmonella* infection. This may have been partly confounded by whether or not the farms included in the study had an all-in/ all-out system. A Portuguese study did not find evidence that an all-in/ all-out system was effective (45) whilst a study from Canada reported that continuous production increased risk (58). A European study that included data from Germany, Greece, Netherlands and Sweden showed an association between all-in/ all-out units and a lower risk of *Salmonella* infection (67). The consensus amongst studies that did investigate an association between cleaning and disinfection and *Salmonella* was that either there was a modest benefit or that no discernible association could be detected (48, 57, 61, 64, 70, 76). A Canadian study reported that cold water washing of housing was associated with higher risk compared to hot water washing (62), which was also reported from GB (95).

Table 1.2. Reported associations between herd size or stocking density and *Salmonella* infection in pigs

Risk Factor		Effect Size	95% ci	Reference
Larger herd/ batch size	Sow herd > 203	1.65	0.83 – 3.44	Correia-Gomes 2012 (75)
	Finisher herd > 400	2.32	1.34 – 4.00	Dorn-In 2009 (74)
	Finisher pig numbers (continuous) per 100	1.01	1.00 – 1.01	Farzan 2006 (58)
	Finisher herd size >1600	5.87	1.59 – 22.72	Mejia 2006 (96)
	Slaughter > 3500 pigs/yr	1.78	0.96 – 3.31	Garcia-Feliz 2009 (65)
Pig Density	< 0.75 /m^2	0.22	0.05 – 0.90	Funk et al 2001 (61)
Pigs / room	Increase per 10 pigs	1.05	1.03 – 1.06	Beloeil 2007 (56)

Studies that examined herd size also showed variation in the reported strength of association. Those studies which provided a quantitative estimate of effect are summarised in table 1.2. Some reported no impact (97), others that increasing herd or group size was associated with increased risk (43, 56, 58, 61, 63, 74, 75) whilst one study from Netherlands found the reverse association, with smaller herds reportedly at greater risk (70). Any association with herd size is likely to be due to management issues associated with the number of pigs present. If the herds are breeder-finishers that do not breed their own replacement gilts, then increasing herd size will be associated with a greater number of introduced animals, which may also be derived from a diverse number of breeding company premises. However, farms that rely on homebred replacements, with new genetics being introduced through artificial insemination, may be at a reduced risk. Specialised finisher units are also likely to be filled from multiple sources as herd size increases and this has been separately reported (53, 67).

Concurrent diseases, especially those that may cause a level of immune impairment such as PRRS and PMWS, may be associated with *Salmonella* infection (47, 56, 57, 86). Although most studies do not report any evidence of

clinical impact associated with *Salmonella* infection, one study in Denmark and a second from Spain do report an increased risk if signs of diarrhoea are present (43, 51) and a prior history of salmonellosis was also associated with an increased risk of infection (43, 70).

Small numbers of studies also reported other factors that were associated with *Salmonella* status. These included: nose-to-nose contact which was associated with an increased risk (67, 72), slatted floors being associated with a reduced risk (71, 90, 98); membership of a QA scheme was associated with either an increased (74) or a decreased risk (99) and rodent problems were associated with an increased risk (39, 43, 63). The reported association with outdoor herds also varied, being associated with reduced risk in one study in Denmark (100) but associated with an increased risk in another from the USA (55). One study associated presence of milk spot liver, due to migrating ascarid larvae which may be more common outdoors, with an increased risk of *Salmonella* infection (70).

In summary, as concluded by Rostagno, Callaway and Todd (2012) (101), there is limited coherent evidence for risk factors for *Salmonella* infection in pigs, despite a sizeable literature that has grown over the past 10 years. The exception to this is with respect to feed, where there is a consistent association with an increased risk amongst those herds using a commercial pelleted ration. The reported risk factors are biologically plausible; however, plausibility in the absence of a robust study design and in particular, the absence of control of potential confounding variables does not yield great confidence in ascribing the reported association to a causal effect. Therefore, in the research that is reported in this thesis, potential exposures that might increase the risk of transmission of infection between batches of pigs, or that may be implicated in the recurrence of infection from environmental sources are considered. The risk of introduction of infection when re-stocking accommodation may be of particular importance.

In addition to the observational studies described and summarised above, there are reports on field interventions against *Salmonella* infection in pigs. A review for the British Pig Executive (34) was consulted alongside two further reviews (102,

103) and a limited number of additional publications were found through this literature review.

These reviews identified 17 papers concerned with vaccination against *Salmonella*. Six of these were concerned with *S*. Choleraesuis, which has little relevance to GB as this serovar is very uncommon here. Three further studies involved experimental challenge with *Salmonella* and thus, were not representative of the field situation. Amongst the remainder, the outcome against which efficacy was measured varied, including for example, clinical disease, MJ ELISA or seroprevalence, shedding during production or lymph node culture at slaughter. Where culture of *Salmonella* was undertaken, the outcome was not serovar-specific. One study (104) compared shedding by piglets from vaccinated sows to shedding by piglets from sows that had been treated with antibiotics but did not include an untreated control group. A large trial reportedly showed a reduction of 86% in the prevalence of infected ileocaecal lymph nodes at slaughter. However, this study did not use contemporaneous controls, but relied on a comparison of prevalence prior to and after implementation of the vaccination programme (105). A reduced prevalence of infected ileocaecal lymph nodes was also reported by two further studies (106, 107). Other studies reported a reduced shedding load but not prevalence of shedding amongst weaners (2 studies), a reduced clinical impact or a reduced seroprevalence at slaughter. However, one paper that was not included in either review showed that the odds of shedding of *Salmonella* was greater in vaccinated pigs than in a control group (108). At present, the lack of a licensed *S.* Typhimurium vaccine for pigs in GB and the costs of administration do not make this a feasible intervention for widespread adoption.

The review by Friendship et al (34) noted that the limited number of studies of probiotic use or competitive exclusion gave equivocal results, with no consistent beneficial effects being reported. Results from field trials contradict the frequently positive responses seen in experimental infections (109-111). This review also presented data to show that only 2 of 11 interventions using antimicrobials produced a benefit; such practice is in any case contra-indicated as it may lead to the selection of *Salmonella* strains that carry antimicrobial resistance.

The use of acidified feed or water has been promoted to stimulate a reduced pH in the gut, which is protective against *Salmonella*. However, the reported association between use of acidified feed or water and *Salmonella* infection is not consistent. In one review, only 4 of 9 studies showed a benefit (34) whilst in the other review, 2 of 4 additional studies showed a beneficial effect (102). However, the authors of these reviews did not quote any estimates of association made in the studies that they included. In a review of field experience with farms with a high seroprevalence in GB, acidified feed was not shown to be beneficial (47). Interventions that involved use of coarsely ground feed or liquid feed reportedly reduced the prevalence of *Salmonella* when measured by serology or culture (34, 102, 103), which is consistent with the reports from the observational studies considered earlier.

Other interventions that may be effective but that are seldom feasible include the use of isolated weaning, in which piglets are removed from sows at around 10 days of age and then reared at a remote nursery site (112, 113) or by complete depopulation and repopulation of a breeder-finisher herd after extensive cleaning and disinfection (114, 115). Another review suggests that efforts to reduce the prevalence of infection at weaning may bring benefits during the finisher period (116).

A recent paper suggests that manipulation of the gut microbiota may afford a novel means to modulate the suitability of the gut environment for the growth of *Salmonella* and thus to reduce the incidence of infection (117). In the future, it is possible that breeding for resistance may be possible (118) but there is no immediate expectation that this potential is likely to be exploited.

Summary

Human salmonellosis continues to be an important public health burden, with some 50,000 cases each year in UK alone. *Salmonella* infections occur in all domesticated livestock and are especially prevalent in poultry and pigs. Almost a quarter of UK pigs carry *Salmonella* at slaughter, including *S*. Typhimurium, which is the second most frequent cause of human disease. A reduction in prevalence would potentially bring significant public health benefits, although it is recognised that only a minority of human cases originate in pigs.

Effective intervention strategies to control *Salmonella* infection in GB pig farms are required if the public health benefits that are anticipated from the introduction of the ZAP scheme and its successors are to be realised. The evidence for costly interventions such as competitive exclusion, vaccination or the use of organic acids in feed or water is equivocal. These measures may offer promise for some farms, particularly those with a high prevalence of infection. Use of fermented liquid feed has proved to be very effective but the cost of changing from a traditional system to liquid feeding is unlikely to be justified economically solely for the purpose of controlling *Salmonella*. Interventions that aim to improve farm hygiene and biosecurity offer promise as a feasible means for producers to reduce moderate levels of *Salmonella* infection and to minimise the risk of the prevalence of infection increasing on those farms where it is currently low.

The ultimate aim of the research in this thesis is to test the hypothesis that a hygiene and biosecurity protocol would be adopted by pig farmers and would deliver an important reduction in incidence amongst finisher pigs. Before this hypothesis could be tested, it was necessary to evaluate the 2 main approaches that are employed to measure *Salmonella* in pigs – culture to isolate the bacteria or serology using the MJ ELISA test. Furthermore, the limited literature available at the inception of this research offered little insight into important risk factors that were associated with *Salmonella* infection in GB and no farm-level survey to estimate prevalence was available. Therefore, a national farm survey for

Salmonella in finishing pigs was conducted to firstly, estimate the variation in prevalence amongst farms and secondly, investigate putative risk factors. This evidence was supplemented by analysis of routine data acquired from the ZAP scheme and Quality Assurance schemes. The evidence from these two cross-sectional studies and the literature review were used to design the hygiene and biosecurity intervention. The hypothesis that the intervention would be adopted and effective in reducing the incidence of *Salmonella* infection was tested through a randomised controlled study on an intent-to-treat basis.

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Chapter 2. Laboratory Methods

The research described in this thesis required two principal tests to establish the status of individual pigs with respect to *Salmonella* : culture to isolate and identify *Salmonella* bacteria and enzyme-linked immunosorbent assay (ELISA) to detect circulating antibodies against *Salmonella.* The methods that were employed are described and discussed below. Whilst there are many approaches to the isolation of *Salmonella*, the choice of method here was limited by the need for the National Reference Laboratory for *Salmonella* at AHVLA-Weybridge to deliver results that were firstly, comparable to previous surveys (1-3) and secondly, conformed to requirements for the European baseline surveys on *Salmonella* in pigs (4, 5).

Isolation of *Salmonella* by culture

A wide range of methods for isolating *Salmonella* are described in the literature (6). The most appropriate approach for a particular situation will depend upon a number of factors (7), including:

- The source of the sample
- The nature of the matrix within which *Salmonella* may be contained
- The amount of material to be cultured
- The abundance of *Salmonella* within the sample.

Isolation generally requires a number of steps:

- Pre-enrichment
- Selective enrichment
- Inoculation of solid media
- Screening of suspect colonies
- Confirmation of identity

The time between sample collection and the start of the isolation process and the temperature to which samples are exposed during this period will also impact on the likelihood of successfully growing *Salmonella* in the laboratory.

Pre-enrichment is used where the numbers of *Salmonella* are likely to be low and where the bacterial cells are liable to have been stressed (8) and is conventional practice with pig faeces, despite limited evidence for its benefits (6). A range of pre-enrichment media are available (9) and the currently accepted ISO method recommends Buffered Peptone Water although other alternatives have been shown to be more effective (10). Briefly, the pooled pen faecal samples were subjected to pre-enrichment in buffered peptone water (BPW) (sample to BPW ratio 1:10) for 18 hours at 37^{o} C.

There are 3 major types of selective enrichment media for Salmonella: tetrathionate, selenite and Rappaport-Vassiliadis media. The latter is deemed to be the most sensitive for use with pig faeces (7, 11). In the studies reported in this thesis, the BPW culture was then subcultured onto Diassalm Modified Semi Solid Rappaport Vassiliadis medium (MSRV) - 0.1ml as 3 equal and equidistant drops on the surface of the MSRV plate, which was then incubated at 41.5o C and examined after 24 hours (h) and 48h. MRSV contains malachite green oxalate, magnesium chloride and novobiocin, which are selective compounds that inhibit growth of other bacteria. The semi-solid nature of MSRV enables the motile Salmonella to move beyond the point of inoculation, which separates it from other bacteria. Additionally, two indicators are included – saccharose with bromocresol purple and ferro-iron with thiosulphate. Putative Salmonella colonies are identified as dark areas on a turquoise background (12). MSRV has been recommended as a medium for the isolation of Salmonella from the intestinal content of pigs (13).

Selected colonies were inoculated onto a Rambach agar plate (Merck) for 24 hours at 37^{o} C. This media contains sodium desoxycholate which inhibits gram-positive bacteria. It also contains propylene glycol with which *Salmonella* forms acid and the inclusion of a pH indicator produces *Salmonella* colonies with a characteristic red colour.

A single presumptive *Salmonella* colony was selected from each plate and these were subjected to a slide agglutination test using a range of typing sera and to the minimum phenotypic criteria for identification to *Salmonella* species (14). A subculture of each confirmed *Salmonella* isolate was submitted for full serotyping

and suspect *S.* Typhimurium isolates were also subjected to phage typing. This remains a useful tool for differentiating amongst strains of this common serovar (15-17).

Meat Juice ELISA

The MJ ELISA was first developed and adopted in Denmark for use in the surveillance programme that was instituted in the late 1990s (18) and was adopted by the British ZAP and ZNCP programmes (19-22).

The test is an indirect ELISA, in which multiple *Salmonella* antigens are bound onto the walls of the wells in a micro-well plate. These bind with any antibodies against the O antigens of commonly encountered Group B and C_1 serovars that are present in the MJ sample and these antibodies are thus attached to the well surface. The plate is washed and in the subsequent step, an enzyme-linked second antibody is added, which is species-specific and thus binds to any porcine antibodies present on the well wall, sandwiching the sample antibodies between the bound antigens and the enzyme-linked test antibodies. Finally, a chromogenic substrate is added and the bound enzyme brings about a change in optical density which is detected by the ELISA reader. There is a correlation between the degree of absorption that is measured by the reader and the antibody titre in the sample (23).

Pigs produce antibodies after infection with *Salmonella* and the titre typically peaks at around 3 weeks post infection (24) although this varies according to the serovar and dose involved (25). Longitudinal studies suggest that in sub-clinically infected herds, most pigs are serologically positive by the last third of the finisher period (26, 27). Serological studies conventionally use serum samples to test for antibodies. However, whilst sera that are collected for other purposes have been tested for *Salmonella* antibodies in some countries e.g. Netherlands (28), elsewhere meat juice samples are favoured, since these can be collected at slaughter. The titre of antibodies in meat juice is generally lower than in serum (29) and this may lead to an under-estimation of the true seroprevalence (30) but nevertheless the MJ ELISA is sufficiently sensitive for surveillance purposes (31).

There is no universal standard for MJ ELISA tests at present and ring trials have shown that there is significant variation between laboratories (32, 33). In the studies described within this thesis, the MJ ELISA test employed in the GB ZAP/ZNCP scheme was used. Chapter 5 describes the analysis of the ZAP scheme data; the tests in this case were carried out by a commercial laboratory. MJ ELISA results reported in Chapters 4, 7 and 8 were obtained using the same commercial test and were conducted by the AHVLA Regional Laboratory in Bury St. Edmunds. The only difference to the approach used by ZAP was that AHVLA tested each sample in duplicate and the mean OD values were used to calculate the s:p ratio. Chapter 3 investigates the relationship between *Salmonella* culture and MJ ELISA test results in more detail.

In the abattoir, small pieces of skeletal muscle approximately 1 cm^3 were cut from either the neck muscle or diaphragm and placed into the collection chamber of specialised meat juice sampling tubes, which were promptly frozen and transported in insulated containers with an ice pack to the testing laboratory. After thawing, the meat juice extracts were tested using a commercially available *Salmonella* lipopolysaccharide (LPS) ELISA kit (Guildhay Vetsign kit). Test optical density (OD) results were converted to sample-to-positive (s:p) ratios and samples defined as negative or positive according to the s:p ratio. Two cut-off points were used: a s:p=0.25 (MJ25), which was used in the British Pig Executive's (BPEx) Zoonoses Action Plan (ZAP) and s:p=0.10 (MJ10), which was employed in the successor BPEx Zoonoses National Control Plan (ZNCP).

In addition to the Bayesian analysis that evaluates the performance of these tests in relation to each other, the implications of test methods for the interpretation of results are discussed where relevant in each Chapter of the thesis.

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Chapter 3. A Bayesian analysis to estimate sensitivity of detection of *Salmonella enterica enterica* in pigs with multiple tests

Introduction

As discussed in Chapter 1, *Salmonella* infection is an important cause of human foodborne infection and has an important public health cost (1). The most frequent serovar in human disease is *S*. Enteritidis and the second most frequent isolate has consistently been *S*. Typhimurium. *S*. Typhimurium is found in all British livestock species and is the most common *Salmonella* serovar to be isolated from pigs (2, 3) (see Chapters 1 and 4). *Salmonella* has been isolated from the caecal contents of almost one quarter of British finisher pigs at slaughter (2, 3) and the prevalence in the United Kingdom (UK) was amongst the highest in Europe (4). The EU Zoonoses Order requires that livestock producers accept responsibility for safe food production. Under this order, a series of baseline surveys of *Salmonella* in poultry and pigs were conducted and the results from these surveys are intended to be used to set targets for reduction of prevalence in order to protect public health. In Great Britain, the British Pig Executive initiated the Zoonoses Action Programme for Salmonella in pigs (ZAP) in 2001. ZAP utilised a meat juice (MJ) ELISA to estimate the prevalence of *Salmonella* infection in pigs at slaughter (5, 6). The intention was that herds with a high prevalence (>50%) would implement control measures and that this would lead to a reduced incidence of human salmonellosis from home-produced pig meat and pig meat products.

Infection with *Salmonella* in pigs may be routinely detected either by isolation of bacteria or by detecting antibodies against *Salmonella*, which is indicative of previous exposure but not necessarily of current infection (7). Infection may be acquired through ingestion of contaminated material including food or faeces and results in rapid dissemination in a matter of hours (8, 9). This acute infection will occur before the immune response and such animals are therefore antibody-negative. Infection is followed by a varying period of intermittent excretion during which *Salmonella* can be present in high numbers in the faeces. Circulating antibodies can be found after around 10 days and these persist for many weeks (10). Monitoring of *Salmonella* contamination of carcasses within an abattoir

requires the use of carcass swabs and this may represent the most relevant measure of the threat to public health. Under the EU microbiological criteria for *Salmonella* in pork, all abattoirs that slaughter pigs, regardless of throughput, must take carcass swabs from 5 carcasses each week (Commission regulation EC 2073/2005).

The validity of a diagnostic test is classically estimated through comparison with a "Gold standard" in order to estimate sensitivity and specificity. However, it has been recognised that there may be no authoritative gold standard and mathematical approaches have been developed to enable results from two or more tests applied simultaneously to subjects to be combined in a statistical model. The output from such a model can include estimates of the sensitivity and specificity of each test. Such approaches were reviewed by Enoe *et al* (11) and have been applied, for example, to tests for *Mycobacterium avium paratuberculosis* in dairy cattle (12) and *Mycoplasma hyopneumoniae* infection in pigs (13). In surveys of farmed livestock to estimate prevalence, the farm (herd or flock) is commonly the primary sampling unit. Since animals within farms are more alike than those selected at random, the distribution of estimates of within-farm prevalence will be subject to a "cluster effect" (14, 15). The intracluster correlation coefficient (ICC) is a measure of infection/disease clustering between the individuals from a group (such as a herd) that has application in the design and statistical analysis of epidemiologic studies (14, 16-18). As an analytical tool, ICC has been used to adjust for infection clustering as part of a variance inflation factor for estimators of prevalence (14, 18). Analytical methods that account for infection clustering but do not rely on ICC are also being used (19-21), including Bayesian hierarchical models presented in Suess *et al* (22) and Branscum *et al* (23). The ICC is usually estimated by a maximum likelihood approach (18). A Bayesian approach has also been proposed for estimation of the intracluster correlation coefficient where imperfect tests are employed and multiple animals within a farm are sampled (24).

In a survey using a single test, the observed prevalence p is estimated as the number of test positive animals divided by the total number of animals sampled.

The probability of an individual animal testing positive is given by:

$$p = P(T^+) = TP*Se + (1-TP)(1-Sp)$$

Where TP = true prevalence
Se=sensitivity
Sp=specificity

In other words, the observed prevalence is the sum of the number of true positive animals that give a positive test result and the number of true negative animals that give a positive test result divided by the number of animals tested. It is not possible to estimate the sensitivity, specificity or true prevalence from these data alone. If valid estimates of the sensitivity and specificity of the test are available, for example in the published literature, then the Rogan Gladen estimator (25) can be applied.

Hui and Walter (26) proposed an alternative approach, based on latent class analysis, in which two tests are applied to a random sample of animals drawn from two or more distinct populations (eg herds). It is assumed that the two test outcomes are independent, conditional on the true status of each animal, and the sensitivity and specificity of each test are constant. However, the true prevalence must vary between populations and may also be zero. The data from each herd can be presented as a 2x2 table, showing the number of animals that are positive or negative by each test. Thus, one of four possible outcomes is observed for every animal within each herd and the probability of each will depend upon test sensitivity, test specificity and true prevalence (TP):

1. Positive to test 1 and positive to test 2

$P_{11} = TP*Se_1*Se_2 + (1-TP)\ (1-Sp_1)\ (1 – Sp_2)$

2. Positive to test 1 and negative to test 2

$P_{12} = TP*Se_1\ (1 – Se_2) + (1 - TP)\ (1-Sp_1)\ Sp_2$

3. Negative to test 1 and positive to test 2

$P_{21} = TP*(1 – Se_1)\ Se_2 + (1 - TP\ )\ Sp_1\ (\ 1 – Sp_2)$

4. Negative to test 1 and negative to test 2

$P_{22} = TP\ (1 – Se_1)\ (1 – Se_2) + (1 - TP\ )\ Sp_1\ Sp_2$

A maximum-likelihood estimate can then be obtained for the value of each of the six parameters i.e. the prevalence, sensitivity and specificity in each herd. However, the assumption that the tests are conditionally independent is open to challenge (27). An alternative Bayesian approach to estimate these parameters has been described (28). Briefly, a Bayesian approach requires that prior values are specified for the parameters of interest based, for example, on existing literature, data from other studies or expert opinion. A probability distribution is associated with each of these prior values to account for uncertainty and these prior distributions are combined with the likelihood for the observed data to obtain a posterior distribution. A measure of central tendency, usually the median, can then be used as an estimate of the parameter of interest and a credible interval about that estimate generated by Markov Chain Monte Carlo (MCMC) simulation using thousands of iterations of the model.

Branscum *et al* (29) reviewed the use of Bayesian models for computing test validity and extended previous approaches to estimate the sensitivity and specificity of two conditionally dependant tests combined with a third independent test. Conditional dependence may arise, for example, if two different tests are intended to detect circulating antibodies against a specified antigen. If conditional dependence is present and is not taken into account then the uncertainty associated with the estimated values of the parameters of interest is likely to be under-estimated.

In this study, data from an abattoir-based survey for *Salmonella* infection in UK pigs that was conducted as part of an EU baseline study were used to compare the results of three different samples that were cultured and the results from meat juice (MJ) enzyme-linked immunosorbent assay (ELISA) tests to detect antibodies against Group B and C_1 *Salmonella*. A Bayesian analysis has been adopted, so that the observed results can be considered in a single model that also incorporates prior information. Bayesian models may include quantitative data derived from other studies or may utilise formalised approaches to express expert views or opinions for example, from the so-called Delphi approach (30). Such approaches are finding increasing popularity for risk analysis in veterinary public health (31-33). In the analysis presented in this Chapter, a Bayesian approach provides estimates of test validity and of prevalence that are mutually coherent.

Surveillance strategies will be required to monitor progress towards targets for reduction in the prevalence of *Salmonella* in pigs that are anticipated to be agreed by the EU during 2014. The results of this study will be valuable in informing sample size estimation and in deciding testing strategy. For example, although culture of mediastinal lymph nodes might be the stipulated measure against which performance is to be judged, it may be cheaper to use MJ ELISA results for continuous monitoring in-between formal surveys.

Materials and Methods

Data for this analysis were collected during the EU baseline survey for *Salmonella* in pigs at slaughter. The survey design is described in detail in a technical specifications document (SANCO/40162/2006) annexed to Decision 2006/668/EC. The sample size for UK was estimated as 600 pigs and an additional 10% was added to compensate for any problems that may have arisen during the survey. Eighteen abattoirs that had received 81.8% of the pigs slaughtered in UK in the previous year were selected. The number of pigs sampled per abattoir was proportional to the estimated annual throughput and was equally distributed across 12 calendar months beginning on October 1st

2006. Sampling days were randomly selected for each abattoir for each month and only one pig per day was selected. The approximate daily kill was recorded and the pig to be sampled was chosen at random from that number. The selection of sampling days was managed by CERA-VLA and the actual pig to be sampled was selected according to the study protocol by a designated Meat Hygiene Service operative at each abattoir, who had been trained by CERA-VLA.

Four samples were collected from each carcass: the aggregate of ileo-caecal lymph nodes (25g), a muscle sample, carcass swab and sample of caecal contents. A minimum of five lymph nodes were bluntly harvested with gloved fingers in order to reach 25g. In some cases, mesenteric lymph nodes were collected in addition to ileo-caecal lymph nodes in order to ensure 25g of material. The caecum was ligated and removed at the abattoir after the lymph nodes had been removed and it was sent to the laboratory where 10g of its content was milked for testing. The carcass swab was taken on the left or right side of the carcass using one single sponge for all fours sites designated in Annex A of Standard ISO 17604 (hind Limb, Abdomen, Mid-dorsal region, Jowl). Two sites were swabbed with one side of the sponge, which was turned over and used to swab the remaining two sites as detailed in the sampling protocol. A muscle sample was taken from the diaphragm muscle (not fat, gristle or membrane) or occasionally from the neck muscle.

Carcass samples taken at the abattoir were packed in an insulated box together with an ice pack and sent on the same day to the Veterinary Laboratories Agency.

Bacterial examination of samples was begun within 24 hours of arrival at the laboratory and no later than 96 hours after being taken at the abattoir. The isolation method used for *Salmonella* was a modification of that described in the ISO6579 2002 Annexe D and is described in Chapter 2.

On receipt at the laboratory, muscle samples for the MJ ELISA tests were stored at -20^{O}C until analysis using the method described in Chapter 2.

All data were entered into a Microsoft Access database, and checked for data entry errors. Preliminary analyses were carried out using Stata10 (Statistical Software Release 10; StataCorp). New variables were generated so that each pig was classified according to the result from each of the four tests; namely, culture of caecal content (cc), culture of pooled lymph nodes (ln), culture of the carcass swab (cs) and MJ ELISA test at s:p 0.10 (mj10) or s:p 0.25 (mj25). Thus, there were 16 different possible combinations of test results at mj10 and a further 16 at mj25 as shown in tables 3.1 and 3.2. Bayesian analysis was conducted using WinBugs v14 (http://www.mrc-bsu.cam.ac.uk/bugs/winbugs) and was based on code from Branscum *et al* (24). BetaBuster software (http://www.epi.ucdavis.edu/diagnostictests/betabuster.html) was used to estimate prior distributions, which were based on previous studies or other data sources as described below. Models were created assuming no conditional dependency or allowing conditional dependency between cc and ln, cc and cs or ln and cs. Model outputs were compared to assess the impact of modelling conditional dependency.

Previous studies have shown that there is a poor correlation between the presence of *Salmonella* on the exterior of a carcass and isolation of the bacterium from the caecal content or lymph nodes, although the surface of an infected pig is more likely to yield a positive carcass swab than a swab from a negative pig (34-36). The exterior of an infected pig may be contaminated, especially if there is leakage of faecal material or if the gut is damaged during evisceration. However, cross-contamination may also occur following contact with an adjacent carcass or via contamination of abattoir equipment and one study estimated that 30% of contaminated carcasses arose due to cross-contamination (37). *Salmonella* has been detected within the environment and in sampled air in pig abattoirs (38). Since a *Salmonella* positive carcass swab may arise from an uninfected pig, an analysis based on three-test models (cc, ln and mj10 or mj25) was also developed. Finally, since MJ ELISA test results are poorly correlated with culture for *Salmonella*, a three-test model using caecal content (cc), lymph node (ln) and carcass swab (cs) was created.

Results

Samples from a total of 640 pigs were submitted to VLA for testing. However, the source abattoir for samples from one pig was not identified and one or more of the four required samples was missing for a further 17 pigs. Meat juice samples were missing from 2 pigs, there was neither a lymph node sample nor a caecal content sample from one pig and caecal content samples were missing from a further 14 pigs (see table 3.1). Thus, full test data were available from 622 pigs (97%).

Table 3.1 Number of pigs and number of caecal contents sampled in a UK abattoir survey (October 2006 – September 2007).

Abattoir	Caecal samples		Total pigs sampled
	Submitted	Missing	
1	34	0	34
2	43	0	43
3	66	3	69
4	62	0	62
5	20	0	20
6	12	1	13
7	26	0	26
8	31	0	31
9	24	1	25
10	58	1	59
11	12	0	12
12	45	0	45
13	30	0	30
14	49	1	50
15	21	0	21
16	10	0	10
17	19	1	20
18	63	7	70
Total	625	15	640

After slaughter, the carcass is eviscerated and typically, the gastro-intestinal tract is dropped onto a tray which moves along the slaughter line in parallel with the carcass until a meat inspection point is reached. Here, the meat inspector removed the lymph nodes into a container and also had to retain the caecum and contents. The gut then slides down a chute and it is unsurprising that

occasionally, the meat inspector was unable to remove the caecum before the gut left the slaughter line. Five abattoirs each failed to submit a caecal content sample from a single pig; one abattoir failed on 3 occasions and one abattoir failed to submit a total of 7 caecal samples.

Four-test models

Tables 3.2 and 3.3 show the 16 test result combinations that could be encountered for any individual pig and the number of pigs observed with each test combination in each abattoir using the mj10 and mj25 cut-off points respectively.

The probability of each of the 16 different test combinations in each of the 18 abattoirs was expressed in terms of the sensitivity and specificity of each test and the true prevalence of *Salmonella* infection in each abattoir. These probabilities, for models for both conditional dependence and no conditional dependence, are shown within the model in the appendix (see enclosed CD).

Table 3.2 Number of pigs with each combination of test result (caecal content, lymph node, carcass swab and meat juice ELISA s:p 0.10) in a UK abattoir survey for *Salmonella* in pigs (October 2006 – September 2007)

Caecal content	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Lymph node	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Carcass swab	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
MJ10	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Abattoir																
1	0	0	0	1	0	0	0	2	1	0	1	3	0	1	7	18
2	3	1	3	1	0	0	1	3	0	0	5	1	1	3	9	12
3	5	0	5	0	1	2	4	3	0	0	4	2	7	3	14	16
4	5	1	6	0	1	0	4	0	2	0	7	0	5	5	11	15
5	0	0	0	2	0	0	0	4	0	0	0	0	0	0	4	10
6	0	0	0	0	0	0	2	1	0	0	1	0	1	1	2	4
7	1	0	1	0	0	0	3	1	0	0	0	0	1	2	2	15
8	0	0	3	0	0	0	2	0	3	0	0	1	0	1	6	15
9	2	0	1	0	0	1	0	1	0	0	1	2	1	1	3	11
10	0	0	5	1	0	1	3	4	1	0	5	3	3	3	14	14
11	1	0	0	0	0	0	1	1	0	0	1	0	0	0	3	5
12	1	0	4	2	1	0	1	0	1	0	2	3	3	2	12	13
13	0	0	2	1	0	0	1	0	0	0	3	0	1	0	12	9
14	1	1	2	2	0	0	5	2	0	2	1	0	0	1	13	18
15	0	0	0	0	0	1	3	0	0	0	2	1	0	1	4	9
16	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2	6
17	0	0	0	0	0	0	0	1	0	0	0	1	0	1	4	12
18	2	1	2	1	1	0	2	2	0	0	3	4	1	2	13	29
Total	**21**	**4**	**34**	**11**	**4**	**5**	**32**	**26**	**8**	**2**	**36**	**22**	**24**	**27**	**135**	**202**

Table 3.3 Number of pigs with each combination of test result (caecal content, lymph node, carcass swab and meat juice ELISA s:p 0.25) in a UK abattoir survey for *Salmonella* in pigs

Caecal content	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Lymph node	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Carcass swab	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
MJ25	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Abattoir																
1	0	0	0	1	0	0	0	2	1	0	1	3	0	1	0	25
2	1	3	2	2	0	0	1	3	0	0	3	3	0	4	5	16
3	4	1	4	1	1	2	2	5	0	0	3	3	4	6	8	22
4	5	1	4	2	1	0	3	1	0	2	4	3	1	9	7	19
5	0	0	0	2	0	0	0	4	0	0	0	0	0	0	0	14
6	0	0	0	0	0	0	1	2	0	0	1	0	1	1	0	6
7	0	1	1	0	0	0	2	2	0	0	0	0	1	2	1	16
8	0	0	2	1	0	0	1	1	1	2	0	1	0	1	3	18
9	0	2	1	0	0	1	0	1	0	0	1	2	1	1	0	14
10	0	0	5	1	0	1	2	5	1	0	2	6	1	5	9	19
11	1	0	0	0	0	0	0	2	0	0	1	0	0	0	0	8
12	1	0	3	3	0	1	0	1	1	0	1	4	1	4	7	18
13	0	0	2	1	0	0	0	1	0	0	3	0	1	0	3	18
14	1	1	2	2	0	0	4	3	0	2	1	0	0	1	6	25
15	0	0	0	0	0	1	2	1	0	0	2	1	0	1	2	11
16	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	7
17	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	15
18	2	1	2	1	0	1	0	4	0	0	1	6	1	2	7	35
Total	**15**	**10**	**28**	**17**	**2**	**7**	**18**	**40**	**4**	**6**	**24**	**34**	**12**	**39**	**60**	**306**

Data from tables 3.2 and 3.3 were entered into the WinBugs programmes (see appendix).

Priors

The evidence used to select the distribution of each of the priors required for the Bayesian models is summarised below.

Prevalence of MJ ELISA positive pigs

A prior for the prevalence of MJ ELISA positive pigs was based on data from the ZAP scheme (6), which has shown a consistent prevalence of approximately 26% (6). The variation was modelled using a beta distribution with 95% certainty that the median was greater than 8% (dbeta (1.73, 2.71).

Sensitivity of MJ ELISA (Semj)

Despite the widespread use of the MJ ELISA test in monitoring programmes in several EU Member States, including Denmark, Germany, Eire and UK, there is a paucity of published data on sensitivity or specificity. Studies that relate test performance to that of a serum ELISA (eg Nielsen 1998 (39)) are inappropriate for judging a prior for Semj, since these use the serum ELISA as a Gold standard and do not consider that the serum ELISA sensitivity is less than 100%. A further issue is that published data either relate to experimental studies, in which case the "gold standard" is considered to be having been infected, or from observational studies in abattoirs, when concurrent demonstration of infection by culture is taken to reveal the "true" status of the pigs. The first studies were from Denmark, where experimental infection of 43 pigs and use of an equivalent to the mj10 cut-off provided an estimated sensitivity of 67% (7). In one experimental study, 16 pigs received an oral dose of 4×10^9 organisms and later, MJ ELISA tests were conducted (40). Sensitivity was estimated as 87.5% - 94.0% using an equivalent to the mj10 cut-off and from approximately 81.5% - 90.5% using an equivalent to the mj25 cut-off. In an abattoir study in Germany, Steinbach (41) used a 0.3 s:p cut-off and compared MJ ELISA results to faecal and lymph node culture. The sensitivity of the MJ ELISA was estimated as 35.7% - however, the test method was poorly described. Furthermore, the relatively high cut-off employed would predictably reduce sensitivity and this evidence was given little weight in determining Semj. Korsak *et al* (42) used abattoir data to compare the MJ ELISA with culture of 5g of caecal content and estimated sensitivity as 80% (59.8%-100%) for a mj10 cut-off and as 40% (15.2% - 64.8%) using a mj25 cut-off. In a paper describing an adaptation of the Danish monitoring programme, Alban *et al* assume a sensitivity of 50% for an equivalent to the mj10 cut-off (43). In this analysis, a conservative approach was adopted and the prior for Semj was assumed as a beta distribution with a mode of 60% and with 95% confidence that it was greater than 30% whether the mj10 or mj25 cut-off was used. The impact of changing the prior according to cut-off was investigated through a sensitivity analysis.

Specificity of MJ ELISA (Spmj)

Similar issues to those discussed with respect to Semj arise. Nielsen *et al*'s experimental study in Denmark estimated Spmj as 100% (7) and Alban *et al* assumed a value of 98% (44). Steinbach assumed a specificity approaching 100% but did not provide evidence to support this contention (41). Korsak *et al* in contrast estimated Spmj as 50% (35.2% - 64.8%) using a cut-off equivalent to mj10 and 84.1% (73.3% - 94.9%) using a cut-off equivalent to mj25. For this Bayesian analysis, a beta distribution was used with a mode of 84% and 95% confidence that Spmj was greater than 70%.

Sensitivity of culture (Secc, Seln, Secs)

Sensitivity of culture is dependent on the number of *Salmonella* bacteria per gram of material, the amount of material that is cultured, the presence of any competitive bacteria or inhibitory substances within the material and the particular culture methods that are used. A study in Denmark that employed pooled caecal samples estimated Secc as 50%-60%, with 95% confidence it was greater than 30% (45). Steinbach *et al* estimated the sensitivity of faecal culture as 13.8% - 23.3%; faecal material, being waste gut content, could be considered a proxy for caecal content (41). The same study estimated Seln as 13.2% - 20.4%. However, Steinbach *et al* assumed that all pigs with a positive MJ ELISA result should have yielded a positive culture and thus, sensitivity may have been underestimated. Korsak *et al* estimated faecal culture sensitivity as 13.3% but only used a 1g sample (42) whilst Baggesen *et al* (46) estimated culture sensitivity as approximately 40%. In this study, priors of culture sensitivities were modelled using beta distributions. For Secc, the mode was 30% with 95% confidence it was greater than 20%. For Seln, the assumed mode was 45% with 95% confidence it was greater than 30%. A non-informative prior was assumed for Secs, using a uniform distribution from 0%-100%.

Specificity for all culture was assumed to be 100%, since any suspect colony was subject to confirmatory tests and it was assumed that no cross-contamination of samples occurred.

All models were also run using non-informative priors.

Model outputs

The following tables (3.4 and 3.5) present output derived from the Bayesian models shown in the appendix.

Table 3.4. A comparison of Bayesian models assuming independence of tests applied to test data from a random survey for *Salmonella* infection in 622 pigs slaughtered in UK abattoirs.

		Informative priors			Non-informative priors[1]		
		Median	95% credible interval		Median	95% credible interval	
			Lower	Upper		Lower	Upper
mj10	Secc	0.3798	0.3284	0.4339	0.3740	0.3136	0.4391
	Secs	0.2707	0.2225	0.3229	0.2603	0.2116	0.3129
	Seln	0.3962	0.3431	0.4539	0.3762	0.3140	0.4404
	Semj10	0.6279	0.5696	0.6835	0.6192	0.5603	0.6768
	Spmj10	0.7417	0.6736	0.8097	0.7384	0.6585	0.8248
mj25	Secc	0.4610	0.4059	0.5182	0.3662	0.3092	0.4277
	Secs	0.3361	0.2830	0.3922	0.2546	0.2085	0.3068
	Seln	0.4851	0.4257	0.5442	0.3695	0.3134	0.4315
	Semj25	0.4101	0.3498	0.4686	0.4061	0.3506	0.4659
	Spmj25	0.8541	0.8114	0.8918	0.9577	0.8948	0.9971

Secc = Sensitivity (caecal content)
Seln = Sensitivity (lymph node)
Secs = Sensitivity (carcass swab)
Semj = Sensitivity MJ ELISA
Spmj = Specificity MJ ELISA
[1] Non-informative priors uniform distribution 0% - 100%

Table 3.4 shows that the choice of informative or non-informative priors has negligible impact on the results of the mj10 models. However, there was some evidence that the estimated sensitivity of culture (Secc, Seln and Secs) was lower in the mj25 models with non-informative priors, although the 95% credible intervals overlapped. Sensitivity of the MJ ELISA was substantially greater using the mj10 cut-off and specificity was correspondingly reduced. This may reflect the reduction in circulating antibody titres with increasing time from infection so that a proportion of infected pigs produce a MJ ELISA result with a s:p ratio between 0.1 and 0.25.

Table 3.5. A comparison of Bayesian models assuming conditional dependence between tests applied to test data from a random survey for *Salmonella* infection in 622 pigs slaughtered in UK abattoirs

	Assumed conditional dependence: Caecal content: lymph node			Assumed conditional dependence: Caecal content: carcass swab			Assumed conditional dependence Lymph node: carcass swab		
	Median	95% credible interval		Median	95% credible interval		Median	95% credible interval	
Mj10		Lower	Upper		Lower	Upper		Lower	Upper
Secc	0.3648	0.3138	0.4152	0.3850	0.3335	0.4414	0.3827	0.3303	0.4396
Secs	0.2567	0.2112	0.3073	0.2744	0.2276	0.3273	0.2735	0.2263	0.3269
Seln	0.3765	0.3257	0.4308	0.4001	0.3455	0.4572	0.4004	0.3455	0.4590
Semj10	0.6283	0.5706	0.6842	0.6279	0.5700	0.6852	0.6284	0.5685	0.6819
Spmj10	0.768	0.6958	0.8411	0.7384	0.6710	0.806	0.7378	0.6726	0.8076
CovDp	0.0511	0.0257	0.0757	-0.0091	-0.0330	0.0143	-0.0066	-0.0312	0.0169
Mj25									
Secc	0.4504	0.3989	0.5043	0.4527	0.3970	0.5116	0.4507	0.3925	0.5116
Secs	0.3347	0.2813	0.3934	0.3194	0.2675	0.3774	0.3191	0.265	0.3745
Seln	0.4832	0.4249	0.5422	0.4601	0.4011	0.518	0.4613	0.4034	0.5194
Semj25	0.4091	0.3505	0.4711	0.4121	0.3544	0.4715	0.4130	0.3523	0.4708
Spmj25	0.8550	0.8128	0.8924	0.8689	0.8242	0.9081	0.8698	0.8252	0.9094
CovDp	0.0116	-0.0177	0.0417	-0.0308	-0.0576	-0.0045	-0.0286	-0.0559	-0.0026

Secc = Sensitivity (caecal content)
Seln = Sensitivity (lymph node)
Secs = Sensitivity (carcass swab)
Semj = Sensitivity MJ ELISA
Spmj = Specificity MJ ELISA
CovDp = covariance term

The mj10 models show no evidence that allowing for conditional dependence alters predicted parameter estimates importantly compared to the model which assumes conditional independence because the point estimates do not differ substantially. The 95% credible intervals are similar and their width is dependent upon sample size. However, the credible interval of the covariance term (CovDp) did not include zero when conditional dependence between caecal content and lymph node culture was assumed. There is some evidence of conditional dependence between the results from culture of the caecal content and carcass swab and between lymph node and carcass swab samples when the mj25 cut-off was used, since the credible intervals for CovDp do not include zero (table 3.5). However, the 95% credible intervals associated with adjusted estimates of test sensitivity and specificity showed no important difference compared to the independent model (see table 3.5) and therefore, the independent model has been preferred for further analysis.

The estimated carcass swab sensitivity was lower (33.6%) than that of the caecal content or lymph node (46.1% and 48.5% respectively), which may reflect the lower *Salmonella* burden generally found on the carcass compared to the lymph node or caecal content. There was no convincing evidence of any important difference in the sensitivity of culture of caecal content or lymph node since point estimates are very similar and the credible intervals overlap. Unsurprisingly, altering the cut-off for the MJ ELISA has a profound effect on that test's validity. The s:p 0.25 cut-off showed a lower sensitivity (41.0%; cri_{95} 35.0% - 46.9%) but a higher specificity (85.4%; cri_{95} 81.1% - 89.2%) than the s:p 0.10 cut-off where sensitivity and specificity were estimated as 62.8% (cri_{95} 57.0% - 68.4%) and 74.2% (cri_{95} 67.4% - 81.0%) respectively.

The model was used to predict the number of pigs in each of the 16 different test combinations and these were compared to the observed data (see figures 3.1 and 3.2). The number of pigs in most classes was relatively small (<40) but in the negative to all tests class (cc- ln- cs- mj-) there were more than 300 pigs. Therefore, figures 3.1 and 3.2 display the natural logarithm of the observed or predicted results, to facilitate visual examination. There were some differences between the predicted and observed results. Visual examination of the model output with the observed data (figures 3.1 and 3.2) shows that whether the s:p 0.10 or 0.25 cut-off was used, there were differences in the number of pigs in each of the 16 test combinations. For example, using the mj25 cut-off, 39 pigs were observed to be carcass swab positive and negative in all other tests, whilst the model with assumed independence amongst the parameters predicted only 10 pigs with this combination of test results, suggesting that there is a poor fit of the model to the data. Table 3.6 shows the observed and predicted number of pigs in each class using the MJ25 cut-off – these data are displayed in figure 3.1.

These data were summarised to provide a comparison of the estimated and observed prevalence of *Salmonella* infected pigs by each test individually. The model output included the number of pigs that were predicted to give a positive result to each test and this was divided by 622 (the total number of pigs tested) to estimate prevalence. For example, 294/622 pigs (47.3%) gave

a positive MJ ELISA result using the mj10 cut-off and the model predicted that 288/622 (46.3%) would present a positive result. Comparing the observed results with the model outputs in table 3.6 showed that the goodness of fit was poor in all cases (chi-square test p-value <0.01).

Table 3.6: A comparison of the observed and predicted number of pigs in each class of test result (MJ25 cut-off) from a Bayesian analysis of tests for Salmonella in a sample of GB finisher pigs

Class	Observed	Independent	Independent non-informative priors	Dependent Caecal Content & Lymph node	Dependent Caecal Content & Carcass swab	Dependent Lymph node & carcass Swab
cc+ ln+ cs+ mj+	15	6	5	6	6	6
cc+ ln+ cs+ mj-	10	8	7	8	8	8
cc+ ln+ cs- mj+	28	11	15	11	17	16
cc+ ln+ cs- mj-	17	16	22	17	24	23
cc+ ln- cs+ mj+	2	6	9	6	7	10
cc+ ln- cs+ mj-	7	9	13	8	9	14
cc+ ln- cs- mj+	18	12	26	11	20	16
cc+ ln- cs- mj-	40	17	38	16	28	23
cc- ln+ cs+ mj+	4	7	9	6	10	7
cc- ln+ cs+ mj-	6	10	13	9	14	10
cc- ln+ cs- mj+	24	13	26	13	17	20
cc- ln+ cs- mj-	34	19	38	18	24	29
cc- ln- cs+ mj+	12	7	15	7	12	12
cc- ln- cs+ mj-	39	10	22	11	17	17
cc- ln- cs- mj+	60	78	57	78	67	67
cc- ln- cs- mj-	306	393	304	394	341	342

Figure 3.1 Comparison of model output and observed data from a UK survey of *Salmonella* infection in UK pigs, using four tests (MJ ELISA s:p 0.25 cut-off) and models with or without conditional dependence.

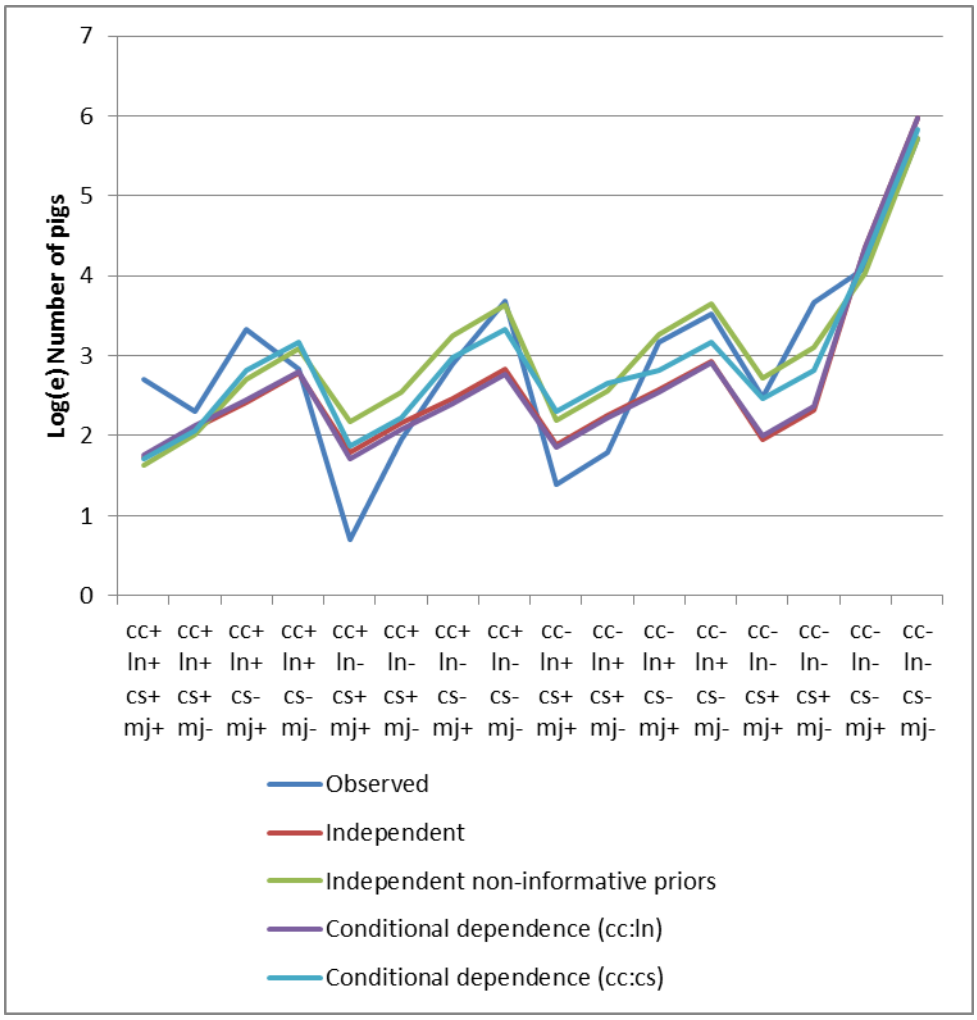

Figure 3.2 Comparison of model output and observed data from a UK survey of *Salmonella* infection in UK pigs, using four tests (MJ ELISA s:p 0.10 cut-off) and models with or without conditional dependence

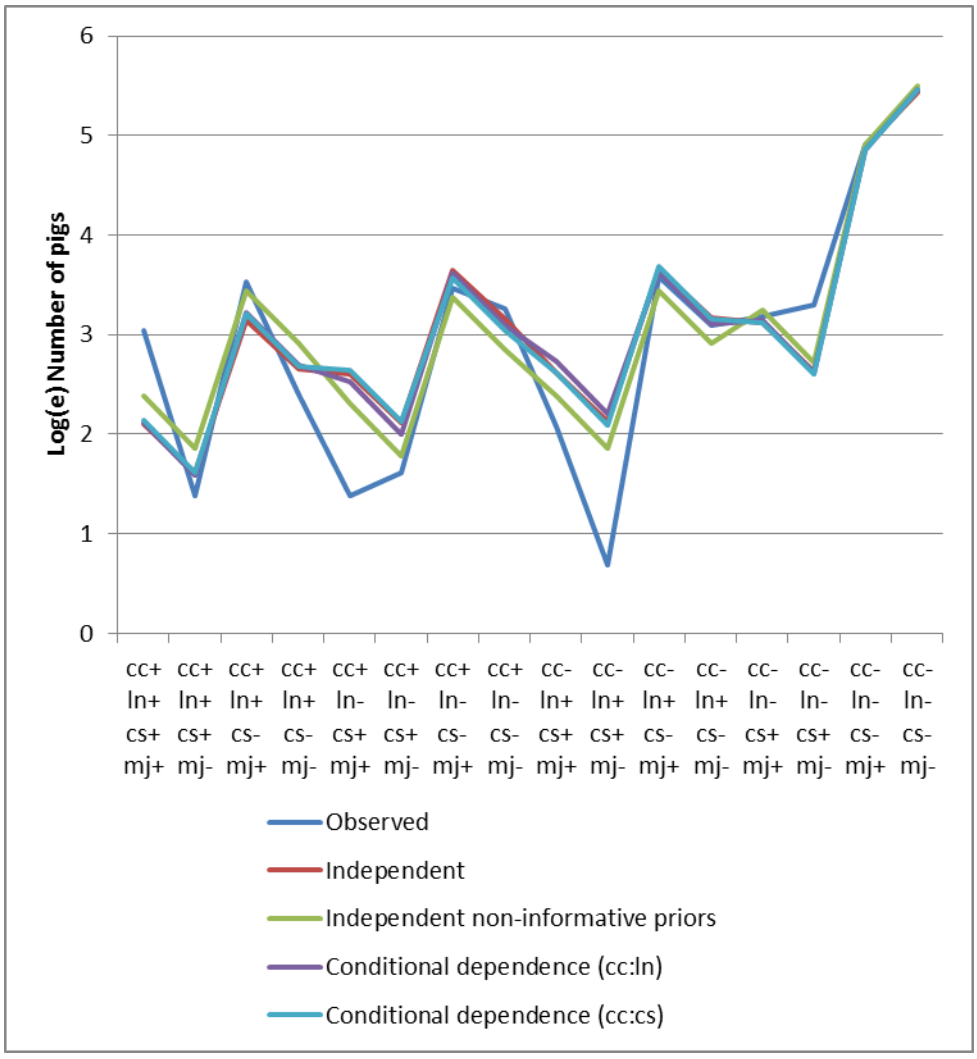

Table 3.7. A comparison of the predicted and observed prevalence of *Salmonella* infection as measured by 4 different tests in a survey of UK finisher pigs at slaughter using models without conditional dependence.

	MJ10				MJ25			
	Observed		Predicted		Observed		Predicted	
	No.	%	No.	%	No.	%	No	%
Cc	137.0	22.0	131.6	21.2	137.0	22.0	84.7	13.6
Ln	138.0	22.2	137.3	22.1	138.0	22.2	89.1	14.3
Cs	95.0	15.3	93.6	15.0	95.0	15.3	61.7	9.9
Mj	294.0	47.3	288.2	46.3	163.0	26.2	139.0	22.3

There was a greater discrepancy between the observed and predicted results when the s:p 0.25 cut-off was used. Since the predicted number of culture positive (cc, ln and cs) samples is less than that observed and the specificity of all culture methods was fixed as 100%, the model predicts that some of these true positive samples would have yielded false negative results. Table 3.7 shows the predicted and observed frequency of each combination of test result. A goodness of fit chi-square test was used to test the hypothesis that the model output would be close to the observed values. The model provides a poor fit to the observed data (chi-square p-<0.001).

As shown in table 3.8, although the mj10 model provides a closer fit to the observed data, it remains substantially different (p=0.059).

Table 3.8 A comparison of observed and predicted test results, MJ25 cut-off from a Bayesian analysis of tests for *Salmonella* in a sample of GB finisher pigs

	cc+ ln+ cs+ mj+	cc+ ln+ cs+ mj-	cc+ ln+ cs- mj+	cc+ ln+ cs- mj-	cc+ ln- cs+ mj+	cc+ ln- cs+ mj-	cc+ ln- cs- mj+	cc+ ln- cs- mj-	cc- ln+ cs+ mj+	cc- ln+ cs+ mj-	cc- ln+ cs- mj+	cc- ln+ cs- mj-	cc- ln- cs+ mj+	cc- ln- cs+ mj-	cc- ln- cs- mj+	cc- ln- cs- mj-
Observed	15	10	28	17	2	7	18	40	4	6	24	34	12	39	60	306
Predicted*	6	8	11	16	6	9	12	17	7	10	13	19	7	10	78	393

Pearson chi2(15) = 65.2396 Pr < 0.001

*Predicted values have been rounded

Table 3.9 A comparison of observed and predicted test results, MJ10 cut-off from a Bayesian analysis of tests for *Salmonella* in a sample of GB finisher pigs

	cc+ ln+ cs+ mj+	cc+ ln+ cs+ mj-	cc+ ln+ cs- mj+	cc+ ln+ cs- mj-	cc+ ln- cs+ mj+	cc+ ln- cs+ mj-	cc+ ln- cs- mj+	cc+ ln- cs- mj-	cc- ln+ cs+ mj+	cc- ln+ cs+ mj-	cc- ln+ cs- mj+	cc- ln+ cs- mj-	cc- ln- cs+ mj+	cc- ln- cs+ mj-	cc- ln- cs- mj+	cc- ln- cs- mj-
Observed	21	4	34	11	4	5	32	26	8	2	36	22	24	27	135	231
Predicted*	9	5	24	14	13	8	36	22	14	9	39	23	22	13	130	237

Pearson chi2(15) = 24.3996 Pr = 0.059

* Predicted values have been rounded

Three-test models

Interpretation of the results from the four test model is challenging, since the model fit was poor and as anticipated, many anomalous results were observed in which pigs were positive in some tests but negative in others. Culture of a carcass swab differs from the other tests, since the carcasses from pigs that were truly negative in MJ ELISA and culture before slaughter may have been contaminated during post-slaughter processing. In addition, the risk of carcass contamination varied amongst the 18 abattoirs. Therefore, the data were subjected to further analysis but excluding the carcass swab results. The code for each model is provided in the appendix.

Table 3.8 shows the output from 6 models and figures 3.3 and 3.4 show compare the predicted and observed outcomes for each of the 8 possible test combinations.

Table 3.10 Output from Bayesian models applied to three tests for *Salmonella* infection in a sample of GB finisher pigs

	Informative priors			Non-informative priors			Conditional dependence – cc:ln		
	Median	95% credible interval		Median	95% credible interval		Median	95% credible interval	
mj10		Lower	Upper		Lower	Upper		Lower	Upper
Secc	0.4052	0.3473	0.4672	0.4489	0.3625	0.5405	0.38	0.3268	0.4414
Seln	0.4243	0.3636	0.489	0.452	0.3633	0.5448	0.3948	0.3385	0.4581
Semj10	0.667	0.6009	0.7266	0.6635	0.5941	0.725	0.6664	0.6023	0.7269
Spmj10	0.7521	0.6829	0.8261	0.7119	0.6381	0.8039	0.7898	0.7122	0.8684
mj25									
Secc	0.5403	0.4776	0.6023	0.4496	0.3735	0.5346	0.5981	0.528	0.6633
Seln	0.5744	0.5098	0.6387	0.4515	0.3758	0.5391	0.6531	0.5732	0.7184
Semj25	0.4505	0.3847	0.5173	0.4526	0.3885	0.5174	0.4484	0.386	0.5175
Spmj25	0.8477	0.8088	0.8835	0.9216	0.858	0.9847	0.8321	0.793	0.8671

There is evidence for conditional dependence between the caecal content and lymph node sample in the mj10 model, since the 95% credible interval for the covariance term (covDp) does not include zero (0.0187 – 0.0707). Nonetheless, the 95% credible intervals for all parameters substantially overlap and thus, there is no evidence that adjustment for the conditional dependence has any important effect on parameter estimation. In contrast, the mj25 model did not show evidence of conditional dependence (covDp -0.1307 – 0.0518). Therefore, the independent models have been preferred for further discussion.

The estimate of the sensitivity of the MJ ELISA was increased (66.7%; cri_{95} 60.1% - 72.7%) and the MJ ELISA specificity was reduced (75.2%; cri_{95} 68.3% - 82.6%) when the mj10 cut-off was used compared to the mj25 cut-off (45.1%; cri_{95} 38.5% - 51.7% and 84.8%; cri_{95} 80.9% - 88.4% respectively). The sensitivity of culture of the caecal content and lymph node was also increased in the model using the mj25 cut-off.

As shown in table 3.10, the mj10 model assuming that all tests are independent fits the observed data (p=0.241) reasonably well. However, neither the independent nor the conditional dependence models with the mj25 cut-off predict results that can be judged to be similar to those observed (p=0.001 and p=0.043 respectively), as shown in tables 3.11 and 3.12. As described in the introduction, the titre of circulating antibodies declines after infection. The mj25 cut-off will result in a greater number of false negative test results, since a proportion of truly positive pigs will have an insufficient antibody titre to be classified as positive.

Figure 3.3 A comparison of predicted and observed results for three tests for *Salmonella* infection in pigs, using a s:p cut-off of 0.1 in the MJ ELISA.

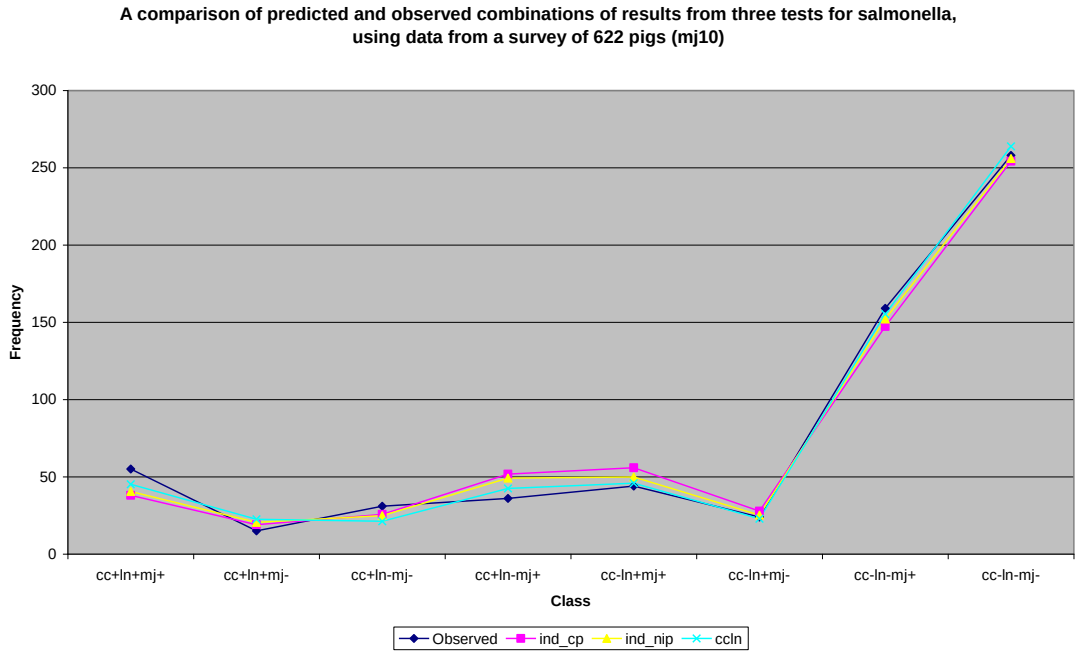

Figure 3.4 A comparison of predicted and observed results for three tests for *Salmonella* infection in pigs, using a s:p cut-off of 0.25 in the MJ ELISA

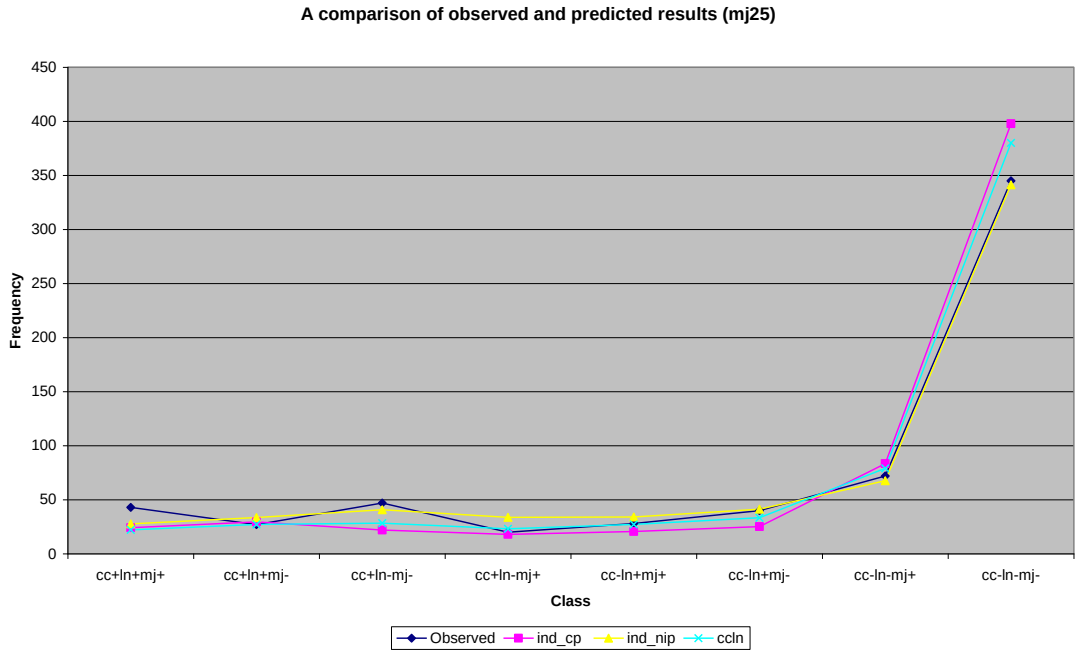

Table 3.11 A comparison of the observed and predicted prevalence of test results using the s:p 0.1 cut-off for the MJ ELISA test from a Bayesian analysis of tests for *Salmonella* in a sample of GB finisher pigs

	cc+ ln+ mj+	cc+ ln+ mj-	cc+ ln- mj-	cc+ ln- mj+	cc- ln+ mj+	cc- ln+ mj-	cc- ln- mj+	cc- ln- mj-
Observed	55	15	31	36	44	24	159	258
Predicted*	38	19	26	52	56	28	147	254

Pearson chi2(7) = 9.1721 Pr = 0.241
* Predicted values have been rounded

Table 3.12 A comparison of the observed and predicted prevalence of test results using the s:p 0.25 cut-off for the MJ ELISA test (all tests independent) from a Bayesian analysis of tests for *Salmonella* in a sample of GB finisher pigs

	cc+ ln+ mj+	cc+ ln+ mj-	cc+ ln- mj-	Cc+ ln- mj+	cc- ln+ mj+	cc- ln+ mj-	cc- ln- mj+	cc- ln- mj-
Observed	43	27	47	20	28	40	72	345
Predicted*	24	30	22	18	21	25	83	398

Pearson chi2(7) = 23.7312 Pr = 0.001
* Predicted values have been rounded

Table 3.13 A comparison of the observed and predicted prevalence of test results using the s:p 0.25 cut-off for the MJ ELISA test (conditional dependence between cc and ln) from a Bayesian analysis of tests for *Salmonella* in a sample of GB finisher pigs

	cc+ ln+ mj+	cc+ ln+ mj-	cc+ ln- mj-	cc+ ln- mj+	cc- ln+ mj+	cc- ln+ mj-	cc- ln- mj+	cc- ln- mj-
Observed	43	27	47	20	28	40	72	345
Predicted*	22	27	28	23	27	33	79	380

Pearson chi2(7) = 14.5037 Pr = 0.043
* Predicted values have been rounded

In all cases, models were also tested with a range of other plausible prior values. However, these did not provide any better fit.

Table 3.14. A Bayesian prediction of the sensitivity of culture for *Salmonella* from a three-test model: caecal content (Secc), carcass swabs (Secs) and ileocaecal lymph nodes (Seln) using data from an abattoir survey of 622 pigs in UK.

	Median	95% credible interval	
		Lower	Upper
Secc	0.8048	0.7742	0.8329
Secs	0.7482	0.7139	0.7807
Seln	0.8683	0.8407	0.8932

The sensitivity of the carcass swab (74.8%; cr_{95} 71.4%-78.1%) is lower than that of culture of lymph node (86.8%; cr_{95} 84.1%-89.3%) or caecal content (80.5%; cr_{95} 77.4%-83.3%). The greatest sensitivity is observed using culture of the lymph node. These estimates of sensitivity are far greater than those predicted in models that incorporated data from the MJ ELISA test.

Discussion

All of the tests considered in this study are in use today for monitoring *Salmonella* infection in pigs in abattoirs. It is widely recognised that there is imperfect agreement amongst these tests which has led to some loss of confidence amongst stakeholders in the pig sector (47) and this is particularly the case for the comparison of MJ ELISA and culture at slaughter (34, 42, 48, 49). For example, in one study, fewer than 25% of culture-positive pigs in an abattoir were MJ ELISA positive (50). This is also a challenge for policy-makers, since measuring progress towards a target for reduction in prevalence demands that reliable quantitative data are gathered. This study provides evidence for test selection and of the extent to which the different tests can or cannot be compared.

The tests that have been considered in this chapter are all used to evaluate the status of individual pigs and groups of pigs with respect to *Salmonella*. However, they each ascertain a different condition:

- Culture of caecal content aims to detect *Salmonella* bacteria within the lumen of the gut. This may following ingestion of contaminated material

resulting in transient passage of bacteria through the gut or may be due to an active infection.

- Culture of the mesenteric lymph nodes aims to detect current infection and *Salmonella* may or may not be present concurrently in the caecal content
- Culture of the carcass swab aims to detect surface contamination, which may or may not originate from the sampled pig
- The MJ ELISA test aims to detect antibodies against *Salmonella*, which are indicative of a prior infection and immune response. Thus, this test may be positive after infection has been eliminated from the individual pig.

It is therefore important to appreciate that these tests, even if they each performed perfectly on their own terms (i.e. with sensitivity and specificity of 100%), would not always all yield consistent results (all tests positive or all tests negative). Each test reflects events that may have occurred during the life of the pig and up to the moment that the carcass swab is collected – from ingestion of *Salmonella* through active infection, an immune response and potentially becoming a carrier status through to contamination after slaughter. Thus, dependence may be considered as reflecting the relationship between different stages in this history. For example, the lymph node result from a pig may have some dependence on the caecal culture result, since it is possible that recent ingestion of *Salmonella* has resulted in active infection. It is also possible that a lymph node positive pig has coincidentally ingested contaminated material shortly before slaughter and these two events in the life of a particular pig are not causally related. The benefit of combining these tests in a single model lies in enabling the prediction of the likely result from one test given the result of another. In routine surveillance, it is not cost-effective to employ all of these methods and this research will help to inform decisions regarding future tests.

In a comparison of serological samples tested by ELISA and faecal samples from pigs close to slaughter, it was noted that test cut-off had an important effect on sensitivity and specificity. However, the authors did not choose to

apply a Bayesian analysis to their data (45). Other authors have also commented on the significance of the test cut-off eg (35, 40). A Danish study of more than 1600 pigs from 167 herds compared results from culture of caecal samples, tonsils, mesenteric lymph nodes, carcass swabs and MJ ELISA tests, using two cut-offs similar to those in the current study (51). They found a positive correlation between the prevalence of MJ ELISA positive pigs and the prevalence of positive caecal contents, tonsils and carcass swabs for pigs but not for mesenteric lymph nodes from the participating farms. They also demonstrated correlations amongst all the sites that were cultured. However, although their data would have been appropriate for the Bayesian approach used here, the authors did not report test sensitivity.

Every one of the 16 different test combinations is biologically plausible and was observed in the field data. Results with respect to the sensitivity of culture of any of the three sample types used were in broad accordance with the published literature. The probability that any sample that is tested for *Salmonella* by culture yields a positive result depends upon a number of characteristics: the true prevalence of infection, the probability that a particular sample contains viable bacteria, the number of clusters of viable organisms within the sample and the presence of any inhibitory organisms or substances, e.g. alcohols, copro-antibodies etc (52). In addition, the handling of the sample between collection and onset of culture can lead to a loss of sensitivity and the steps in the isolation process are themselves prone to some error (53). For example, as the duration of culture increases, the medium tends to become more acidic, which inhibits growth of *Salmonella* (54). The published evidence for the sensitivity and specificity of the MJ ELISA is sparse. Several authors report on the validity of the MJ ELISA compared to a serum ELISA and have shown a good correspondence (39). However, there are only two limited experimental studies that offer a comparison with *Salmonella* infection (7, 55). The results reported here suggest that the MJ ELISA may have a lower sensitivity and specificity than previously anticipated and are in agreement with the frequent observation of a poor association between MJ ELISA and results of culture at slaughter (56). It has been reported that pigs may become infected with *Salmonella* during

transport from the farm and in the lairage prior to slaughter (9, 57-61). These pigs would not have detectable circulating antibodies and would therefore be MJ ELISA negative.

A ring trial of MJ ELISA tests available in 2000 concluded that there was a need for harmonisation (62) and the results from the EU MS that volunteered to use MJ ELISA tests in the baseline survey showed that no meaningful between-country comparison could be made. In the ZAP or ZNCP monitoring schemes in UK, the small sample size has already been shown to limit the value of testing to individual pig producers (6). The relatively poor sensitivity and specificity reported here further limit the value of the monitoring process and may confirm the anecdotal scepticism of some producers with respect to the test, which might lead to a state of complacency rather than a stimulus for change. Despite these issues, there may be some value for MJ ELISA testing as a relatively cheap and technically simple means of monitoring change at a national or regional level.

There is widespread recognition that the sensitivity and specificity of a test has an important impact on interpretation of results, especially when these are related to a herd level classification based on a relatively small number of individual samples. However, in a literature search few published papers relating to *Salmonella* infection in pigs were identified which proffered any estimate or that took account of these test characteristics (7, 39, 42, 46, 63). The Danish *Salmonella* control programme initially assumed that the MJ ELISA was perfect (64) although when it was revised, some account was taken of test validity. Results from a survey in Northern Ireland suggested that recent infection, for example during transport, could explain the disparity between MJ ELISA and culture (43) although the authors did not consider the sensitivity or specificity of the tests that were employed.

The validity of the output from the Bayesian models depends upon the underlying assumptions, which inform the priors. The 4 test models did not fit the observed data as closely as the 3 test models. It has been pointed out that the assumption that test sensitivity will not vary between populations may be

violated if, for example, one population has a very high prevalence of infection which may be associated with a greater number of bacteria per gram (65). However, the authors conclude that representative estimates will be provided by Bayesian models if at least one test has a true specificity of 100%. This is a valid assumption for microbiological culture where suspect colonies are subject to confirmatory tests and assuming that no cross-contamination of samples occurs, as was done in this study.

The interpretation of the estimated prevalence of *Salmonella* infected pigs from the Bayesian 4 test model is not straightforward. It is proposed that this might be defined as the proportion of pigs within a population that have ever been infected with *Salmonella* during life or have been contaminated after slaughter. Thus, the prevalence that is observed represents the combined effects of on-farm and abattoir factors. Since intervention to protect public health may involve both farm and abattoir practices, it may be considered that this is a useful measure. However, it may be a difficult measure to communicate and decision-makers may prefer to rely on estimates that are distinct for the pre and post harvest stages in the pig chain. The 3 test model, which considers caecal content and lymph node culture together with the MJ ELISA is more straightforward to interpret, since the prevalence that is estimated can be defined as the proportion of pigs that were ever infected with *Salmonella* up to the moment of slaughter. Test selection should primarily be driven by the purpose for which the results are required. Possible recommendations are listed below:

1. To measure the impact of an intervention on farm – use MJ ELISA and lymph node culture at slaughter. Culture alone may fail to identify a reduced prevalence, since only a proportion of pigs that have been infected during their lifetimes will be positive. Regular monitoring of pooled pen faecal samples during production would be more informative but would be prohibitively expensive for individual producers and is likely to be restricted to funded intervention studies.
2. To determine whether the public health threat from *Salmonella* in the pork chain has reduced – use culture of carcass swabs

3. To monitor progress towards a national reduction in *Salmonella* infection in pigs at slaughter over a period of 5 years – use lymph node culture at the start and finish, since this is required by the EU and use MJ ELISA (MJ10 cut-off) as a cost-effective on-going measure. Whilst it would be feasible to use lymph node culture as a monitoring measure, the costs of sample collection and of testing lymph node samples are greater.

Conclusion

This analysis has estimated the sensitivity and specificity for each of the four commonly-used methods to detect *Salmonella* infection in individual pigs. The results support previous studies that described the imperfect correlation between sample types and enable predictions to be made about the likely outcome from future UK surveys that use multiple tests.

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Chapter 4. A survey of *Salmonella* infection of finishing pigs in farms in Great Britain

Introduction

As described in Chapter 1, an outbreak of *Salmonella* Infantis in Denmark in 1994 was attributed to infection that originated in pigs (1). In addition to the public health impact and damage that the outbreak had upon consumer trust in Danish pigmeat, exports of pigmeat and pig meat products were important to the Danish economy. Therefore, a monitoring and control scheme was introduced by the Danish pig industry to safeguard public health and protect the reputation of the industry in the European and international markets (2). These events alerted policy-makers in other countries to the potential threat to public health posed by *Salmonella* infection in pigs. Knowledge of the prevalence of infection within a country was fundamental to making informed, evidence-based decisions on whether control was necessary. In Great Britain (GB), the Government funded a survey of pigs, cattle and sheep in 1999-2000 to estimate the prevalence of foodborne zoonoses including *Salmonella*, *Yersinia* and verotoxigenic *E. coli*. This showed that *Salmonella* was present in the caecal content of approximately a quarter of GB pigs (Davies *et al*., 2004), which was a higher prevalence than had been observed in an earlier survey in Denmark, in which approximately 6% of pigs carried *Salmonella* infection in the caecal content (3). As a direct response to the GB survey, the GB industry, supported by the Food Standards Agency (FSA) initiated the Zoonoses Action Plan *Salmonella* monitoring programme (ZAP) in 2001 (4). This was based on Danish experience and utilised the meat juice ELISA test (MJE) to place herds into one of three “ZAP” categories. Initially, farms with a prevalence greater than 85% were defined as ZAP level 3, those with a prevalence of 65% - 85% were defined as ZAP level 2 and those below 65% were classed as ZAP level 1. Later, the cut-off for ZAP level 1 was reduced to 50%. ZAP only applied to farms that sent pigs for slaughter to British Quality Assured Pork (BQAP) abattoirs – however, these accounted for more than 80% of all pigs slaughtered in GB. Farms with a ZAP 2 or ZAP 3 score were required to act to reduce the prevalence of *Salmonella* or

eventually face loss of their quality-assured status. ZAP data were available at the individual farm level but since the number of samples per farm varied considerably, ZAP only provided a limited picture of the variation in prevalence of infection within GB pig herds (5). In addition, as discussed in Chapter 3, there is a weak association between MJE and caecal culture. Finally, there was scant evidence of risk factors that were associated with infection on GB farms. Information on these risk factors was necessary to inform the design of interventions that could be applied by those farms shown by ZAP to have a high MJE prevalence.

The cross-sectional study reported in this chapter was due to be conducted in 2001-2002 but was delayed by the 2001 Foot and Mouth Disease epidemic, which precluded unnecessary visits to livestock farms. The aims of the study were:

- To estimate the prevalence of *Salmonella* infection in a random sample of GB farms rearing pigs to slaughter, using bacteriological and MJE tests;
- To investigate the association between culture and MJE results at a farm level;
- To investigate the association between putative risk factors and *Salmonella* infection at farm level

It was anticipated that the results from this survey would inform the design of interventions and also be important for quantitative risk assessments.

After the survey had been approved but before it was started, Defra identified an additional requirement to investigate post-weaning multisystemic wasting syndrome (PMWS) and it was decided to recruit farms in parallel for the *Salmonella* and PMWS surveys.

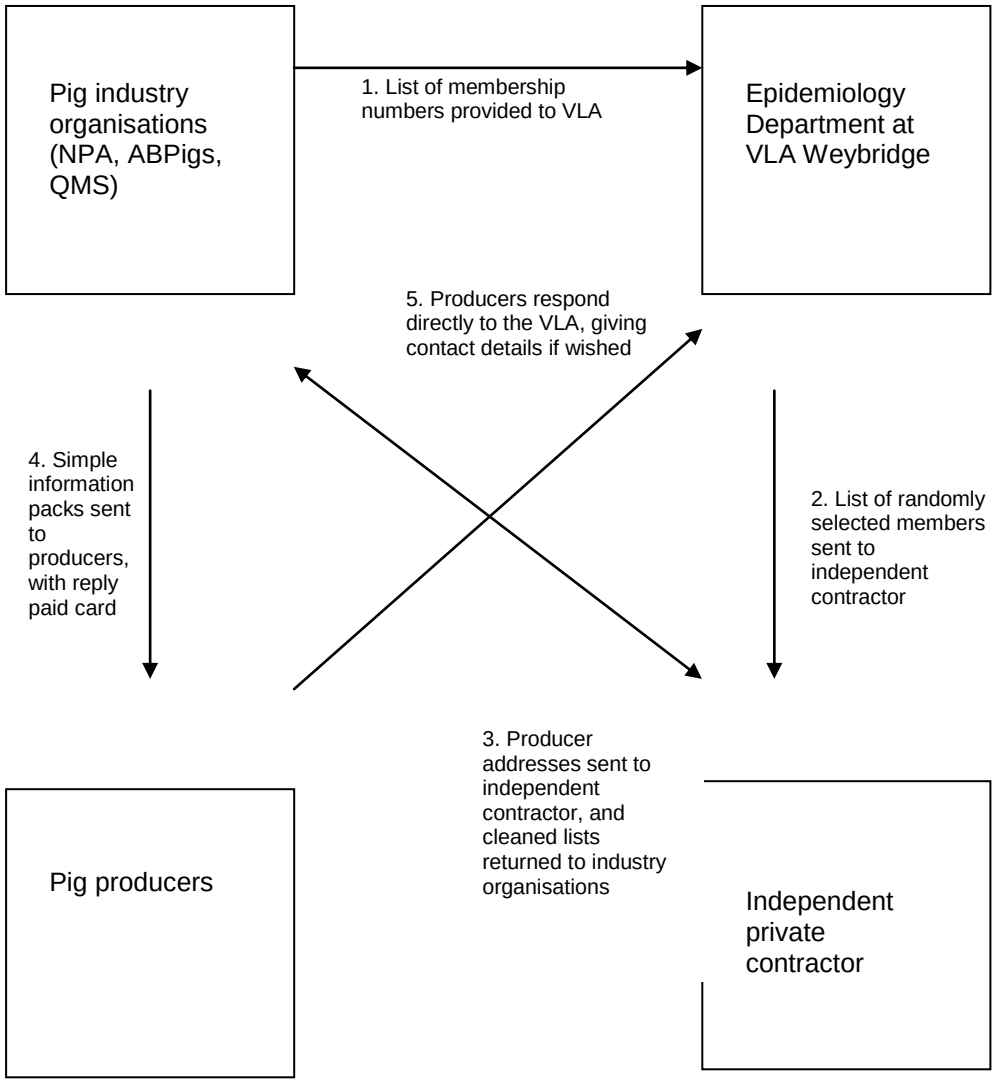
Materials and methods

In advance of study recruitment in late 2002, this project was publicised through articles published in the agricultural press (Farmers Weekly and Farmers Guardian); and through the websites and newsletters of the National Pig Association and the Pig Veterinary Society.

Discussions were held with representatives of three organisations that cover a large majority of GB pig production – the National Pig Association (NPA), Assured British Pigs (ABPigs), and Quality Meats Scotland (QMS). While many small-scale producers were not associated with these bodies, it was estimated that approximately 86% of pigs slaughtered in the UK were raised on farms which were members of these quality assurance schemes (6).

To safeguard their members' anonymity, lists of membership numbers alone were released to the VLA in the first instance and a sample of membership numbers was selected at random from each list. An independent private contractor received the selected membership number from VLA and the farm contact details from the 3 schemes. This company 'cleaned' the final lists to avoid the same farm being contacted twice, and to ensure that we contacted a sufficient number of producers. The VLA provided information packs to each of the organisations to send to the selected members, along with a reply paid card with space for the producer to supply us with their contact details. Thus, each selected farm was initially contacted via their assurance scheme and invited to contact us if they wished to participate. The recruitment process is summarised in figure 4.1.

Figure 4. 1: The recruitment process for a survey of British finisher pig producers



Invitations were sent to over 400 farms, in a phased recruitment process over 5 months commencing in December 2002. Lists of non-responding producers were compiled by comparing the membership numbers of selected members with respondents and the industry organisations sent a single reminder letter to the non-responders; this was done in collaboration with the industry to assure anonymity. Criteria for joining the study were as follows:

1. They did not sell produce directly to the public – the isolation of *Salmonella* from such a premises might lead to further action if it were deemed on this evidence alone that an important public health threat existed. Such action might jeopardise the commercial interests of the producer.

2. They must have at least 100 breeding females if breeder/finishers, or 200 finisher places if specialist finishers – this condition was designed to exclude small-scale producers that represent a large proportion of farms but that rear a relatively small proportion of all pigs.

On joining, farmers were sent an initial farm questionnaire and asked to provide their private veterinary surgeon's (PVS) contact details. Farmers received two additional questionnaires after the first had been completed, one for information in respect of PMWS and a second on feeding practices. The PVS was also asked to complete a farm health questionnaire and to arrange to visit the farms to collect 30 pooled pen floor faecal samples for *Salmonella* culture. A standardised protocol for sample collection was issued to all of the PVSs involved and the first visits commenced in December 2002. Veterinary costs were paid by VLA Weybridge; no compensation was offered to participating farmers for their time but they did receive the results from the samples collected from their own farms, including tests for PMWS (not reported here) which was a very topical issue at the time. All samples were posted to the National *Salmonella* Reference Laboratory at the Food & Environmental Safety Department at VLA Weybridge to be cultured and serotyped according to VLA Standard Operating Procedures (SOPs). Details are given in Chapter 2 (Laboratory Methods).

The farmers were contacted again to obtain details of the abattoir to which the sampled batch of pigs was being sent. Using address lists supplied by the Meat & Livestock commission (MLC), all the British Quality Assured Pork (BQAP) abattoirs in the country had previously been sent details of the study. The abattoirs were then contacted with details of the individual batches of pigs from which samples were required. Samples of the neck or diaphragm muscle were collected, normally by abattoir staff, though sometimes with the support of VLA Epidemiology or Regional laboratory staff or the farmers' own private veterinary surgeon. These samples were frozen and sent to the VLA Regional Laboratory at Bury St Edmunds where they were tested for antibodies to *Salmonella* using the Guildhay Vetsign Meat-Juice ELISA kit following the manufacturer's instructions (see Chapter 2).

Salmonella prevalence on each farm was assessed by collection of 30 pooled pen floor faecal samples for culture and 40 meat juice samples collected after slaughter for testing by MJ ELISA to detect antibodies against Group B and C_1 *Salmonella*. Pooled pen samples were preferred to individual faeces because: 1) stress to the pig was minimised 2) *Salmonella* excretion is intermittent and a negative sample may be obtained from an infected individual pig; 3) there was a reduced cost, since pooled pen sampling is quicker and farm staff can feasibly be trained in such sample collection for large scale studies. Subsequent research showed that pooled sampling was a sensitive method to detect pen level infection (Arnold *et al* 2005).

Contact was maintained with farmers throughout by telephone and post to check any information which was missing or unclear on the questionnaires. Copies of their farm results were sent to the farmers and their PVSs, and at the end, a summary of the overall results was sent to every participant.

All data were collected in the Epidemiology Department at VLA Weybridge for entry onto a bespoke MS Access database by staff trained in data entry. All data analysis was performed using STATA releases 8 and 9 (Statacorp). Initially, descriptive statistics were used to summarise the distribution of recorded variables, using the mean and standard error for normally distributed variables and the median and inter-quartile range for other continuous variables as appropriate. 2 x n tables were used to summarise categorical variables. Univariable and multivariable analyses were conducted using either the pooled pen culture status or the individual pig MJE result as an outcome. All analyses were adjusted for clustering by farm by using generalised estimating equations (GEE).

Results

The survey was conducted between December 2002 and August 2003. A total of 416 farmers were invited to participate. Responses were received from 345 (82.9%) and 107 of these (31%) agreed to participate. Of the farms which gave a reason for not taking part 78 were ineligible; 2 because they sold produce direct to the public, 11 did not produce finisher pigs, and 65 no longer kept pigs or had too few animals. The GB pig industry was undergoing a significant contraction during this period and many farmers were leaving the industry. A further 34 farms stated that they were unable to take part due to a lack of time and/or money, and 24 gave a range of other reasons. Thus, 107 of 267 eligible farms participated (40%).

Descriptive analysis

A total of 107 farms were enrolled into the survey. A veterinary questionnaire was received for all 107 farms. However, only 99 respondents submitted a farm questionnaire and only 90 respondents submitted a feed questionnaire.

Eighty-seven farms (81%) submitted 30 pen faecal samples as requested; 18 farms submitted fewer samples than requested (8-29 samples) and 2 farms submitted more samples than requested (31 and 40). There were no *Salmonella* positive pens on 31 farms and the number of positive pens on the remaining 76 farms ranged from 1-29.

A total of 3062 pooled pen floor faecal samples were collected and *Salmonella* was isolated from 651 (21.3%) of these. A positive Salmonella sample was detected on 76 of the 107 farms (71.0%; 95% confidence interval 62.3%-79.8%). Figure 4.2 shows the observed distribution of pen prevalence amongst the 107 farms that participated in the survey and Table 4.2 shows the different serovars that were identified. Some samples yielded more than one serovar, hence the total number of serovars in table 4.2 (679) exceeds the number of positive pens.

Figure 4.2 – Distribution of farm-level pooled pen prevalence of *Salmonella* infection amongst 107 GB pig farms sampled in 2002-2003

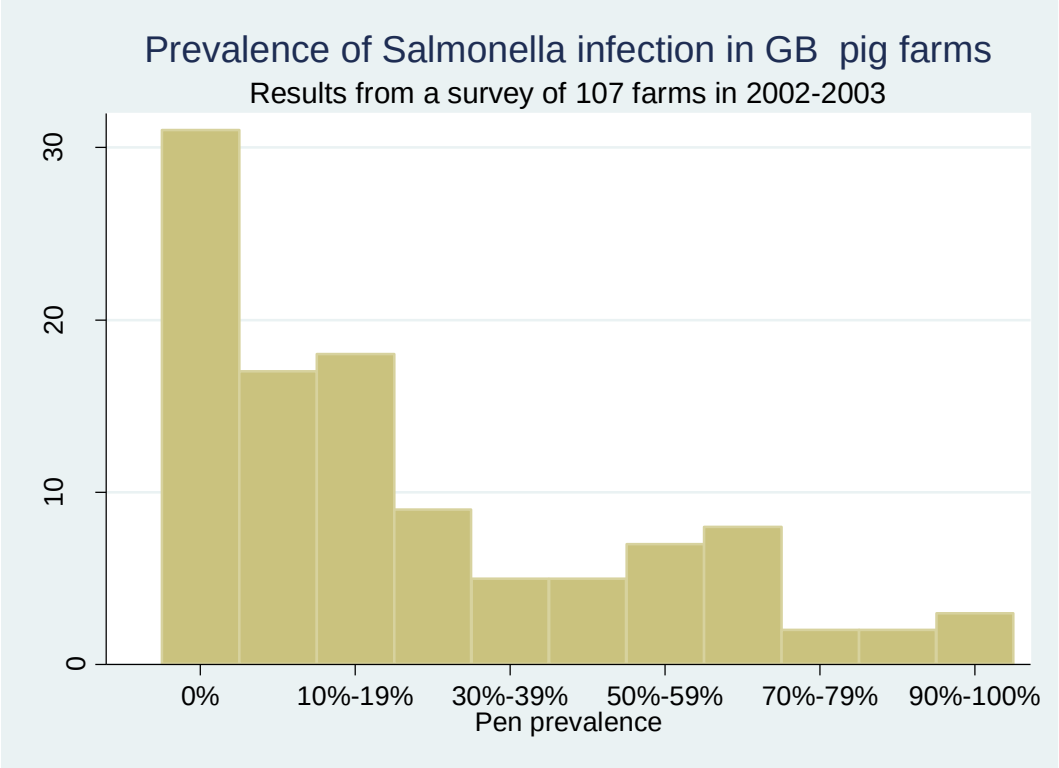


Table 4.1: Serovars of *Salmonella* identified in a survey of 107 pig farms in GB

Serotype	Number of Samples (N=3062)	Number of farms (N=107)
BINZA	1	1
DERBY	136	20
GOLDCOAST	14	4
KEDOUGOU	42	11
LONDON	10	7
MANHATTAN	4	2
MONTEVIDEO	1	1
MUENCHEN	1	1
READING	21	6
SCHWARZENGRUND*	16	2
TAKSONY	5	2
THOMASVILLE	2	1
TYPHIMURIUM*	426	60

** The following partial classifications were included with Typhimurium: 4,12:-:1,2 4,12:I:- ORough:I:- ORough:I:1,2.*

4,12:D:- was included with Schwarzengrund

Up to 5 isolates of *S*. Typhimurium were phage typed per farm - if more than 5 isolates were found, then the 5 to be tested were selected at random. A total of 225 *S*. Typhimurium isolates were tested and the phage types that were found are shown in table 4.2.

Table 4.2: Phagetypes of *S*. Typhimurium identified in a survey of GB finisher pig farms

Phagetype	Number of Samples (N=225)	Number of farms (N=107)
104	31	12
104B	4	3
120	3	1
167 variant	7	2
170A	2	1
193	41	20
193A	5	3
195	3	1
204 variant	3	1
208	5	2
40	1	1
RDNC*	16	5
U288	81	21
U302	9	7
UNTY*	14	5

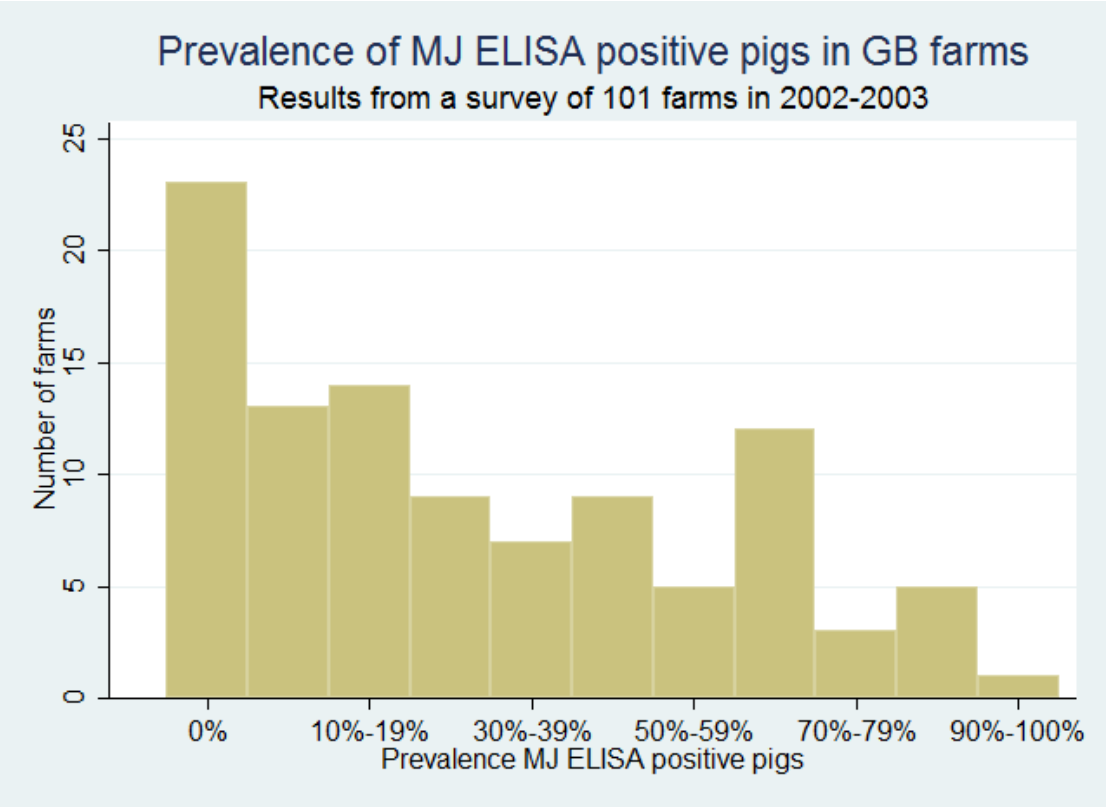
* *RDNC – reacts with the phages but does not conform to a recognised pattern of lysis*

* *UNTY – A culture which does not react to any of the phages in the typing scheme.*

A total of 3300 MJ samples from 85 farms were collected and tested. Using the s:p ratio cut-off of 0.1 (mj10), 1,551 of these were positive (47%; 95% confidence interval 45.3%-48.7%). Six farms did not submit any MJ samples; 3 farms submitted more than 40 MJ samples (80, 43, 41 respectively) and 27 farms submitted fewer than 40 MJ samples (17-39). Overall, 80 of these farms (94.1%; 95% CI 89.0% - 99.2%) had at least one MJ ELISA positive pig, using the 0.10 s:p cut-off (mj10). The s:p ratio cut-off of 0.25 (mj25) was used by ZAP at the time of the survey and 819 of the 3300 samples were positive on this basis (24.8%; 95% confidence interval 23.3%-26.3%). This result is very similar to the national results from ZAP at this time.

Figure 4.3 shows the proportion of samples that tested positive for antibodies to *Salmonella* in the MJ ELISA test with a cut-off SP value of 0.10. This cut-off is recommended for research purposes, as it is more sensitive than the 0.25 cut-off used for monitoring purposes.

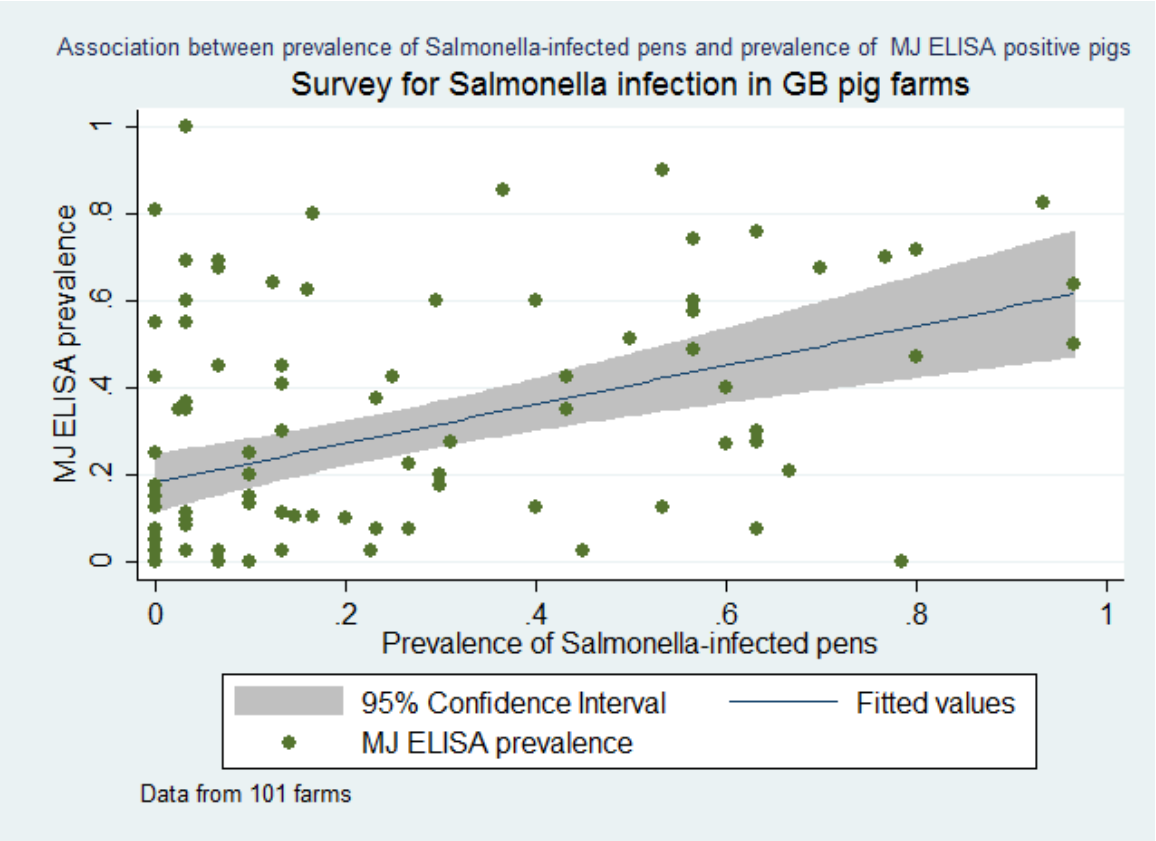
Figure 4.3: Prevalence of MJ ELISA positive pigs within pig farms recruited to a survey in GB, using a s:p 0.10 cut-off



On 58 farms, both the mj10 and pen status were positive for *Salmonella*. There were only 4 farms that were negative in both tests whilst 22 farms were positive by MJ ELISA but negative by pen culture and just one farm was positive by pen culture but negative by MJ ELISA.

The association between the prevalence of positive pooled pen floor faecal samples and the prevalence of MJ ELISA positive pigs within farms was investigated using linear regression. The results are shown in Table 4.3 and illustrated in Figure 4.4.

Figure 4.4: Scatterplot of prevalence of *Salmonella*-infected pens of pigs and MJ ELISA positive pigs in a GB survey showing the best-fitting regression line and 95% confidence interval.



It is apparent from figure 4.4 that there are many paradoxical results at a farm level, where the prevalence of positive pooled pen samples is high and the prevalence of MJ ELISA positive pigs is low or *vice versa*. Some of this variation may be explained as firstly, the MJ ELISA only detects antibodies against Group B and C_1 *Salmonella*. Whilst these groups contain the most prevalent serovars including *S*. Typhimurium and *S*. Derby, they do not include eg *S*. Goldcoast, *S*. London or *S*. Kedougou. Secondly, since the pigs from which the MJ ELISA samples were obtained were not necessarily those from the sampled pens, it is possible that they had not been infected or that they had been infected either much earlier and so their antibody titres had waned or they may have been infected very recently, so that a detectable titre had not yet been reached. Finally, both of these measures are indicators of the *Salmonella* status of the farm although one is based on infected pens whilst the MJ ELISA is based on individual pigs. A single actively-excreting pig within a pen may be sufficient to

yield a positive result and the prevalence of infection within pens of pigs in a single farm can vary significantly. Thus, it would be expected that the individual pig prevalence varied amongst farms with the same pen-level prevalence, as suggested by figure 4.4.

Table 4.3. Results for the linear regression of pen prevalence (PenPrev) on MJ ELISA (mj10 cut-off) prevalence (MJE prev) in a survey of GB pig farms for *Salmonella* infection.

PenPrev	Coefficient	Standard Error	*T*	P >|*t*|	95% CI
MJE prev	0.5428	0.1124	4.82	<0.001	0.3190-0.7666
Constant	0.3214	0.0408	7.87	<0.001	0.2402-0.4026

The square of the correlation coefficient (r^2) equals 0.2190, indicating that about 22% of the total variation in MJ ELISA prevalence might be accounted for by the variation in pen prevalence. As discussed above, there are other important factors including the serovars present on a farm and the within-farm variation in within-pen prevalence that will also influence this association. Therefore, these results indicate that the association between the prevalence of *Salmonella* through culture of pooled pen floor faeces and MJ ELISA prevalence is limited.

Table 4.4 Univariable analysis, showing the association between recorded variables and *Salmonella* status of either pens of pigs (faecal culture) or individual pigs (Meat Juice ELISA) adjusted for clustering by GEE.

Variable	Class	No. of Farms	No. of Pens		Odds Ratio (ci)	No. of Pigs		Odds Ratio (ci)
			Salm+	Salm-		MJ+	MJ-	
QA scheme	No	21	201	357	1.00	326	184	1.00
	Yes	78	440	1824	0.24 – 0.90	1280	1480	0.48 (0.21 – 1.08)
Farm boots provided	No	8	43	175	1.00	72	88	1.00
	Yes	89	598	1946	0.32 (0.12 – 0.84)	1488	1522	0.54 (0.20 – 1.44)
Farm overalls provided	No	11	26	276	1.00	124	196	1.00
	Yes	86	615	1845		1456	1414	1.81 (0.67 – 4.85)
Contractor vermin control	No	69	496	1437	1.00	1124	1106	1.00
	Yes	28	129	708	0.53 (0.27 – 1.02)	441	519	0.84 (0.44 – 1.61)
Mortality group	<= 2%	60	1454	359	1.00	1170	943	1.00
	>2%	19	302	199	2.48 (1.19 - 5.19)	319	442	1.71 (0.83 – 3.52)
Home mix ration	No	52	456	100t6	1.00	958	792	1.00
	Yes	36	132	938	0.31 (0.15 – 0.62)	504	816	0.53 (0.29 – 0.96)
Pelleted feed used	No	11	25	294	1.00	143	217	1.00
	Yes	78	570	1681	4.20 (0.97 – 18.20)	1357	1353	1.47 (0.50 – 4.29)
Written biosecurity plan	No	17	69	392	1.00	194	286	1.00
	Yes	79	545	1726	1.85 (0.96 – 3.53)	1330	1340	1.39 (0.57 – 3.38)
Salmonella action plan	No	60	294	1418	1.00	990	1050	1.00
	Yes	34	314	646	2.22 (1.24 – 3.98)	563	517	1.05 (0.57 – 1.95)
Rotavirus	Negative	100	628	2232	1.00	1606	1584	1.00
	Positive	7	23	179	0.43 (0.20 – 0.92)	75	165	0.48 (0.16 – 1.48)
PCV2	Negative	22	595	41	1.00	590	281	1.00
	Positive	85	1816	610	(1.82 – 13.72)	1159	1270	2.28 (1.17 – 4.43)
Systemic salmonellosis	Negative	103	634	2338	1.00	1651	1659	1.00
	Positive	4	3	107	0.12 (0.03 – 0.43)	30	90	0.37 (0.06 – 2.31)

A large number of potential risk factors were investigated through the questionnaires. Table 4.4 shows the results for those variables that were associated with either the MJ ELISA using a cut-off signal:positive ratio of 0.10 or the pen culture outcome in a univariable analysis. The estimated standard error of the odds ratio must be adjusted to account for clustering by farm. Various approaches are available to correct for within-farm clustering, including use of robust standard errors, random effects models and generalised estimating equations (GEE). In this case, GEE has been used as this approach adjusts both the standard errors and parameter estimates. It has been recommended for logistic regression analyses, especially where the cluster effect itself is not of major interest (7). Furthermore, the univariable association between one explanatory variable and the outcome of interest may be confounded by other variables or exhibit collinearity with one or more variables. Therefore, a multivariable analysis must be conducted. As noted, there was a modest correlation between the two outcome variables. The MJ ELISA result for an individual pig represents a retrospective measure of whether the pig was infected with *Salmonella* at any point in its life, subject to a sufficient concentration of antibodies being present in the meat juice at the time of slaughter. In contrast, the result of the pooled pen floor faecal sample culture demonstrated whether one or more pig within a pen was infected with *Salmonella* at the moment that the sample was collected, subject to the sensitivity of the test. Since the questionnaire also measured farm status with respect to the explanatory variables at the time of the visit, it was decided to use the pooled pen sample result as the primary outcome variable. At the end of the multivariable analysis, the final model was repeated but using MJ ELISA result as the outcome of interest.

A large number of variables were studied in this survey and some associations could occur by chance. Therefore, those variables that did not show any evidence of an association with pen *Salmonella* status in the univariable analysis were not considered further. In addition, any variable where more than 30% of the values were missing was dropped. The explanatory variables were considered in three groups: farm characteristics including management, feed variables and disease variables. Each group was considered separately and

those variables that showed an important association with presence of *Salmonella* in the pooled faecal sample were then combined to create a final multivariable logistic regression model that could include variables from all three groups. An important association was defined as having an odds ratio and 95% confidence interval that did not include 1.00 and that was biologically plausible on the basis of consistency with the published literature. At each stage, individual variables were reintroduced to multivariable models to ensure that they did not exert any important confounding by identifying whether inclusion of a potential confounding variable resulted in any significant change in the odds ratio for the other variables in the model. The final model was tested for biologically plausible interactions by adding the relevant interaction term to the multivariable model

.

Previous experience whilst developing this study had shown that farmers frequently under-estimate the scale of the mouse and rat population on their farms. In this study, two variables were used: the farmers' opinion as to whether vermin were a problem and whether or not vermin control was conducted by a contractor. Only the use of a contractor was associated with pen *Salmonella* status (OR 0.53; 95% CI 0.27-1.02; p=0.058).

Feed was known to have an important effect on *Salmonella* infection in pigs as it mediated the gut environment. In this study, univariable analysis showed evidence that several feed-related variables were associated with pen *Salmonella* status. These included use of wet feed, compound feed and home mix rations. Many of these variables exhibited considerable collinearity, which is unsurprising since, for example, wet feed is by necessity home mixed and if a home mixed ration is being fed then commercial compound feed is less likely to be used. There was evidence of a strong association between use of a home mix ration and pen *Salmonella* status (adjusted OR 0.31; 95% CI 0.15-0.62) and once this variable was included in any model, the addition of other feed-related variables did not demonstrate any important additional effect.

Information on pig diseases was provided by the farmers' PVS. Two conditions – post weaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS) – have previously been associated with *Salmonella* infection in pigs (8). The aetiology of both conditions is linked to

infection with porcine circovirus type 2 (PCV2) (9), so a new variable (PCV2) was created and farms were classed as PCV2 positive if either condition were reported by the vet. This was strongly associated with pen *Salmonella* status (OR 5.00; 95% CI 1.82-13.72) and there was no evidence that other disease variables were importantly associated with the outcome if models contained this PCV2 variable. A second indicator of the health of the pig herd was provided by the reported mortality rate amongst finisher pigs. These data were provided by the farmer and are frequently used in farm health management, so farm records were generally of good quality. Mortality data were dichotomised to above 2% or at or below 2%, since this was the accepted industry standard at the time. Pens on farms with a higher mortality were more likely to be *Salmonella* positive (adjusted OR 2.48; 95% CI 1.19-5.19).

A multivariable model comprising PCV2, home mix, finisher mortality group and vermin control by a contractor was tested. Data from 2053 pen samples collected on 70 farms were included in the final model. The results from this final model are shown in table 4.5 below.

Table 4.5. Risk factors for *Salmonella* infection in pens of pigs in a GB survey: a multivariable logistic regression analysis adjusted for clustering using generalised estimating equations (GEE).

Variable	Odds Ratio	95% CI	p-value
Home mix	0.41	0.21 – 0.80	0.009
PCV2	4.20	1.45 – 12.13	0.008
Finisher Mortality	2.00	0.93 – 4.28	0.075
Contracted vermin control	0.41	0.20 – 0.83	0.014

The association between pen status and finisher mortality did not demonstrate conventional statistical significance (p=0.075) and the model was refitted without this variable. This analysis included data from a total of 85 farms and the associations observed between home mix, PCV2 and contracted vermin control and pen *Salmonella* status remained, with no important change in the estimated strength of each association (OR and 95% CIs were 0.39 (0.20-0.76), 4.04 (1.49-

10.93) and 0.49 (0.25-0.97) respectively). The model that included finisher mortality was preferred as this association has relevance to farmers. Mortality is an important proxy for the general health status of the farm and losses through mortality can have an important effect upon the financial profitability of an enterprise.

The final model was repeated but using mj10 as the outcome variable. The only variable that showed an important association with this outcome was PCV2 (0R 2.38; 95% CI 1.15 - 4.93). A model using mj25 as the outcome variable did not provide evidence of any important association with any explanatory variables.

Discussion

This survey showed that 71.0% (95% confidence interval 62.3%-79.8%) of 107 GB finisher pig farms contained pens of pigs that were infected with *Salmonella* and 94.1% (95% CI 89.0% - 99.2%) of 85 farms produced finisher pigs that were MJ ELISA positive (mj10 cut-off) at slaughter. This is the lower bound of the prevalence of infected farms, since a sample of pens and pigs from each farm was tested. It is possible, especially where prevalence is low, that by chance no positive MJ ELISA pigs or *Salmonella* positive pooled pen samples were collected from farms that were infected.

The survey provided unique evidence of the prevalence of *Salmonella* infection in finisher pigs in GB at a holding level. Previous GB surveys of finisher pigs were conducted at the abattoir (10-12) and thus were not intended to evaluate the variation in prevalence amongst holdings nor to investigate risk factors at a farm level that might be associated with prevalence. A subsequent GB survey conducted as a part of an EU-wide baseline study of infection in finisher pigs was also abattoir-based (12) and therefore, this remains the only GB farm-level survey of finisher pigs. In 2008, a further EU baseline survey of breeding pig holdings was conducted and this was farm-based (13, 14). However, finisher pigs were not included in this survey and it did not estimate within-herd prevalence, instead categorising holdings as positive or negative where it was assumed that on a positive holding, at least 10% of breeding pigs were infected with

Salmonella. Monitoring of quality-assured pigs at slaughter for the BPEx ZAP/ ZNCP programmes provided evidence of the prevalence of MJE positive pigs. However, the ZAP programme reported the mj25 cut-off and would, therefore, under-estimate the true prevalence of pigs that had been exposed to *Salmonella* infection during their lifetimes. The ZNCP reported the prevalence of positive pigs using the mj10 cut-off but a reduced number of samples were tested. Furthermore, although there are now data for several years for many finisher pig holdings, the number of samples collected from any particular batch of pigs was typically insufficient to enable any precise estimate of prevalence (5).

The MJE results from this survey were comparable with the ZAP/ ZNCP results using the mj25 cut-off. Overall, 24.8% (95% CI 23.3% - 26.3%) of 3300 samples were positive and these samples originated from 64 of the 85 farms (75.3%; 95% CI 65.9% - 84.7%) that submitted MJ samples. The mean prevalence of MJE positive (mj25) pigs in GB has been consistent throughout previous surveys and in the ZAP/ ZNCP programmes.

Overall, 107 of 416 invited farms (25.7%) participated in the survey. No response was received from 71 farms. Of the 345 that did reply, 65 farms no longer kept pigs and a further 13 were ineligible to participate. Amongst the remainder, 34 declined due to a lack of time and 24 reported a diverse range of reasons for rejecting the invitation. The remainder simply sent a negative response with no explanation.

This survey provided additional evidence to the EFSA surveys (12, 13) that the prevalence of *Salmonella* infection in GB pigs is greater than that in most European countries. One feature of GB pig production is that there is a greater preponderance of outdoor breeding units than in most EU MS (http://www.bpex.org.uk/prices-facts-figures) and this may be associated with an increased risk of *Salmonella* infection (15). Furthermore, the GB pig industry has been contracting as a result of poor margins (16). Consequently, it has proved difficult for individual farmers to raise capital for basic maintenance and improvements to the pig accommodation. Many farms have damaged floors that trap faeces and other materials and insulation panels are cracked or broken,

providing a haven for vermin. Amongst large integrated enterprises, finisher pigs are often raised to slaughter weight by contractors who own the facilities but not the pigs within. The contracted farmers typically receive pigs from several sources and this mixing both increases stress and the risk of introducing *Salmonella* into the finisher unit. Many of these units are solid-floored systems, which may also confer a greater risk of transmission of *Salmonella* (17).

Serological surveys from Belgium (18), Denmark (19, 20), Greece (21), Eire (19), Netherlands (22) and Canada (23, 24) and the US states of Wisconsin, North Carolina, Ohio (15) and Iowa (25) have focused at the abattoir level. A comparison of results is hampered by the different ELISA methods and cut-offs that have been employed. Whilst MJ samples are most commonly used, some countries, e.g. Belgium and Netherlands, have used serum derived from whole blood as the test substrate. Notwithstanding these methodological differences, the picture in GB is consistently poor by European and international standards.

Microbiological culture has been employed by several authors in studies of *Salmonella* infection of pigs in abattoirs and farms. Sources of variation include the nature and weight of the sample and the isolation methods employed (26-32). The EU baseline surveys had the advantage that the same methods were employed across all EU Member States (12, 13). On farm, faecal sampling has been employed most frequently. However, this has the disadvantage that the pig must be restrained before a rectal swab can be inserted and the pig may be provoked to sudden and vigorous reaction against the procedure, risking injury to itself or the handler. In GB, the procedure is subject to Home Office regulation when conducted for research purposes, which introduces an important added difficulty for recruitment of farms and expense through bureaucracy and a restricted pool of licensed staff. The quantity of faeces that may be recovered is often small and since excretion is intermittent, the sensitivity of this approach is relatively poor (33). Pooled pen floor faecal sampling was employed in this study to obviate these issues and has subsequently proved a robust and sufficiently sensitive method for farm-level surveys (34, 35), being selected as the method of choice for the EU Baseline survey for *Salmonella* in pig breeding herds. Although environmental sampling alone or in combination with other approaches has been

used by some researchers, none of these have been employed in a national survey but rather in studying smaller numbers of herds, often repeatedly (23, 36-49). In addition, the details of how the pooled or environmental samples have been collected are often absent from published reports. Thus direct comparison of the results of this survey and others is not possible.

The predominant serovar isolated in this survey was *S.* Typhimurium. This serovar has consistently been the most frequently identified in surveys and other studies of pig herds in GB as well as in clinical samples examined in VLA's Regional Laboratories (10, 11, 50-52). Although some of the phage types that were isolated (e.g. U288) are strongly associated with pigs, many are widespread amongst all domestic livestock species. *S.* Typhimurium is also the second most frequent cause of human salmonellosis in GB (53) and Europe (54). The second most frequent serovar isolated in this survey was *S.* Derby, which was also as expected from previous surveys and passive surveillance. This serovar has a more limited distribution although it is not uncommon in turkeys and whilst it is very uncommon in human salmonellosis in GB, when it does occur, the disease may be clinically severe.

A modest correlation was observed between the prevalence of *Salmonella* infected pooled pen samples and the MJE results. In the study, the MJ samples were collected from the abattoir when the pigs in the sampled pens were due to be slaughtered. However, it was not possible to confirm that the pigs from which the MJ samples were collected had been raised in the sampled pens. Therefore, by chance more pigs from truly positive or truly negative pens might have been selected at the abattoir. Circulating antibodies against *Salmonella* continue to be detected after excretion has diminished or ceased (55, 56). Therefore, it is plausible that the pens in which MJE positive pigs were reared were positive prior to the day on which they were sampled. In contrast, infection late in the rearing period, within 1-2 weeks of slaughter, may provide insufficient time for a detectable titre of antibodies to appear. Since there is relatively little cross-protection between *Salmonella* serogroups, infection e.g. with *S*. Enteritidis or *S.* Panama may not provoke any serological response that could be detected by the MJE test. The poor correlation between MJE and culture has been observed

previously and it has been suggested that both tests are better interpreted at a herd level (33). The results from this survey indicate that great care should be taken in interpreting the MJE results. In practice, the majority of producers would only have the MJE results available via ZAP/ ZNCP. Where there is evidence of a high (>50%) prevalence and the producer with his or her private veterinary surgeon intends to invest in a control programme, then use of pooled pen samples represents a cost-effective approach to determining a baseline against which progress may be monitored. The comparison of MJE and culture for *Salmonella* at the individual pig level was discussed in greater detail in Chapter 3.

The variation in prevalence amongst the farms in this survey has some important implications. Firstly, it is notable that 31 farms (29.0%) had no positive pens and a further 17 farms (15.9%) had a prevalence of less than 10%. These results suggest that all farms might aspire to achieve a similar prevalence through control of *Salmonella*. Secondly, there were 22 farms (20.6%) where the prevalence was at or above 50% and adopting control measures would be particularly appropriate to reduce the risk that *Salmonella* infection derived from these farms should enter the food chain. Finally, although the risk of human infection occurring from a higher prevalence farm is greater than that from a lower prevalence farm, there were 261 *Salmonella* positive pens (38.4% of all positive pens) on farms where the pen prevalence was less than 50%. In order to protect public health, these farms should also be encouraged to adopt *Salmonella* control measures.

The final aim of this study was to investigate risk factors for *Salmonella* infection in GB pig farms. The data were analysed at the individual pen sample level, adjusting for clustering by farm through the use of GEE. A multivariable model enabled simultaneous consideration of confounding amongst putative risk factors. The final model showed that there was a reduced risk of pooled pen faecal samples yielding *Salmonella* in culture where farms reportedly used home mixed rations (OR 0.41; 95% CI 0.21 – 0.8) or used a contractor for vermin control (OR 0.41; 95% CI 0.20 – 0.83). The presence of PCV2 infection and a reported finisher mortality greater than 2% were associated with a greater risk of isolating

Salmonella from pooled pen faecal samples (OR 4.20; 95% CI 1.45 – 12.13 and OR 2.00; 95% CI 0.93 – 4.28 respectively).

The perception that *Salmonella* originating from pigs represented an important public health threat led to the initiation of control programmes and research in several European countries and in North America. The published literature were reviewed to inform the design of a GB intervention study that is described in Chapter 7 and so here, discussion will be restricted to the main findings from this GB finisher farm survey.

Growth of *Salmonella* bacteria is inhibited by an acidic environment and the pH of the gut content is influenced by the diet. Dry, commercial compound pelleted feed has been associated with an increased risk of *Salmonella* infection in several studies (23, 39, 57-60) and conversely, use of a wet or liquid diet has been associated with a reduced risk (24, 61-66). Coarse feed structure was shown to promote production of organic acids (lactate, butyrate and propionate) in pig gut contents (67, 68).

The observation that an acidic environment is unfavourable for *Salmonella* has led to the addition of organic acids to compound feeds for pigs. The reported outcomes have been variable. A reduction in faecal excretion, in the prevalence of infection in mesenteric lymph nodes and in MJE results has been reported (69, 70) and provision of organic acids via drinking water has also been reported to reduce the prevalence of *Salmonella* infection (71). However, carefully monitored field studies in GB have not shown any beneficial effect (50).

A later cross-sectional study of PMWS in England also showed an association with *Salmonella* infection (72) and an outbreak of *Salmonella* in pigs in Korea has been associated with PMWS infection (73). Infection with porcine respiratory & reproductive syndrome virus (PRRS) causes suppression of the immune system and was associated with an increased risk of *Salmonella* infection in a longitudinal study in France (74, 75) and the USA (76) although the authors did not report the PMWS status of the herd. Concurrent PMWS and PRRS infection has been associated with *Salmonella* infection in Japan (77) This survey did not

demonstrate an association between PRRS and *Salmonella* (OR 1.11; 95% ci 0.62 – 1.98). However, one experimental study failed to demonstrate that PRRS infection altered the risk of *Salmonella* infection in pigs (78). There are few reports of any association between *Salmonella* infection and clinical disease in pigs, except with respect to *S.* Choleraesuis, which is highly pathogenic for pigs but is seldom isolated in Europe. However, an experimental study showed that acute *S*. Typhimurium infection was associated with weight loss due to decreased appetite and the acute phase immune response (79).

Mice have been linked to *Salmonella* infection in pigs in the past (80). *Salmonella* infection may persist in mice on poultry units in the absence of any birds for many months (81) and chicks have been infected following ingestion of infected faeces (82). Poor rodent control measures have been associated with an increased risk of *Salmonella* infection in sows in Spain (41) and *Salmonella* has been isolated from wildlife in contact with pigs in Denmark (76).

Summary

This survey indicates that most GB finisher farms were infected with *Salmonella* in 2002-2003 since 71% yielded positive pooled pen faecal samples and up to 94.1% had at least one MJE positive pig, using the mj10 cut-off. The overall prevalence of infected pigs, using the MJE test with a mj25 cut-off, was 24.8%, which was very similar to that observed in previous and subsequent abattoir surveys and with results from national monitoring (ZAP/ ZNCP). Importantly, this farm-based survey showed that there was considerable variation in the within-farm prevalence and a substantial minority of farms (45%) had a low (0%-10%) prevalence of *Salmonella* infected pens. These farms present a target to which other producers may aspire.

The *Salmonella* serovars that were isolated were also typical of those reported in other surveys and in routine passive surveillance. *S.* Typhimurium was the predominant serovar and this has potentially important public health implications.

There was a weak correlation between the prevalence of infected pens and the prevalence of MJE positive pigs at slaughter, which concurred with previous studies. British producers sending their pigs to BQAP abattoirs received MJE results routinely. Although the number of samples collected per batch is small and thus provides only a weak indication of the true prevalence, where the farm is a breeder-finisher, a rise from a consistently low MJE prevalence may provide early warning of a rapid increase in incidence; for example, due to introduction of a new strain of *Salmonella* to a naïve herd. Where the true status of the herd must be known then microbiological tests to isolate *Salmonella* are recommended and pooled pen faecal samples offer a simple and sufficiently sensitive method for most circumstances.

The survey provided evidence that four risk factors were important with respect to *Salmonella* infection. Use of home mix rations, including liquid feeding and of a contractor for vermin control were both protective whilst PMWS and finisher mortality greater than 2% were both associated with an increased risk. The costs of changing a feeding system could not be justified simply as a control measure for *Salmonella*. However, where a new finisher building is planned then *Salmonella* control could be just one advantage from installing a liquid feed system. Rodent control is a worthwhile investment in its own right, as mice and rats may cause financially significant damage to buildings and fittings. They may also consume a surprising amount of feed. However, many farmers fail to realise the true rodent burden on their farms and are reluctant to invest in either a rigorous control programme themselves or in paying a contractor for their services.

PMWS may cause significantly increased mortality up to around 10 weeks of age. Recommended control measures include improving biosecurity, reducing stress and minimising mixing of pigs. These measures would be expected to bring direct and indirect benefits with respect to *Salmonella* control. Reported finisher mortality of more than 2% is unlikely to be due to *Salmonella* infection. However, improved herd health management with the aim of reducing mortality may bring about a coincidental reduction in the prevalence of *Salmonella* infection.

The primary motive for *Salmonella* control in pigs is to protect public health by reducing the risk of introducing infection into the food chain. Therefore, all producers should be encouraged to adopt control measures. The evidence from this study was used in the design of the intervention study described in Chapter 7.

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Chapter 5. Risk factors for a positive meat juice ELISA result – an analysis of routine data.

Introduction

Conducting a cross-sectional survey to estimate the prevalence of *Salmonella* infection at a farm or individual pig level and to investigate potential risk factors is expensive and the sample size is inevitably constrained by the available research budget. In Great Britain, the prevalence of Salmonella infection in finisher pigs has been measured in abattoir surveys conducted by Defra (1-4) which may also provide some evidence of association with a limited range of putative risk factors (1, 5). However, these surveys did not collect data at the farm level.

Chapter 4 describes a voluntary survey of 107 GB finisher farms, in which questionnaires were used to gather information on environmental, management and health-related variables. Within-farm prevalence was estimated and to a multivariable analysis was performed to look for evidence of associations with *Salmonella* infection at a farm level. However, in common with other voluntary surveys of livestock holdings, the participation rate was modest. Disincentives to participation commonly include lack of time, lack of interest and in the case of *Salmonella* infection, concern that disclosure of infection, which has to be reported under the Zoonosis Order, may impact negatively upon their business. However, where data exist that were collected for other purposes, it may be possible to utilise these for research purposes. The analysis reported here describes an exercise performed in 2002-2003 to access routine data from different sources for research purposes. Further studies were conducted afterwards, as described in the discussion.

From 2001 to 2009, all farms that sent pigs for slaughter through a British Quality Assured Pork (BQAP) abattoir in England and Wales were required to be members of the Assured British Pigs (ABP) quality assurance scheme. This scheme was incorporated into GB's Assured Food Standards as the Red Tractor

Farm Assurance Pigs Scheme in 2009 and current standards can be accessed via their website:
http://assurance.redtractor.org.uk/rtassurance/farm/pigs/pg_about.eb.
Quarterly audits of the farm were conducted by independent assessors and routine data were submitted to ABP. These data included information on potential risk factors for *Salmonella* infection, including the type of enterprise, feed use and herd size, as listed in table 1. A sample of three pigs from every batch delivered to any BQAP abattoir was tested by the MJ ELISA for the British Pig Executive's Zoonoses Action Plan *Salmonella* monitoring scheme (ZAP) and these results were stored on a dedicated database. A batch of pigs comprised all those selected for slaughter and sent to a specific abattoir on a particular day. These would usually be transported as a single lorry load. The total number of samples tested per farm in three months therefore depended upon the number of batches that were sent to slaughter. Each farm was designated a "ZAP level" on the basis of the prevalence of MJ ELISA positive pigs over the preceding 3 months. At the time of this study, farms with a prevalence greater than 85% were assigned ZAP level 3; those between 65% and 85% were ZAP level 2 and those less than 65% were ZAP level 1 (6). Under the conditions of BQAP, farms assigned ZAP level 2 or 3 were required to act to reduce the prevalence to ZAP 1 within 12 months or else face loss of their quality-assured status and hence access to BQAP abattoirs.

The purpose of this analysis was to discover whether the ABP and ZAP data could be linked and then analysed using conventional epidemiological approaches to investigate possible risk factors for a positive MJ ELISA result. The results would be of interest firstly, to increase knowledge of the epidemiology of *Salmonella* infection on pig farms and secondly, to demonstrate the value of combining routine data for surveillance purposes.

Material and methods

The ABP quality assurance scheme database recorded information collected during routine herd inspections by approved auditors. These confidential data were provided by farms in order to meet the scheme's requirements. Under the Data Protection Act (http://www.legislation.gov.uk/ukpga/1998/29/contents) data provided for one purpose cannot be used for another without the explicit permission of the person who originally provided it. However, ABP were themselves permitted to make use of the data under the terms of the scheme and agreed to provide a download of their dataset provided that no individual farm could be identified. Likewise, the ZAP scheme data were made available on the proviso that no individual holding could be identified. Therefore, the two datasets were combined into an MS ACCESS database by a third party. Any records that could not be matched were excluded from the final dataset. The number of failed matches was not reported. Each pig holding registered with ABP was given a unique identifier. ABP also held the County Parish Holding (CPH) number, which is the unique identifier required by UK law for all agricultural holdings. There was also a legal requirement that pigs from individual units were identified by a unique slapmark that was applied before slaughter (The Pigs (Records, Identification and Movement)(Interim Measures) (England) (No. 2) Order 2002. As well as enabling the identification of the holdings of origin for individual carcasses, the slapmarks are used to ensure slaughter data, including weight and depth of back fat, are assigned to individual producers. Since these data are used to determine the price paid for the carcasses, the data are accurately recorded by dedicated staff. In the ZAP scheme database, unique slapmarks were recorded and associated with the CPH number. Thus, these data were linked by CPH and a new sequential study number was assigned to each holding prior to transfer of the Access database. Data were then imported into Stata version 8 (www.stata.com). The test results were coded as positive or negative, using the S:P ratio cut-off value for the MJ ELISA test that has been defined for the ZAP scheme (0.25). In the ZAP programme, at least 15 samples from one farm had to be tested in any quarter for a ZAP score to be determined. For this study, all data from the whole study period were analysed.

Breeding herd size was dichotomised as 50 – 299 or >= 300 breeding females – there were no breeding herds with fewer than 50 females – and feeding herd size was dichotomised as <=1,800 or >1,800. In each case, the division was approximately the median point of the frequency distribution. In the original dataset, many variables were coded as 1 for “yes” or 0 for “no”. However, it was apparent that 0 also included missing values. For example, one farm was coded as 0 for “any finisher pigs kept outdoors” and 0 for “any finisher pigs kept indoors”, yet had several hundred finishers on site. Therefore, the original data were used to generate new variables. In the preceding example, a new variable “finout” was defined and given the value 1 if any finisher pigs were kept outside and 0 if finisher pigs were reportedly only kept indoors. The farm that did not report whether pigs were kept indoors or outdoors was thus coded as a missing value. Certain variables proved not to be amenable to analysis, including abattoir supplied, distance to abattoir, time to abattoir, haulier used, feeding herd supplied, distance to feeding herd, time to feeding herd, weaner source and teeth clipped, tails docked, ears notched and ears tattooed – these have not been listed in Table 1. These variables operate at the individual pig or batch level, but were reported at the farm level. For example, one farm might have despatched pigs to several abattoirs during the study period but the ABP data only listed the abattoir that had been used most recently at the time that the Quality Audit was conducted. Although batches of pigs from a farm could have been identified through use of the date of test field, it was not possible in the available data to relate this to a specific abattoir.

Each potential explanatory variable was considered independently for evidence of an association with the test result. Clustering at the farm level was accounted for by using a random effects model, which takes account of the hierarchical nature of the data. Individual observations within clusters are assumed to be independent and the random effect for each cluster is assumed to vary at random (7). Those variables for which there was some evidence ($p<=0.05$) of an association in this univariable analysis were then incorporated into a multivariable random effects logistic regression model and investigated for possible confounding. Variables were considered in groups – for example, relating to herd

size, to diet, to medication and to environment. Within each group, a stepwise approach was followed and those variables that showed some evidence ($p<=0.05$) of an association were selected for inclusion in development of a final model, where the same process was followed. A new variable was defined to simplify categorisation of the type of feed used. Five classes were generated:

1. Home mix ration; no dry co-products in mix; no pellets used
2. Home mix ration; dry co-products in mix; no pellets used
3. Home mix ration; no dry co-products in mix; pellets used
4. Home mix ration; dry co-products in mix; pellets used
5. Only pellets used

Finally, all variables that had shown evidence of an association in the univariable analysis were individually tested against the final model. The final model was examined for evidence of any pre-specified biologically plausible interaction. Tested interactions included: feed (pellets or home mix) and environment (outdoor or indoor and solid floor); feed and herd type (breeder-finisher or finisher) and environment and herd type.

Results

MJ ELISA test results from 93,879 samples collected from 1,688 farms between 20 June 2002 and 2 October 2003 were provided. 25,581 (27.3%) samples were positive in the MJ ELISA test using the S:P 0.25 cut-off value. There were 386 farms from which fewer than 15 samples had been tested (see table 5.2) and a total of 2,578 tested samples originated from these farms. Although these farms were not eligible for a ZAP score, since this analysis was undertaken at the level of the individual test, these results were retained in the dataset.

Table 5.1 lists those variables that were derived from the ABP database and were considered in the univariable analysis.

Table 5.1 Variables derived from data recorded by Quality Assurance Audits on GB Pig Farms with a potential association with *Salmonella* infection

Variable	Values	Variable	Values
Type of Breeding herd	Indoor Outdoor	Number of gilts	Integer
Weaner herd	Indoor Outdoor	Number of sows	Integer
Grower herd	Indoor Outdoor	Number of boars	Integer
Finisher herd	Indoor Outdoor	Breeding herd size	<= 299 sows >=300 sows
Feeding herd (weaner-grower-finisher)	All Indoor Any Outdoor	Number of weaners & growers	Integer
Home mix feed	Yes/ No	Number of finishers	Integer
Home mix feed medicated	Yes/ No	Number of feeding pigs (weaner-finisher)	Integer
Dry co-products fed (in home mix)	Yes/ No	Growth promoter weaners	Yes/ No
Dairy liquid co-products in home mix	Yes/ no	Growth promoter growers	Yes/ No
Non-dairy liquid co-products in home mix	Yes/ No	Growth promoter finishers	Yes/ No
Prescription medication in weaner feed	Yes/ No	Probiotics weaners	Yes/ No
Prescription medication in grower feed	Yes/ No	Probiotics growers	Yes/ No
Prescription medication in finisher feed	Yes/ No	Probiotics finishers	Yes/ No
Any prescription medication in feeding herd	Yes/ No	Enzymes in weaner feed	Yes/ No
Weaner feed pellets	Yes/ No	Enzymes in grower feed	Yes/ No
Grower feed pellets	Yes/ No	Enzymes in finisher feed	Yes/ No
Finisher feed pellets	Yes/ No	Any growth promoter	Yes/ No
Weaner floor	Solid Semi-slat Slatted	Any probiotics	Yes/ No
Grower floor	Solid Semi-slat Slatted	Any enzymes	Yes/ No
Finisher floor	Solid Semi-slat Slatted	Grower-finisher floor all solid	Yes/ No

Table 5.2 Number of farms in England and Wales for which fewer than 15 MJ ELISA test results were available between June 2002 – October 2003

Number of samples	Number of farms	Total number of samples
1	41	41
2	33	66
3	37	111
4	34	136
5	34	170
6	27	162
7	25	175
8	19	152
9	25	225
10	20	200
11	21	231
12	22	264
13	27	351
14	21	294
Total <15	**386**	**2,578**

The median number of samples collected from the 1302 farms submitting at least 15 samples was 53 (interquartile range 31 - 87). There were 200 or more samples tested from 49 farms; the maximum was 1062 samples. The mean within-farm prevalence from the 1302 farms with 15 or more samples was 27.7% (95%ci 26.7% - 28.7%) and the range was from 0.0% - 85.7%. There were 1255 farms which were ZAP level 1, 44 farms that were ZAP level 2 and 3 farms which were ZAP level 3 in this dataset. As noted previously, the ZAP scheme did not classify herds from which fewer than 15 samples were tested (see Table 2). These results are not entirely comparable with those reported by the ZAP programme, as the ZAP results are based on the preceding 3 months data whilst these results are based on all samples submitted.

Table 5.3 gives the results of the univariable analysis. The table shows the number of pigs in each class of each variable according to test result. The odds ratio and 95% confidence interval is given, adjusted for the cluster effect at farm level since each variable describes a farm-level effect.

Table 5.3. Univariable analysis of herd level risk factors for *Salmonella* infection (MJ ELISA test s:p ratio 0.25 cut-off) in pigs tested in England and Wales

Variable	Value	No. pigs[1]		Odds Ratio	95% ci[3]
		Total	MJ ELISA +ve (%)		
Any breeding pigs	No	45,822	14,960 (32.7%)	1.00	
	Yes	48,057	10,621 (22.1%)	0.40	0.35 – 0.45
	Missing Values	*0 (0%)*			
Breeding herd outdoors	No	35,206	7,407 (21.0%)	1.00	
	Yes	6,014	2,161 (35.9%)	2.47	1.81 – 3.37
	Missing Values	*52,659 (56.1%)*			
Any weaners outdoors	No	89,558	23,912 (26.7%)	1.00	
	Yes	4,321	1,669 (38.6%)	1.88	1.32 – 2.67
	MissingValues	0 (0%)			
Any growers outdoors	No	68,053	16,523 (24.3%)	1.00	
	Yes	2,830	1,405 (49.7%)	2.44	1.60 – 3.72
	Missing Values	22,996 (24.5%)			
Any finishers outdoors	No	90,597	24,327 (26.9%)	1.00	
	Yes	1,164	591 (50.8%)	2.63	1.46 – 4.73
	Missing Values	2,118 (2.3%)			
Any feeding herd outdoors[2]	No	64,733	15,499 (23.9%)	1.00	
	Yes	5,494	2,297 (41.8%)	2.44	1.76 – 3.37
	Missing Values	23,652 (25.2%)			
Any feed home mixed	No	61,526	19,538 (31.8%)	1.00	
	Yes	32,353	6,043 (18.7%)	0.34	0.29 – 0.40
	Missing Values	0 (0%)			
Dairy liquid co-products fed	No	57,314	22,901 (28.6%)	1.00	
	Yes	13,664	2,680 (19.6%)	0.46	0.36 – 0.60
	Missing Values	22,901 (24.4%)			
Non-dairy liquid co-products fed	No	80,046	22,795 (28.5%)	1.00	
	Yes	13,833	2,786 (20.1%)	0.52	0.40 – 0.68
	Missing Values	0 (0%)			
Dry co-products fed	No	86,951	23,886 (27.5%)	1.00	
	Yes	6,928	1,695 (24.5%)	0.59	0.42 – 0.81
	Missing Values	0 (0%)			
Home mix medicated	No	68,520	20,973 (30.6%)	1.00	
	Yes	25,359	4,608 (18.2%)	0.33	0.28 – 0.39
	Missing Values	0 (0%)			
Antibiotic in feed	No	21,054	5,998 (28.5%)	1.00	
	Yes	72,825	19,583 (26.9%)	0.89	0.77 – 1.04
	Missing Values	0 (0%)			
Weaner feed	Pellet	50,197	13,145 (26.2%)	1.00	
	Meal	8,263	1,254 (15.2%)	0.36	0.28 – 0.48
	Wet	1,244	253 (20.3%)	0.68	0.33 – 1.37
	Missing Values	34,175 (36.4%)			
Grower feed	Pellet	52,139	16,972 (32.6%)	1.00	
	Meal	22,077	4,359 (19.7%)	0.36	0.31 – 0.47
	Wet	15,970	3,132 (19.6%)	0.39	0.23 – 0.42
	Missing Values	3,693 (3.9%)			

Table 5.3 continued

Variable	Value	No. pigs[1]		Odds Ratio	95% ci[3]
		Total	MJ ELISA +ve (%)		
Finisher feed	Pellet	49,004	16,261 (33.2%)	1.00	
	Meal	16,005	4,207 (20.8%)	0.37	0.32 – 0.44
	Wet	17,165	4,015 (19.0%)	0.34	0.28 – 0.42
	Missing Values	11,705 (12.5%)			
Sow feed	Pellet	19,242	4,800 (25.0%)	1.00	
	Meal	13,243	2,286 (14.7%)	0.35	0.27 – 0.44
	Wet	3,323	784 (19.1%)	0.56	0.35 – 0.89
	Missing values	58.071 (61.9%)			
Weaners fed to appetite	No	488	151 (30.9%)	1.00	
	Yes	12,169	2,159 (17.7%)	0.50	0.22 – 1.14
	Missing Values	81,222 (86.5%)			
Growers fed to appetite[4]	No	3,790	1,134 (29.9%)	1.00	
	Yes	1,447	291 (20.1%)	0.52	0.31 – 0.87
	Missing Values	88,642 (94.4%)			
Weaner flooring	Solid	21,403	5,959 (27.8%)	1.00	
	Part slats	3,852	906 (19.0%)	0.50	0.36 – 0.71
	Full slats	28,724	6,057 (21.1%)	0.67	0.54 – 0.82
	Missing Values	39,900 (42.5%)			
Grower flooring	Solid	48,125	13,658 (28.4%)	1.00	
	Part slats	12,879	2,720 (21.1%)	0.53	0.43 – 0.67
	Full slats	16,372	3,650 (22.3%)	0.54	0.44 – 0.67
	Missing Values	16,503 (17.6%)			
Finisher flooring	Solid	50,157	14,634 (29.2%)	1.00	
	Part slats	14,617	3,662 (25.1%)	0.74	0.60 – 0.90
	Full slats	25,099	5,865 (23.4%)	0.46	0.46 – 0.65
	Missing Values	4,006 (4.3%)			
Breeding herd size	50 – 299	9,333	1,633 (17.5%)	1.00	
	>=300	13,530	3,121 (23.1%)	2.02	1.46 – 2.80
	Missing Values	71,016 (75.6%)			
Feeding herd size	<=1800	23,710	6,601 (27.8%)	1.00	
	>1800	25,212	6,150 (24.4%)	0.87	0.71 – 1.07
	Missing Values	44,957 (47.9%)			

[1] Total of 93,879 individual records
[2]Any weaner, grower or finisher outdoors
[3] Adjusted for cluster effect of farm
[4] All finishers are reported to be fed to appetite

Herds that were breeder finishers were at a lower risk than herds that were specialist finishers (odds ratio (OR) 0.40; 95% ci 0.35 – 0.45)). Data on whether the breeding herd was outdoors were available for approximately 44% of pigs (41,220). Only 15% of pigs were derived from farms with outdoor breeding herds and these were at an increased risk of having a positive MJ ELISA test result (OR 2.47; 95% ci 1.81 – 3.37).

Keeping any weaners, growers or finishers outdoors increased the risk of a positive test result (OR 2.44; 95% ci 1.76 – 3.37). However, only 7.8% of pigs, derived from 87 / 1196 farms, were reportedly kept outdoors.

Use of pelleted feed in a herd for all classes of pigs was associated with increased risk of a positive MJ ELISA result compared to either wet feed (OR 0.56; 95% ci 0.35 – 0.89) or meal (OR 0.35; 95% ci 0.27 – 0.44) (Table 5.3). Meal or wet feed is typically home-mixed. Pigs that originated from farms where home mixed rations were prepared were at a reduced risk of being MJ positive. This association was also seen with related variables (dairy liquid co-products fed, non-dairy liquid co-products fed, use of dry co-products and medicated home rations used) in the univariable analysis, as shown in table 5.3. Table 5.4 considers the data from 409 farms (24%) that reported that some home mixed rations were used for growers or finishers and presents univariable analysis for home-mix related variables. These results show that, apart from use of dry co-products, there is no strong evidence for any association with these related variables when the analysis is restricted to farms practising home mixing.

Table 5.4. Feed-related risk factors for *Salmonella* infection (MJ ELISA test) in pigs fed home mixed rations in England and Wales; data reported from 409 farms.

Variable	Value	No. pigs		Crude Odds Ratio	95% ci[1]
		Total	MJ ELISA +ve (%)		
Dairy liquid co-products fed	No	20,282	3,632 (17.9%)	1.00	
	Yes	12,071	2,411 (19.8%)	1.15	0.82 – 1.61
Non-dairy liquid co-products fed	No	20,160	3,585 (17.8%)	1.00	
	Yes	12.193	2,458 (20.2%)	1.35	0.96 – 1.89
Dry co-products fed	No	26,368	4,495 (17.1%)	1.00	
	Yes	5,985	1,548 (25.9%)	1.54	1.03 – 2.31
Home mix medicated	No	6,994	1,435 (20.5%)	1.00	
	Yes	25,359	4,608 (18.2%)	0.72	0.51 – 1.01

[1] accounting for within-herd clustering

As described in the material and methods, a new variable was defined to simplify the type of feed used. Table 5.5 shows the results of a univariable analysis for feed type, accounting for clustering by farm through a random effects model.

Missing values precluded inclusion of data from 731 of the 1688 farms that were included in the study. Home mix rations were associated with the lowest risk of MJE positive pigs and compared to this group, pigs fed solely on a pelleted ration had an approximately threefold increased risk (OR 3.11; 95% ci 2.57 – 3.78).

Table 5.5 A univariable analysis of feed-related variables and their estimated association with *Salmonella* MJ ELISA test results, adjusted for clustering by farm using a random effects model

Feed type	No. farms	No. Pigs		Odds Ratio (95% ci)
		Total	MJ ELISA +ve (%)	
Home mix	317	24,494	3,970 (16.2%)	1.00
Home mix plus DCP[1]	59	5,679	1,374 (24.2%)	1.57 (1.07 – 2.28)
Home mix plus pellets	17	1,137	326 (28.7%)	2.64 (1.34 – 5.20)
Home mix plus DCP[1] plus pellets	1	67	30 (44.8%)	7.95 (0.66 – 95.50)
Pellets	563	29,310	9,204 (31.4%)	3.11 (2.57 – 3.78)

[1] DCP – dry co-products

Amongst weaners, growers and finishers, risk of a positive MJ ELISA test was reduced for pigs kept on partly or fully slatted floors, compared to solid floors. These variables were re-coded as weaners on full slats yes/no and growers and finishers on solid floors yes/no. However, as there were a large number of missing values (42.5%) with respect to weaner floor type, this variable was not included in the multivariable analysis. This may be because, in some systems, pigs are maintained after weaning in the same accommodation until reaching the finishing stage, at around 12 weeks of age.

A larger breeding herd size was associated with an increased risk of a positive test result but feeding herd size was not. Data on the number of breeding females present were available for 22,863 pigs whilst data on whether any breeding animals were kept outdoors were available for 41,220 pigs. Whether any breeding females were present or not was known for all 93,879 pigs. There were a large number of missing values for breeding herd size and breeding pigs

outdoors, so these were excluded from further analysis in order to maximise the number of observations that could be included.

The following categorical variables were selected for investigation in a multivariable logistic regression model (see table 5.6):-

1. Breeding herd or weaner-finisher herd
2. Any feeding herd (weaner or grower or finisher) outdoors or all feeding herd indoors
3. Feed type (as shown in table 5.5)
4. Grower/ finisher floor solid or some accommodation with partial or full slats.

Results are shown in table 5.6.

Table 5.6. Results of a multivariable analysis of possible risk factors for a positive *Salmonella* MJ ELISA test result from pigs from England and Wales (June 2002 – October 2003)

Variable	Category	Total number of pigs	No. of pigs MJ ELISA +ve (%)	Odds ratio adjusted (95% ci)
Breeding herd	No	12,437	3,559 (28.6%)	1.00
	Yes	37,730	8,299 (22.0%)	0.56 (0.45 – 0.70)
Any feeding pigs outdoors	No	46,733	10,538 (22.6%)	1.00
	Yes	3,434	1,320 (38.4%)	2.01 (1.32 – 3.06)
Feed types	Home mix only	24,494	3,970 (16.2%)	1.00
	Home mix plus DCP[1]	5,679	1,374 (24.2%)	1.56 (1.04 – 2.34)
	Home mix plus pellets	1,137	326 (28.7%)	3.20 (1.63 – 6.28)
	Home mix plus DCP[1] plus pellets	67	30 (44.8%)	10.81 (0.93 – 126.03)
	Pellets only	29,310	9,204 (31.4%)	2.86 (2.32 – 3.53)
Grower/ finisher floor solid	No	16,962	3,413 (20.1%)	1.00
	Yes	33,205	8,445 (25.4%)	1.16 (0.94 – 1.44)

Number of pigs = 50167, number of farms = 957

This model shows that pigs from breeder-finisher farms were at a reduced risk of a positive MJ ELISA results (OR 0.56; 95% ci 0.45 – 0.70) compared to finisher

only herds, whilst keeping weaners, growers or finishers outside increased the risk of a positive MJ ELISA test result (OR 2.01; 95% ci 1.32 – 3.06). Pigs that only received a home mix feed that did not contain dry co-products had the lowest risk of a positive MJ ELISA result. If home mix feed contained dry co-products, or if pellets were also used, or if both of these situations were present, then the risk of a positive MJ ELISA increased. The greatest risk of a positive MJ ELISA result was observed amongst those pigs that only received pelleted feed (OR 2.86; 2.32 – 3.53). There were 67 pigs from one farm that were reportedly fed with rations that comprised home mix with dry co-products and pellets; the odds ratio for this group was 10.81 and the 95% confidence interval was from 0.93 – 126.03. All of these pigs originated from a single farm, which had a high prevalence (44.8%) of MJ ELISA positive pigs. The results from this farm are consistent with the observation that inclusion of dry co-products or pellets increases the risk of a positive test result. In the multivariable model, the odds ratio for a positive MJ ELISA for pigs that had been housed on solid floors at any time was 1.16 (95% ci 0.94 – 1.44; p=0.172). In the univariable analysis (see table 5.3), semi-slatted or slatted flooring showed a reduced risk of a positive MJ ELISA for each class of pig – weaners, growers or finishers. However, incomplete data precluded inclusion of these variables in the final model.

Discussion

This analysis of routine data identified important, biologically plausible associations between feed and environmental exposures and MJ ELISA results. As discussed in Chapter 3, a positive MJ ELISA result does not necessarily indicate that the pig was actively infected with *Salmonella* at slaughter. A dynamic susceptible-infected-recovered model, adapted to include a carrier phase and to estimate duration of a serological response to *Salmonella* infection in pigs predicts that most pigs that have been infected with *Salmonella* will be MJ ELISA positive at slaughter and that most MJ ELISA positive pigs are not infected at slaughter (8). This is consistent with results from a previous pilot study (9) that showed that there is important unexplained between farm variation in the prevalence of *Salmonella* infection, as indicated by the MJ ELISA result.

Data from more than 93,879 MJ ELISA tests were available but a high frequency of missing values results in data from 50,049 samples that originated from 832 individual farms being included in the final multivariable model. Similar risk factors were identified in the formal cross-sectional studies reported in chapter 4 – use of home mixed rations and breeder finisher herds were protective. There is also an indication that solid flooring may have been associated with an increased risk and this observation would merit further investigation in future studies. Similar results have been reported from Denmark, USA and The Netherlands (10-12). The impact of home mixing may be associated with a number of factors, including the size of the particles ingested by the pig, the degree of acidity induced within the stomach and intestinal tract and specific ingredients which may induce or repress growth of *Salmonella* (13). This is discussed in more detail in Chapter 1. The observation that inclusion of dry co-products, such as biscuit waste or maize gluten, in home mix feed increases the risk of a positive MJ ELISA result has not been reported before. Use of alternative feed ingredients takes advantage of the omnivorous nature of the pig. Feed is a substantial part of the cost of raising pigs and thus, there is a stimulus to seek cheaper materials.

These results could be used to identify farms that are likely to have a higher risk of *Salmonella* infection, where other control measures might bring the greatest

benefits. Differentiating the risk of infection that is associated with type of farm is also useful for the development of farm to fork risk assessment models. The large number of observations enabled risk factors to be considered within a sub-set of farms. However, it is also evident that data on important *a priori* confounders, such as the herd health profile, particularly PMWS status (14), or veterinary treatments, is lacking and this does limit interpretation.

The large number of observations allows detection of relatively weak associations, e.g. with type of flooring, that would be unlikely to be observed in an analytical epidemiological study, since the cost of obtaining data from such a large sample would be prohibitive. Furthermore, there is always some reluctance amongst farmers to participate in voluntary studies, not least because of the additional demands upon limited time.

The value of these data would be greatly increased if firstly, all farms submitted complete and accurate records; secondly, if a uniform set of questions were used in all quality assurance schemes and thirdly, if additional questions were included concerning, for example, PMWS status. As discussed below, these were addressed in a later study in which QA data were supplemented by a questionnaire at the time of an audit visit. From the epidemiological point of view, there were many questions that were of no value since it was impossible to relate the responses to individual pigs. These included, for example, distance from farm to abattoir and sources of pigs that entered a farm. It is appreciated that these questions may be important for quality assurance purposes and that these data are primarily collected for this purpose and not for epidemiology. This study was very well received by the industry and led to a number of follow-up projects utilising MJ ELISA data.

In collaboration with colleagues from the University of Liverpool, a further dataset was created, in which the farms' County-Parish-Holding (CPH) number was provided. This enables farm location to be determined and so enabled investigation of spatial effects, for example, due to shared risk factors (15). Due to the considerable variation in pig farm density across England and Wales, the analysis was restricted to two areas – East Anglia and Yorkshire and

Humberside. There are differences in pig husbandry systems between these two areas, with outdoor breeding herds and raising pigs to slaughter weight in specialist finisher units being more frequent in East Anglia whereas single-site breeder-finisher units were more frequent in Yorkshire and Humberside. Two approaches were used. Firstly, each holding was classified as either high or low risk, where farms that were in ZAP level 2 or 3 were high risk. The hypothesis that high risk farms were clustered differently to low risk farms was formally tested by calculating a test statistic (D), which provides an aggregate measure of excess clustering (16). This analysis did not provide strong evidence of a difference in spatial clustering between the high and low risk farms (P=0.160). However, this approach, in which each farm is designated as positive or negative based upon a single cut-off did not take account of the full range of variation in MJ ELISA prevalence amongst the farms. Therefore, a second geostatistical approach was employed to investigate whether the prevalence estimates from farms in close proximity was more similar than those from more distant farms. The results were expressed as a sample variogram which "summarises the similarity of observations as a function of the distance between them" (15). This analysis showed that there was evidence that farms in closer proximity were likely to have a similar MJ ELISA prevalence. This correlation may be explained by the similarity in husbandry and management practices of farms that are near each other.

A further study was also initiated (17). With support from ABP, all pig farms were invited to complete a questionnaire during a routine veterinary inspection visit. The questionnaire gathered supplementary information on farm-level risk factors that were not available from the ABP or ZAP database. Additional data were also accessed from the Meteorological Office to examine first-order as well as second-order effects – a first order effect occurs within a fixed boundary such as a farm perimeter whilst a second order effect such as rainfall could theoretically be measured at any point in space. A total of 566 farms volunteered to complete the questionnaires. Data were collected over two years and showed both seasonal and year by year variation. Other factors, including feed and overall mortality, were shown to be associated with MJ ELISA prevalence (17). Further work (unpublished) has shown that inclusion of these covariates explained the spatial

relatedness of farms with respect to MJ ELISA prevalence. Benschop and co-authors subjected MJ ELISA data from 2280 pig farms in Denmark to spatial analysis (18-21), taking into account farm-level covariates. They noted that in two areas, Jutland and Funen, there was evidence of some spatial over-dispersion.

MJ ELISA and farm level data have also been used to explore the impact of social networks upon *Salmonella* status amongst these holdings (22). The results showed that there was considerable inter-connectivity between farms and a greater degree of clustering than would have been expected by chance. It is suggested that farms within large companies could act in unison to reduce interconnectivity – for example, by minimising both the number of farms exchanging pigs and the number of farms sharing hauliers.

In summary, the results from the analysis of routine data have proved to be useful, especially where supplemented by additional information as was done in our follow-up study and by Benschop and colleagues in Denmark (20). These data enabled spatial analysis to be conducted, which demonstrated that there was generally relatively little unexplained variation once other covariates were taken into consideration. However, there may still be some benefits in considering different geographical groupings of pig farms that share distinct patterns of management in order to enhance surveillance or to adapt advice with respect to control measures for a particular regional sub-population.

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Chapter 6. The occurrence of *Salmonella* serovars in a longitudinal study of finisher pigs in Great Britain

Introduction

Previous studies that have collected data on the occurrence of *Salmonella* in pigs have generally been cross-sectional surveys that assess status at a moment in time and are often abattoir-based (1-13). Information on the serovars that occur may also be found from routine scanning surveillance (14-17). Previous chapters of this thesis describe the results of a national survey of pig finisher units (Chapter 4) and an analysis of serological data derived from the industry-led Zoonoses Action Plan (ZAP) programme (Chapter 5) (18). Elsewhere, longitudinal studies on the occurrence of *Salmonella* in pigs have been reported although these typically include either a small number of units (19-27) or are repeated visits to farms but without identification of the specific pigs or pens that have been sampled (28-33). Longitudinal studies have the advantage that the incidence rate of infection can be estimated. In a pig finishing unit, a prevalence estimate alone cannot distinguish between existing infections, for example, at the start of the finishing period and new infections that are acquired during that period.

This chapter describes the microbiological data on *Salmonella* infection acquired in a longitudinal study that was conducted on 48 GB pig finisher units that operated an all-in/ all-out management system. As described in Chapter 7, the study was conceived as an intervention study, in which farms were allocated at random to an intervention or comparison group. The intervention group was intended to follow a more rigorous hygiene and biosecurity programme. Analysis did not show any important difference in behaviour between these groups except a longer time between batches in the intervention group. However, the dataset contained information on reported hygiene and biosecurity actions and a second analysis was conducted to investigate the association between reported behaviour and *Salmonella* infection as described in Chapter 8.

The outcome measures for these studies were:

1. The pen incidence rate for *Salmonella* Typhimurium
2. The pen incidence rate for any *Salmonella* serovar, including *S.* Typhimurium

Potential explanatory variables were recorded using weekly reporting forms. Routine farm data concerning, for example, general health status, weight gain, feed conversion rates etc. were not collected. Previous experience, supported by discussions with major contractors placing pigs in finisher units, indicated that this study would not have sufficient power to detect any important differences in these parameters whilst the additional data collection would be a distraction to participants, who typically dislike completing forms. Furthermore, experimental evidence suggests that growth performance is not significantly affected by acute *S*. Typhimurium infection (34). This chapter documents the results of laboratory tests by farm, sample type and time of sampling.

Materials and Methods

Full details of the recruitment process for farms and for random selection of pens within farms are given in Chapter 7, since these relate to the epidemiological study design. Briefly, a sampling frame that comprised all GB pig farms that were members of industry quality control schemes and that operated on an all in/ all out basis was compiled. All farms were invited to participate in the study and a total of 48 were recruited. On each recruited farm, the study began after a batch of pigs had been despatched to slaughter and the accommodation had been emptied. The study batch of pigs was followed from entry until the end of the finishing period, with these pigs in turn being sent for slaughter. Pooled pen-floor faecal samples were collected and returned to VLA Weybridge for culture, as described in Chapter 2. Pooled samples have been shown to be a sensitive method for the isolation of *Salmonella* for epidemiological studies (35, 36). *Salmonella* isolates were identified to serovar level or designated as partial types or untypeable.

Pooled samples for culture of *Salmonella* were collected from:

1. A random sample of up to 30 pens at a pre-trial visit, before the pigs in the prior batch were sent to slaughter
2. Pen floors after cleaning and disinfection but before re-stocking.
3. The transport that delivered the study batch of pigs. The farmer collected up to 4 pooled samples from one vehicle delivering pigs to the study farm; samples were uniquely identified to each farm.
4. A random sample of 30 pens approximately 3 days after re-stocking and every 4 weeks thereafter until the study pigs reached the age of slaughter. Exact sampling dates were recorded. Some variation in dates arose if, for example, re-stocking occurred on a Thursday so that day 3 would have been at the weekend. Typically, fewer staff were available then and there was a risk of delay in postage of collected samples. One pooled faecal sample was requested from each pen on each sampling occasion.

All farmers received at least one training visit from VLA staff. Pre-labelled collection kits were sent to the farms in advance of each sampling occasion. These customised cardboard boxes contained jars and gauze swabs for pen floor faecal sampling. After sampling was completed, the boxes were sent through the post to VLA Weybridge.

Results

Table 6.1 below summarises the serovars isolated at each sampling occasion. Many farms yielded mixed infections and these results are described in more detail for each sampling visit.

Table 6.1. The number of farms from which *Salmonella* serovars were isolated at each visit during a longitudinal intervention study of GB finisher pig farms

Serovar	# farms sampled	# farms +ve	Typhimurium	Derby	Reading	Kedougou	London	Goldcoast	Bovismorbificans	Manhattan	Agama	Agona	Brandenburg	Indiana	Cerro	St Paul	Panama	Partial Type
Pre-trial	48	33	31	4	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Post C&D	48	32	26	7	7	5	2	4	1	0	0	0	0	1	0	0	0	2
Transport	46	19	7	4	8	3	0	0	0	0	0	1	0	0	0	0	1	1
Day 3	46	37	24	8	12	4	8	8	3	2	1	1	1	0	0	1	1	0
Week 4	45	32	26	6	6	2	4	3	2	1	0	0	0	1	1	1	0	1
Week 8	43	27	22	6	4	1	1	1	3	0	0	0	1	0	0	0	0	1
Week 12	32	18	11	3	2	3	1	2	2	0	0	0	0	0	0	0	0	1
Week 16	14	5	3	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Week 20	3	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

There was substantial loss to follow up after week 8; this is discussed in more detail in Chapter 7. Bacteriology results are discussed below firstly, in relation to each sampling occasion and secondly, with regard to the main serovars that were isolated.

Pre-trial

Samples were collected whilst the preceding batch of pigs was present and 33 of 48 farms were positive (69%). *S*. Typhimurium was isolated from 31 farms together with *S*. Derby on 3 farms and a partial type on another farm. *S*. Derby was the sole isolate from another farm and *S*. Reading was the sole isolate on another farm

Post cleaning and disinfection

After cleaning and disinfection but prior to re-stocking, 16 farms (33%) had no pens from which *Salmonella* was isolated. There were 16 farms from which a single serovar was isolated and a further 16 farms from which two or more serovars were isolated. These results are described further below.

There were 26 (54%) farms with pens contaminated with *S* Typhimurium:

- 13 where it was the sole serovar
- 8 farms where one other serovar was isolated:
    - *S.* Derby from 1 farm
    - *S.* Reading from 5 farms
    - *S.* Kedougou from 1 farm
    - *S.* Indiana from 1 farm
- 3 farms where *S*. Derby and a third additional serovar was isolated:
    - *S.* Reading from 1 farm
    - *S.* Kedougou from 1 farm
    - S. London from 1 farm
- 2 farms where a partially typed isolate and two additional serovars were isolated:
    - *S*. Derby and *S*. Goldcoast from 1 farm
    - S. Goldcoast and S Bovismorbificans from 1 farm

S. Derby was isolated from 7 farms, including the 5 where *S*. Typhimurium was also isolated. It was the sole isolate on 1 farm and was found together with *S*. Kedougou on 1 farm.

S. Reading was isolated from 7 farms, including the 6 farms where *S*. Typhimurium was also isolated. It was found together with *S*. Goldcoast on 1 farm.

S. Kedougou was the sole isolate from 2 farms, in addition to the 3 farms with mixed serovars listed above.

S. London was isolated from 2 farms as a mixed contamination – once as described above and on a second occasion together with *S*. Goldcoast.

S. Goldcoast was isolated together with other serovars from 4 farms, as described above.

Transport

Only one vehicle that delivered pigs to each unit was sampled; it is possible that other vehicles also delivered pigs to some units but that these were not sampled. Samples were submitted from 46 of the 48 farms. *Salmonella* was isolated from 19 vehicles (41%) delivering pigs to the study farms. *S*. Typhimurium was isolated from 7 of these vehicles; in one case, *S*. Agona was also isolated and in another case, *S*. Reading was also isolated. The latter serovar was isolated from another 7 vehicles, together with *S*. Derby in 2 cases. One of these vehicles also yielded *S*. Panama. *S*. Kedougou was isolated from 3 vehicles, in one case in combination with *S*. Derby. In addition to the 3 vehicles with *S*. Derby and another serovar, one vehicle yielded *S*. Derby alone. A partial type *Salmonella* was the only isolate from one vehicle.

Day 3 samples

The first samples after re-stocking were collected on day 3 or as soon thereafter as practically possible. Thirty pooled pen samples were requested and 16 farms submitted exactly 30 samples with the actual number of samples submitted varying from 8 - 50. Follow-up calls to those farms with incorrect numbers of pens were made to ensure participants were clear about which pens to sample on subsequent visits. A single colony was selected from each positive pen sample for serotyping, so mixed infections were not identified at a pen level but were observed at a farm level. Therefore, it is possible that the number of mixed infections is underestimated. Where serial dilution is used to make a semi-quantitative estimate the number of *Salmonella* organisms per gram in a sample, it is not unusual that a serovar that was not identified in the primary culture is detected. However, the cost of this approach is too high for routine survey use. New PCR-based methods may provide a rapid and cost-effective approach to identification of mixed infections. Pooled samples from pens on 9 farms (20%) were all negative whilst 37 farms (80%) yielded at least one serovar.

S. Typhimurium was isolated from 24 farms (52%):

- 9 where it was the sole isolate
- 11 where there was a second serovar
    - *S*. Reading (2 farms)
    - *S*. Kedougou (2 farms)
    - *S*. London (4 farms)
    - *S*. Goldcoast (1 farm)
    - *S*. Agama (1 farm)
    - *S*. Brandenberg (1 farm)
- 4 farms with two additional serovars
    - *S*. Derby and *S*. Goldcoast (1 farm)
    - *S*. Derby and *S*. Kedougou (1 farm)
    - *S*. Reading and *S*. Goldcoast (1 farm)
    - *S*. Reading and *S*. Bovismorbificans (1 farm)

S. Derby was isolated from 8 farms:

- As the sole serovar on 1 farm
- With another serovar on 2 farms:
    - *S*. Kedougou (1 farm)
    - *S*. Goldcoast (1 farm).
- In addition to the 2 farms listed previously where *S*. Derby was isolated with *S.* Typhimurium, there were 3 farms where *S*. Derby was isolated with 2 other serovars:
    - *S*. Bovismorbificans and *S*. Reading (1 farm),
    - *S*. London and *S*. Reading (1 farm)
    - *S*. London and *S*. Goldcoast (1 farm).

S. Reading was isolated from 12 farms:

- As the sole isolate on 2 farms
- With one other serovar on 3 farms; 2 of these are listed above
    - *S*. Goldcoast (1 farm)
- .Five farms had 2 additional serovars; in addition to those listed above:
    - *S*. Bovismorbificans and *S*. Goldcoast (1 farm).
- One farm had three further serovars (*S*. London, *S*. Manhattan, *S*. Agona)

- One farm had 4 further serovars (*S*. London, *S*. Goldcoast, *S*. Manhattan and *S*. Panama).

Week 4 samples

One farm did not submit any samples on week 4. A further 19 farms sampled fewer pens at week 4 than they had done on day 3. In total, *Salmonella* was isolated from 32 of 45 farms (71%) sampled at week 4.

S. Typhimurium was isolated from 26 farms

- As the sole serovar (16 farms)
- With a second serovar on 4 farms:
    - *S*. Reading (2 farms)
    - *S*. London (1 farm)
    - *S*. Bovismorbificans (1 farm)
- With two further serovars on 5 farms
    - *S*. Derby and *S*. Goldcoast (1 farm)
    - *S*. Derby and *S*. Manhattan (1 farm)
    - *S*. Reading and *S*. St. Paul (1 farm)
    - *S*. Kedougou and a partial type (1 farm)
    - *S*. London and *S*. Bovismorbificans (1 farm)
- With 4 further serovars on 1 farm
    - *S*. Derby, *S*. Reading, *S*. London and *S*. Indiana

- *S*. Derby was isolated from 3 farms in addition to those listed above On one of these, it was the only isolate whilst S. Reading was also found on the second farm and *S*. Kedougou with *S*. Cerro were isolated from the third farm.

  S. Reading was the only serovar isolated from one farm.

  One farm gave isolates of *S*. London and *S*. Goldcoast.

  S. Goldcoast was the only serovar isolated from one farm.

Week 8 samples

A total of 43 farms submitted week 8 samples but 26 of these did not submit samples from all of the selected pens. *Salmonella* was isolated from 27 of the 43 farms (63%) that submitted samples.

S. Typhimurium was isolated from 22 farms and 9 of these were mixed infections. *S*. Derby was isolated from 3 of these farms, *S*. Reading was isolated from another 3 farms, both *S*. London and *S*. Goldcoast were isolated from one farm, *S*. Bovismorbificans was isolated from one farm and a partial type *Salmonella* was isolated from one farm.

S. Derby was isolated from 3 farms in addition to those where this serovar was present with *S*. Typhimurium. One of these was a mixed infection with *S*. Reading and another was a mixed infection with *S*. Kedougou.

The sole serovar isolated from 2 farms was *S*. Bovismorbificans. *S*. Brandenburg was the only serovar isolated from one farm.

Week 12

Pooled pen floor samples were submitted by 32 farms although only 2 of these farms submitted a sample from all selected pens. Eighteen farms (56%) were positive by culture for *Salmonella*. *S*. Typhimurium was isolated from 11 farms and 4 of these were mixed infections with *S*. Reading (one farm), *S*. Goldcoast (one farm), *S*. Bovismorbificans (one farm) and a partial type (one farm).

S. Derby was isolated from 3 farms and 2 of these were mixed infections – one with *S*. Reading and one with *S*. Kedougou and a partial type *Salmonella*.

S. Kedougou was the only serovar isolated from 2 farms. *S*. Bovismorbificans was the only serovar isolated from one farm. One farm had a mixed infection with *S*. Goldcoast and *S*. London.

Week 16

Fourteen farms submitted samples 16 weeks after re-stocking of the finisher accommodation; in all cases, the number of pen samples that were submitted was less than the number of pens originally selected. *Salmonella* was isolated from 5 farms (36%). *S*. Typhimurium was isolated from 3 farms and there were no mixed infections. One farm had a mixed infection with *S*. Derby and *S*. Reading and one farm yielded *S*. Goldcoast.

Week 20

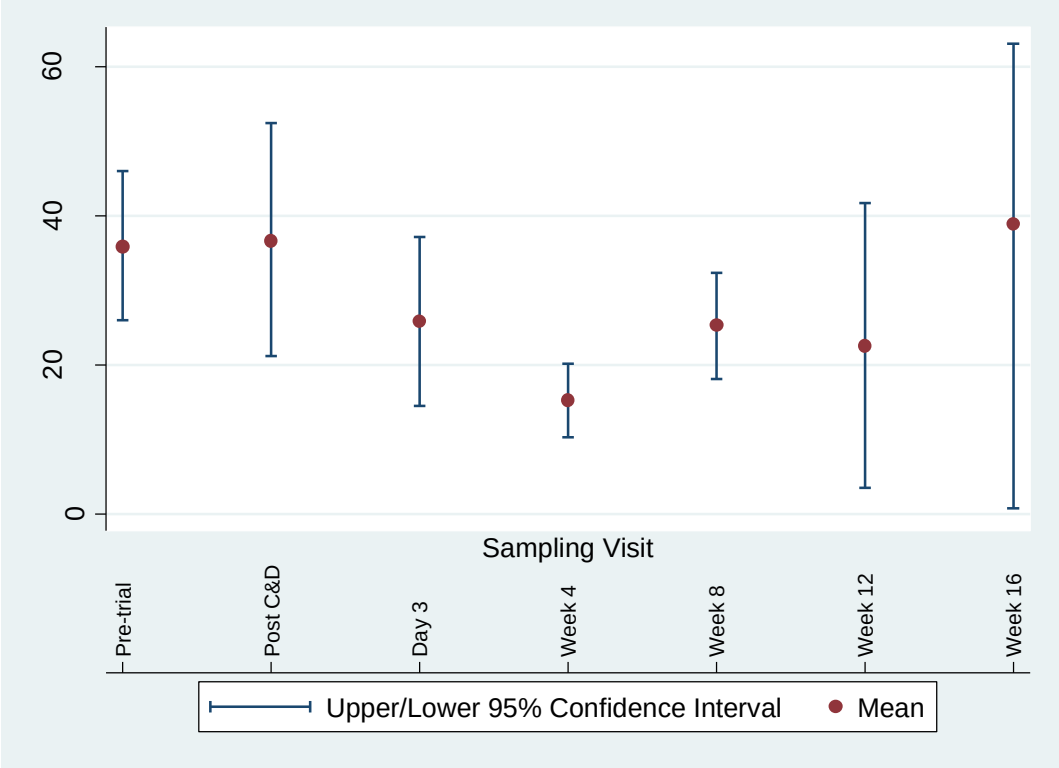
Three farms submitted pen samples on week 20; in all cases from fewer pens than were originally selected. One of these farms was positive for *Salmonella*; this was a mixed infection with *S*. Typhimurium and *S*. Bovismorbificans.

Results are also discussed by serovar in the following paragraphs, in order to examine persistence of a serovar through the study period.

Salmonella Typhimurium

The proportion of pens that were infected with *S*. Typhimurium varied by visit, as shown in figure 6.1 below. The mean and 95% confidence interval is shown; the width of the confidence interval increases with later visits as the number of farms that submitted samples declined. Data for week 20 are not included as only one farm was infected. Note that the pre-trial samples were collected when the preceding batch of pigs were present and the post cleaning and disinfection samples were collected before the study batch of pigs was introduced. The figure suggests that the prevalence of infected pens may have declined from day 3 to week 4 but rose again in week 8, although these differences are not statistically significant (p=0.2). Data in respect of the prevalence of other serovars is too sparse to display in a meaningful graph but is considered further later.

Figure 6.1. The proportion of pens from which *S*. Typhimurium was isolated at each sampling visit during a longitudinal intervention study on GB finisher pig farms

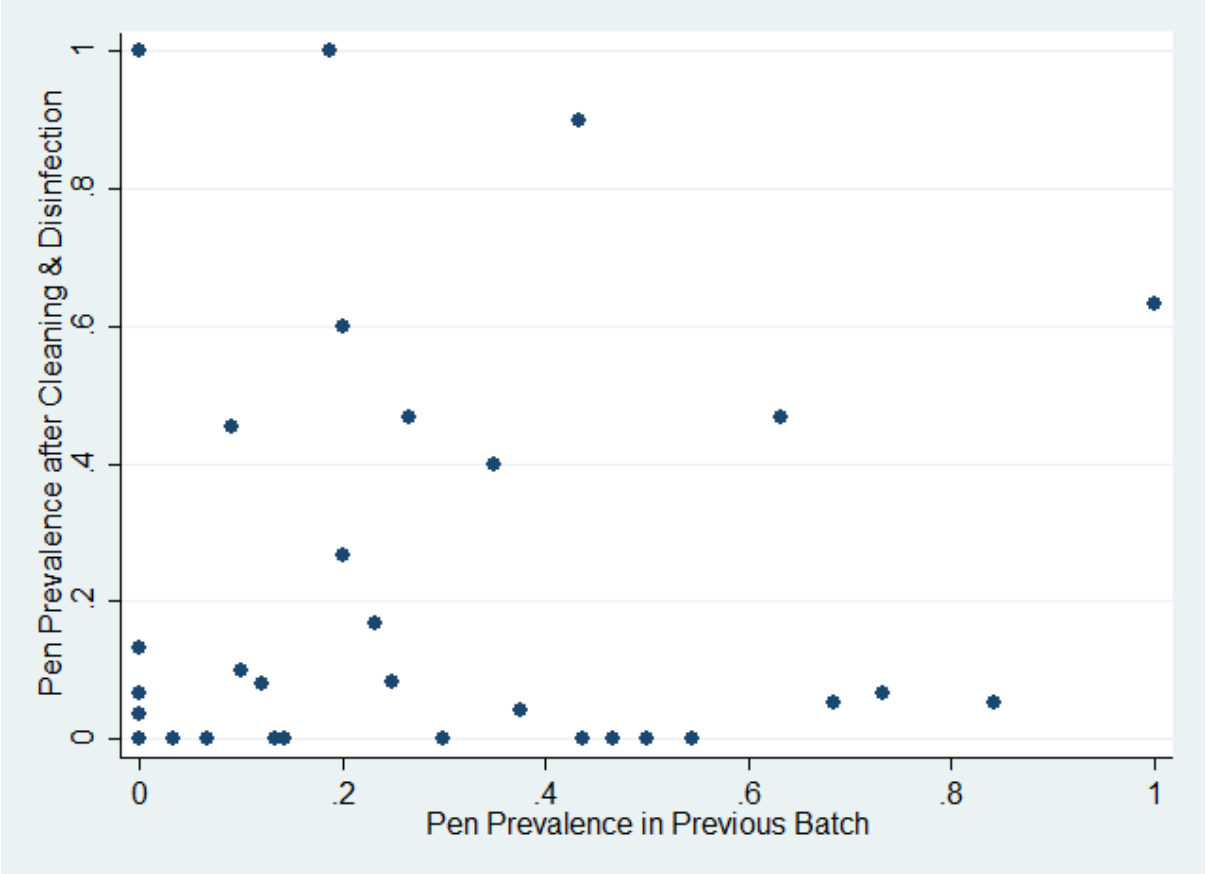


There were 17 farms from which *S*. Typhimurium was not isolated in the pre-trial batch of pigs and none of these had pens that were positive for this serovar after cleaning and disinfection. However, 22 of 31 farms (71%) in which the pre-trial batch was positive for *S*. Typhimurium were also positive after cleaning and disinfection. This may have been due to a failure to achieve a sufficient level of cleaning and/or disinfection to reduce contamination below the detection threshold or due to re-contamination of adequately cleaned pens from the adjacent environment.

There was a strong association between the presence of *S*. Typhimurium in the pre-trial batch of pigs and the presence of this serovar after cleaning and disinfection (OR = 6.42; 95% ci 1.78 – 23.18). However, there was a poor correlation between the prevalence of infected pens in the preceding batch and the prevalence of contaminated pens after cleaning and disinfection (p=0.23; R^2 = 0.03). In some cases, very pen low prevalence during production is associated with a high pen prevalence after cleaning and disinfection whilst in other cases, a high pen prevalence during production is associated with a low pen prevalence

after cleaning and disinfection. Farms where at least one pen was positive for *S*. Typhimurium after cleaning and disinfection were approximately 5 times more likely to be positive for this serovar on Day 3 (OR 5.3; 95% ci 1.4 – 19.7). The results suggest that presence of *S*. Typhimurium on the transport that delivered the study batch of pigs may have been associated with an increased risk of isolation on day 3 although the evidence is weak (OR 2.6 95% ci 0.5 – 15.2; p=0.238). Further pen-level analysis of the association between these factors and *Salmonella* status is presented in Chapter 8.

Figure 6.2. A comparison of the prevalence of pens from which *S.* Typhimurium was isolated 1) during the previous finisher batch and 2) after cleaning and disinfection during a study on GB finisher pig farms



Nineteen (79%) of 24 farms from which *S*. Typhimurium was isolated on Day 3 were positive for this serovar on one or more subsequent sampling date and on 14 (74%) of these 19 farms, *S*. Typhimurium was isolated from at least one pen on every sampling date.

Salmonella Derby

This was isolated from 21 farms (44%) on at least one occasion and was the second most frequently isolated serovar in this study in terms of numbers of positive pens, although more farms were infected on at least one occasion with *S*. Reading (see below). *S*. Derby was isolated from the previous batch of pigs on 7 farms and in 2 cases, it was not found on any subsequent sampling date. *S*. Derby was isolated from 9 farms after cleaning and disinfection and on 3 of these farms it had also been isolated from the previous batch of pigs. There was some evidence that prior infection was a risk factor for contamination after cleaning and disinfection (OR 4.4; 95% ci 0.8 – 24.7; p=0.09). *S*. Derby was isolated from 4 vehicles delivering pigs for re-stocking and on one of these farms it was also isolated from the previous batch and the post cleaning and disinfection samples. Eight farms were positive for *S*. Derby on day 3 after re-stocking and it had been isolated from 4 of these farms on a previous occasion. *S*. Derby was isolated subsequently from 5 of the 8 farms that were positive on day 3 and on 1 farm, it was detected on every sampling date. There were 2 farms that were positive for *S*. Derby at week 4 that were negative on day 3; one of these farms had been positive after cleaning and disinfection. This farm did not yield *S*. Derby at any future sampling date whereas the other farm that was found to be positive at week 4 was then positive at the next 3 monthly sampling dates, up to the time when the pigs were sent for slaughter. There were 2 farms that were positive for *S*. Derby for the first time at week 8; this was the only occasion on which either of these farms yielded this serovar.

Salmonella Reading

S. Reading was isolated from 23 farms (48%). There were 7 farms where the preceding batch was positive for this serovar and in 4 cases, this was the only occasion when positive samples were detected. *S.* Reading was found on 4 farms after cleaning and disinfection, one of which had also been positive in the previous batch. There were 8 vehicles which were positive for *S.* Reading and 6 of these farms were positive on day 3. A further 6 farms were positive for *S.* Reading on day 3. Farms where the transport was positive for *S.* Reading were at

least 2.5 times more likely to be positive on day 3 than other farms (p=0.004; 95% ci for OR 2.5 – 98.4). Six of the farms that were positive on day 3 yielded *S.* Reading on at least one subsequent occasion and 3 of these farms were positive at every sampling point during the finisher period.

Salmonella Kedougou

S. Kedougou was detected on 7 farms (15%). There were 5 farms on which it was isolated from the preceding batch of pigs and on 3 of these, it was also found in samples collected after cleaning and disinfection. There were 2 further farms where S. Kedougou was isolated at this time. Three vehicles yielded this serovar; all of these farms were also positive in a prior sample. Four farms were positive on day 3 after re-stocking and all of these farms had a previous positive sample. Two of the farms that were positive on Day 3 were positive on every subsequent sampling occasion.

Salmonella London

Salmonella London was isolated from 11 farms (23%) in total. It was isolated from pigs in the preceding batch on 2 farms and from pens after cleaning and disinfection on 6 farms. Two delivery vehicles were positive and 8 farms were positive on day 3; 4 of these farms were positive on one or more occasion prior to day 3. *S.* London was detected on a future sampling date on 4 of these farms.

Salmonella Goldcoast

Salmonella Goldcoast was isolated from 11 farms (23%) in total. On 4 farms, it was isolated from the previous batch of pigs and on 1 of these, it was also isolated after cleaning and disinfection. It was not isolated from any vehicle used to transport pigs. There were 8 farms that yielded this serovar on day 3 and on 3 of these, it was isolated on one or more subsequent occasions.

Salmonella Bovismorbificans

This serovar was isolated from a total of 8 farms (17%). It was isolated from the previous batch of pigs on one farm and after cleaning and disinfection on another farm. No vehicles were positive for this serovar. *S.* Bovismorbificans was detected in samples taken on day 3 after re-stocking from 3 farms, none of which were positive on previous sampling dates. Two of these farms were positive for this serovar on at least one subsequent occasion. On one farm, *S.* Bovismorbificans was found for the first time in samples collected at week 4 and it was isolated for the first time in week 8 on another farm.

Other *Salmonella* serovars that were isolated on a small number of occasions are detailed in table 6.1 above and are not discussed further here.

Discussion

This study relied on individual farmers collecting pooled pen floor faecal samples. They were motivated to follow the study directions firstly, through their personal commitment to the study which they made when they provided their informed consent. Secondly, they received compensation for each submitted set of samples and thirdly, they were promised their own results after all sampling had been completed together with an anonymised summary of the whole study once it had been completed. Finally, project staff provided support through a project telephone helpline, through emails and through periodic visits to the farms to help with sampling or to resolve problems. Nevertheless, there was a substantial loss to follow-up after week 8. Reportedly, the main reason for dropping out of the study was lack of time to collect samples and the prioritisation of other tasks that were essential to the effective management of the unit and the pigs. On some farms, problems in pen identification arose when pigs were mixed during production and a decision was taken that only pens that were definitively identified should remain in the study. The losses are unfortunate and in any future study, consideration may be given to other approaches to improve participation. However, the principal of farmer sample collection is necessary to minimise study costs per farm and thus, to enable a larger study to be delivered

within a constrained budget. Generally, farmers did not have any problem with conducting the sample collection correctly – as one participant commented, “putting some **** in a pot is ******* easier than filling in the forms”.

Salmonella incidents that are detected by laboratory diagnosis in livestock in Great Britain are reported to and confirmed by the National *Salmonella* Reference Laboratory at AHVLA-Weybridge (14). These incidents are predominantly disclosed through the investigation of clinical diseases, although *Salmonella* is usually considered to be a secondary or coincidental isolation rather than being a direct cause of the clinical condition. None of the farms in this study reported any disease associated with *Salmonella,* although both Typhimurium and Derby can occasionally cause overt clinical disease, usually diarrhoea and more severe sequelae such as meningitis are reported occasionally (37). *S*. Choleraesuis, which is a host-adapted serovar which is commonly associated with clinical disease (38) and which has been shown in experiments to reduce growth rates without detectable changes in immune parameters (39) is considered to be absent from the UK.

The non-typhoidal *Salmonella* serovars such as S. Typhimurium are highly promiscuous, being found in a wide variety of hosts including the pig. Other host-limited serovars such as *S*. Derby are found in pigs, turkeys and chickens in the UK. The most frequently isolated serovars in this study are also commonly reported from pigs through routine surveillance, with *S*. Typhimurium the most common in both cases. The serovars identified in this study were also frequently found in previous surveys (1, 2). Since 2007, there has been an increasing incidence of monophasic *S*. Typhimurium strains e.g. 4,5,12:i:- and 4,12:i:- . However, these strains were not found during this study. *S*. Newport is also reported in routine surveillance but was not detected during this study. This is an infrequent isolate in routine surveillance so it is unsurprising that it was not found in this relatively small study, despite the intensity of sampling that was conducted compared to the relatively small number of samples usually submitted for diagnostic purposes. Serovars detected on a minority of occasions from study

pigs (*S*. Agama, *S*. Agona, *S*. Brandenburg, *S*. Indiana, *S*. Cerro and *S*. St. Paul) but not reported from routine surveillance from clinical cases in pigs in the UK, were detected in other livestock species between 2007-2011 (14). However, there were no reports of *S*. Manhattan from these species. Detection of *Salmonella* from surveillance of domestic livestock is influenced by the underlying true incidence of infection, the coincidence with clinical disease and the severity of that disease. Crucially, the probability that suitable samples are submitted for laboratory diagnosis has a great impact on detection. The latter is significantly influenced by cost and by farmer and veterinary surgeon behaviour. In addition, serovars including *S*. Typhimurium and *S*. Derby that are able to infect and amplify successfully in the pig host are liable to be maintained in a herd whilst other serovars may be transient infections at a herd level, since the incidence of pig to pig transmission may be lower. Furthermore, the host-adapted serovars are liable to out-compete the others so that these tend to decline to levels that evade detection or even die out completely. Even within serovars, variation in host adaptation may occur (40, 41) and this may be genetically determined by small sets of genes (42). In surveys in other countries, pig-associated serovars including *S*. Typhimurium and *S*. Derby are often found whilst a wide diversity of other serovars are found in small numbers (8, 10, 16, 28, 31, 43-46). More detailed typing methods have been employed to demonstrate persistence of *Salmonella* Typhimurium strains (47). A limited selection of isolates from this study were subjected to typing by variable number tandem repeat (VNTR) analysis that indicated persistence of particular strains in the farm and even to the abattoir (48); this approach has also been used to look at the diversity of strains amongst isolates of different animal origins (49, 50).

Each week, the Health Protection Agency (now Public Health England) reports around 250 human cases of salmonellosis (see:
http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Salmonella/EpidemiologicalData/salmDataHuman/
accessed 7th May 2013). The two most frequent serovars are *S*. Enteritidis, which is commonly associated with poultry and was not isolated from pigs in this study and *S*. Typhimurium. Typically, there are around twice as many cases of *S*. Enteritidis compared to *S*. Typhimurium. There are also a very large number of minority serovars that are detected sporadically or on single occasions and the

other serovars isolated in this study are amongst this group. Risk to human health is associated with the prevalence of infection at the point of slaughter, risk of carcass contamination, weight of infection on the carcass, persistence of the *Salmonella* through the food chain and survival through cooking, processing etc. to present an infectious dose to a susceptible person (51, 52). Whilst theoretically, many serovars could be associated with human salmonellosis, in practice many minor serovars are of little clinical significance (53)

A more complete epidemiological analysis of these results is provided in Chapters 7 and 8. However, there are some interesting conclusions from the results shown here. Firstly, farms where up to 5 different serovars were detected simultaneously were common in this study. Detection of these farms was enhanced in this study compared to routine surveillance or to cross-sectional studies by the number of samples collected at each visit and the longitudinal study design. It is likely that a less intense sampling regime would not have detected all of these serovars, especially since diagnostic submission from clinical disease in the field may be limited to a single animal. The study did not enable detection of mixed infection within an individual pen, since only one colony per plate was selected and since these were pooled samples, there is no opportunity to ascertain whether any individual animal had coincident infection with more than one serovar. The observation that some of these mixed infections persist for several weeks suggests that either there is re-cycling through the pigs in the affected pens or that the serovars can persist in the pen environment at a sufficient level to be detected on repeated occasions despite the probable continued addition of *Salmonella* to the environment from actively infected and excreting pigs. *Salmonella* is known to survive for many months in a suitable environment. It is possible that some serovars, like *S*. Derby and *S*. Typhimurium, are amplified by passage through the pig's gut whilst others, such as *S*. Mbandaka are better adapted to survival on feed or in the environment (54). However, Osterberg and colleagues found few differences in environmental survival between feed or pig-associated serovars in experimental studies (55, 56). The results also illustrate the frequent inadequacy of cleaning and disinfection regimes in ensuring a *Salmonella*-free environment at re-stocking, either through a failure to eliminate *Salmonella* or a failure to prevent re-

contamination afterwards. As noted in the results section, although there was a strong association between the presence of *S.* Typhimurium in the pre-trial batch and after cleaning and disinfection, the correlation between prevalence at these sampling points was weak. This may reflect firstly; the efficacy of between-batch hygiene in diminishing the burden of contamination in pens and secondly, the risk of re-contamination of cleaned pens from other sources on the farm, e.g. rodents or carriage on contaminated boots. Although between-batch hygiene can impact on *Salmonella* contamination (57), a lack of effect has been reported previously (58-60) and this calls into question the practicality of the frequent advice that hygiene can play an important role in *Salmonella* control (61). Studies of farmer's attitudes have also shown that changing hygiene practice is difficult (62, 63). This finding has also been replicated elsewhere (64-67). This study shows strong evidence of an association between presence of *S*. Typhimurium in the preceding batch and after cleaning and disinfection. The results also show a strong association between presence of *S*. Typhimurium after cleaning and disinfection and at Day 3. These observations challenge the conclusions of research where the impact of infected pigs has not been considered (68, 69).

Salmonella serovars are known to differ with respect to factors including host preference, pathogenicity and survival in the environment. However the number of isolations of serovars other than *S*. Typhimurium is too small for meaningful quantitative analysis. Therefore, the results presented in Chapters 7 and 8 use detection of any serovar as the outcome of interest. Since *S*. Typhimurium is the most frequent serovar isolated from pigs and is also of greater importance with respect to public health, analyses are repeated for this serovar alone to investigate whether any different results could be detected.

In summary, these findings show that the *Salmonella* serovars that were isolated from the study farms were typical of those found through general surveillance of pig farms in GB. The duration and intensity of sampling that was undertaken in this study was unusual and provides an exceptional opportunity to observe the frequency and persistence of pen-level infection with *Salmonella*. Unfortunately, the loss of farms to follow up limited the conclusions as sampling time progressed. *S.* Typhimurium was the most frequent serovar and persisted on

throughout the observation period on several farms, appearing to out-compete other serovars where mixed pen infections were present. *S.* Derby was the second most frequent serovar. Amongst the other serovars, some showed a striking persistence throughout the follow-up period on some farms, including *S.* Kedougou, *S.* Reading and *S.* London whist others, for example *S.* Agona, *S.* Brandenburg or *S.* Panama were only isolated sporadically. Many of the minority serovars would not be detected by routine surveillance on clinically diseased pigs, in which *Salmonella* is usually an incidental finding.

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Chapter 7. An intervention study to control *Salmonella* infection amongst finisher pigs by enhanced biosecurity and hygiene.

Introduction

Salmonella infection is seldom reported to cause clinical disease in pigs although infection is widespread. The serovar most frequently isolated from pigs is *S*. Typhimurium, which is found in a wide range of domestic animals and is the second most frequent cause of human salmonellosis in Great Britain (1). The European Commission Zoonoses Regulation requires that "Zoonoses present at the level of primary production must be adequately controlled" and explicitly identifies *Salmonella* as one threat (2). The Regulation presaged the introduction of National Control Plans for *Salmonella* and the setting of Community targets for reductions in prevalence in farmed livestock, specifically poultry and pigs. The UK Food Standards Agency (FSA) Strategy for 2005 – 2010 aimed to achieve a "50% reduction in the incidence of pigs which test positive for *Salmonella* at slaughter by December 2010" (3). This was in order to reduce the burden of human cases of salmonellosis attributable to infection in pigs. The UK pig industry had already initiated the Zoonoses Action Programme (ZAP) in 2001 to monitor *Salmonella* levels in Quality Assured herds in GB, aiming to reduce this by 25% in 5 years. The FSA supported ZAP since it was aligned with their strategy. ZAP covered at least 85% of pigs slaughtered in GB (4). It was superseded by the Zoonoses National Control Plan (ZNCP) in 2009. If these policy aims are to be achieved, then a simple and effective control programme that can be implemented by all farms must be developed.

The *Salmonella* Infantis outbreak in Denmark in the 1990s provoked the introduction of the Danish *Salmonella* control programme for pigs and the development of the meat juice (MJ) ELISA test (5, 6). This also marked the start of an increasing interest in research into the epidemiology of *Salmonella* infection in pigs. Observational studies identified associations between farm-level risk factors such as efficacy of cleaning and disinfection, batch production of finisher pigs and salmonella incidence/prevalence (7). These risk factors are discussed in more detail in the literature review (Chapter 1). Advice to pig farmers has been based upon these observational studies. However, a literature review conducted

for a meta-analysis (8) found that few studies have attempted to evaluate the impact of interventions and no formal randomised controlled trials of hygiene or biosecurity interventions have been reported, apart from a GB pilot study (9, 10). In contrast, there have been four randomised controlled studies to estimate the impact of including organic acids in feed or water although these were conducted within individual farms (11-14). The number of pigs ranged from 20 to 685; only two of these studies found evidence of a beneficial effect. A further three non-randomised studies are reported by Friendship *et al*, two of which were conducted on single farms and found no benefit (15, 16). A single study of 36 farms did report evidence of a benefit (17). Outcome measures were prevalence of either *Salmonella* in faecal culture or MJ ELISA positive pigs at slaughter. Friendship *et al* also quote four field studies that investigated the use of vaccines (18-21). Their review highlighted the impact of various dietary practices, including the generally beneficial effects of coarse meal and home mixed feeds compared to pelleted feeds (8).

This chapter presents a study that aimed to assess the impact on the incidence of *Salmonella* infection of a farm-level intervention based on enhanced hygiene and biosecurity measures. The measures that were included were based on the literature review (Chapter 1), from consideration of the risk factors identified from the farm survey (Chapter 4), the analysis of risk factors from the combined meat juice and quality assurance data (Chapter 5) and experience gained in a pilot study in GB (3, 9). The study was designed to be analysed on an intent-to-treat basis, so that impact would depend upon both the potential efficacy of the proposed measures and the diligence of the participants in the intervention group in their application.

Materials and Methods

Study Design

The study was conceived as a randomised controlled trial to test whether an intervention based on promoting enhanced hygiene and biosecurity would achieve an important reduction in the incidence of *Salmonella* infection. The primary outcome measure was the pen incidence rate of *Salmonella* infection, to be measured by periodic collection of pooled pen floor faecal samples. Compliance with the intervention was measured through self-report by the participants, using forms that were completed on a weekly basis. There is a legal requirement to report the isolation of *Salmonella* to Defra. It was anticipated that most if not all farms would yield a positive culture on at least one occasion during the study. Therefore, to obtain informed consent it was necessary to reveal the purpose of the study to potential participants and a blinded approach, in which participants were not aware of the purpose of the study, was not possible. As the study required changes in management practices, it was not feasible to propose a placebo treatment. Since participating in the study would impact on the normal business of managing the pig herd, a compensation payment was offered, which contributed to covering costs but did not act as a financial inducement to participate. A sampling frame of all farms registered to a quality assurance scheme that granted access to BQAP abattoirs was prepared so that a representative sample of farms could be selected.

To be eligible to participate in the study, a herd had to meet the following criteria:

1. Be an all-in/ all-out producer of finisher pigs. These might originate from a breeding herd that was a part of the same enterprise or be a specialist finisher holding in receipt of pigs from other breeding herds.
2. Supply pigs to a BQAP abattoir and thus be a member of the ZAP scheme.
3. Not be a ZAP level 2 or ZAP level 3 farm – such farms were required to develop an action plan and therefore could not be randomised into a comparison group.
4. Not operate a bed and breakfast or similar business nor offer goods for direct sale to the public. Were *Salmonella* to be isolated from such

premises then this could have a negative impact on their business and cause financial losses.

Intervention protocol

The intervention protocol was developed on the basis of the published literature, experience with a pilot study and expert opinion. The full protocol is provided as an appendix and some details are listed in tables 7.4 and 7.5. Briefly, it promoted actions relating to the following steps:

1. Cleaning and disinfection between batches
    a. Outside the building and surrounding areas
    b. Inside the building
    c. Equipment
2. Cleaning equipment during production
3. Rodent control
4. Biosecurity measures
    a. Boot dips and brushes provided at entry points to all pig accommodation
    b. Personal hygiene, including clean boots and overalls
    c. Pig movements, including avoidance of mixing
    d. Sick pen management, including instruction that pigs should not be returned to the main stream of production after recovery
    e. Visitor management, including restricting access to pig housing unless essential, use of protective clothing
    f. Prevent access to pigs or to feed stores by dogs, cats and wild birds
    g. Feed and water hygiene
5. Disinfectants – advice on products, concentration and application was provided

Background information on herd management and husbandry was collected after recruitment and before the study period began. During the study period, information on adherence to the intervention protocol and on normal practice amongst comparison farms was gathered using the following forms, which are provided as appendices (see enclosed CD):

1. Between-batch cleaning report – completed after the between-batch cleaning and disinfection and before the study batch of pigs were placed in the accommodation
2. Weekly reports – recording adherence to cleaning and biosecurity measures, submitted each week until the end of the study

A pilot visit was carried out on 07/02/05 to a co-operative pig unit to test these forms and the forms were finalised following this visit.

Pooled samples for culture of *Salmonella* were collected from:

1. A random sample of up to 30 pens at a pre-trial visit.
2. Pen floors (30) after cleaning and disinfection but before re-stocking.
3. The transport that delivered the study batch of pigs.
4. Pens (30) approximately 3 days after re-stocking and every 4 weeks thereafter until the study pigs reached the age of slaughter. The date of sampling was recorded as part of the sample submission process.

VLA staff generally collected the samples at the pre-trial visit and the pen floor samples after cleaning and disinfection. A random selection of pens was made for the pre-trial batch. Where it was not possible to use the same pens for the study batch – for example, because the building was not being re-stocked – a second random sample of pens was made after cleaning and disinfection. These pens were then monitored for the duration of the study. Samples from the transport and during the study period were generally collected by farmers, who were provided with a field kit comprising sterile plastic jars containing cotton swabs and buffered peptone water. With respect to transport, farmers were provided with four jars. Once unloading was completed, they were asked to sample at three different points equidistant from the front to the rear of the vehicle and a fourth swab was taken from the unloading ramp. If any one of these samples was positive then the vehicle was classed as positive. Only one vehicle per farm was sampled. The jars were marked with the pen number in advance, to assist the farmer in selecting the correct pens to sample. These were packed into custom-made cardboard boxes which fitted inside a sleeve for despatch to the VLA National *Salmonella* Reference Laboratory in Weybridge. Postage was pre-paid and all packaging etc conformed to safety requirements.

Finally, the study team were notified when the pigs were ready for slaughter and up to 40 meat juice samples were collected from a single batch of pigs at the abattoir for testing in the MJ ELISA test at VLA's Regional Laboratory in Bury St Edmunds.

Contact was maintained with the farmers throughout the study by telephone, and additional visits to assist with sampling were occasionally made if necessary. Farmers received an initial compensation of £100 pounds on joining the project, and £10 for each set of samples which they collected. Table 7.1 summarises the study procedures for each individual farm.

Table 7.1: The stages of study participation for individual farms in an intervention study to control *Salmonella* infection in GB finisher pig farms

Activity		**Payment**
Respond positively to invitation to join study		
VLA staff visit farm to discuss study, complete questionnaire and collect initial samples		£100
Receive notification of group allocation, with intervention protocol if appropriate		
INTERVENTION FARMS: After previous batch ends, begin implementing between batch cleaning regime, and other protocol guidelines	CONTROL FARMS: Follow normal between-batch procedures	
VLA staff visit to collect swabs from cleaned farm before entry of study pigs and complete questionnaire		£10
INTERVENTION FARMS: Ongoing programme of day-to-day hygiene and biosecurity outlined in protocol	CONTROL FARMS: Continue with normal day-to-day management	
Farm staff collect swabs from delivery lorry or lorries		£10
Farm staff begin completing weekly questionnaires		
Farm staff collect pooled pen samples from study pigs around 3 days after arrival		£10
Farm staff collect pooled pen samples from study pigs around 4 weeks after arrival		£10
Farm staff collect pooled pen samples from study pigs around 8 weeks after arrival		£10
Farm staff collect pooled pen samples from study pigs around 12 weeks after arrival		£10
Farm staff collect pooled pen samples from study pigs around 16 weeks after arrival		£10
VLA collect details of abattoir(s) which will be slaughtering study pigs		

Bacteriology

Bacterial examination of samples was begun within 24 hours of arrival at the laboratory and no later than 96 hours after being taken at the farm. Details of the bacteriological methods and the ELISA test were provided earlier, in Chapter 2.

Sample size

The required sample size for the intervention study depended upon the incidence of infection within pens of pigs on farms without the intervention, the variability of this incidence between pig herds and the expected impact of the intervention. Data on expected pen incidence rates were derived from a previous pilot study, in which 30 pens per farm were selected and prevalence of infection was estimated at the start and end of the finisher period. The pilot study also demonstrated a large design effect (deff = 4.4) associated with the use of 30 pens per farm (22). A simulation model was developed and used to determine sample size. Input parameters were the number of pens per farm, the frequency of sampling, the prevalence at the start of the study and the pen incidence rate in the intervention and comparison groups. Table 7.2 shows an example of the model output, based on 100 simulations, for a study with 100 farms per group (200 farms in total) and 30 pens per farm, with pen sampling repeated on four occasions at monthly intervals during a 16 week finishing period. The period prevalence is the estimated proportion of pens from which at least one pooled sample will have been positive for *Salmonella*. It is the sum of the prevalence at the start and the additional number of positive pens, derived from the incidence rate and the duration of the study.

Table 7.2. Output from a simulation model to investigate study power for an intervention study with 100 farms per group

Prevalence at start		Incidence		Period prevalence		%ge of trials significant		
	Sd	Intervention	Control	Intervention	Control	0.01	0.05	0.10
0.20	0.10	0.017	0.072	0.24	0.36	100%	100%	100%
0.20	0.10	0.044	0.072	0.30	0.36	100%	100%	100%
0.20	0.10	0.048	0.072	0.31	0.36	80%	90%	100%
0.20	0.10	0.053	0.072	0.32	0.36	50%	70%	90%
0.20	0.10	0.057	0.072	0.33	0.36	20%	50%	50%

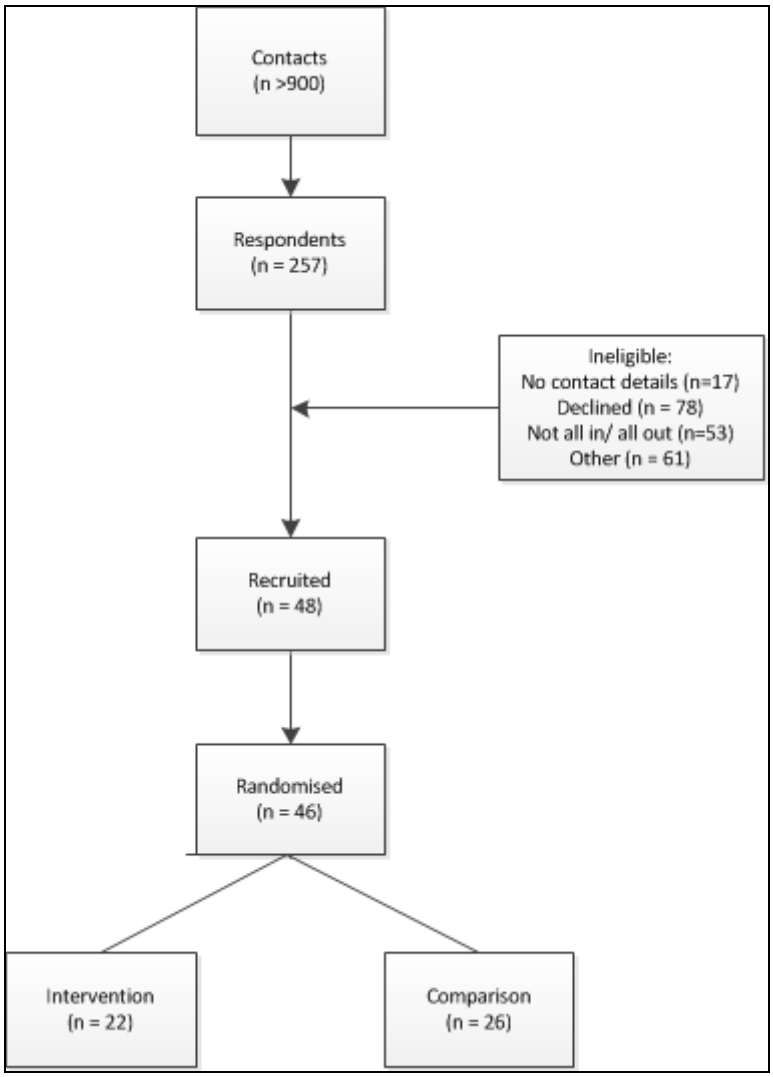
A sample size of 200 farms (100 per group) with 30 pens to be sampled on each farm was estimated to be sufficient to detect a 50% reduction in the pen incidence rate, assuming that the initial prevalence of infected pens is 10% and the incidence rate in the comparison group farms is about 0.07 new pen infections/ 100 pen-days at risk. The between farm standard deviation was assumed to lie between 0.01 – 0.04 and this was taken into account.

Since it was anticipated that there would be a modest rate of acceptance, it was decided to invite all eligible farms in England to join the study. If the number recruited exceeded available resources, then a random sample of eligible volunteers would be drawn.

Recruitment

A letter explaining the purpose of the study was prepared along with a postage-paid reply card addressed to CERA. The respondent could choose to join the study by signing beneath an informed consent statement or could tick a box declining the invitation, in which case a reason for the decision was requested. All letters and reply cards were prepared in CERA and a contact name, telephone number and email address were provided. However, to maintain the anonymity of those contacted, the letters were mailed by BPEx to their members. The study team only learned the contact details of those who chose to send in a reply. A reminder was sent after 2 weeks, having removed the names of anyone who had sent a reply card and the full mailing was sent again after 4 weeks, again excluding those who had responded. Table 7.1 summarises the processes after recruitment. Extensive efforts were made to publicise the project which are summarised in Table 7.3. More than 900 letters were sent out with enclosed pre-paid cards to request enrolment or for farms to decline the invitation. These cards included an informed consent statement and a Data Protection Act statement. Recruitment is summarised in the Consort diagram (Figure 7.1).

Figure 7.1 Consort diagram displaying the results of recruitment for an intervention study to reduce the incidence of *Salmonella* infection in finisher pigs in GB



Eligible farms which agreed to participate were randomly assigned to either the Intervention or Control group, with stratification to ensure that approximately equal numbers with different flooring and feeding systems were randomised to each arm. The random number generator in Microsoft Excel 2000 was used. However, farmers were not informed whether they were in the intervention or comparison group until after a pre-trial visit had been completed. This was done because many farmers were initially reluctant to join the study unless they could join the comparison group and we anticipated a bias if allocation was made prior to the first visit. It was conjectured that farmers would be more reluctant to leave the study after they had received a personal visit and knew that resources had

already been used. Those selected to be in the intervention group were sent the intervention protocol, which was discussed by phone if required. Farms in the comparison group received a shorter booklet detailing their responsibilities and providing instructions on sample collection. Farmers received results from all the laboratory tests conducted on samples from their own pigs at the end of their participation in the study. They also received a brief summary of the full results after the study had been completed.

Pen selection

On each farm, a plan of the finisher pig accommodation was prepared by a member of the study team showing individual pens. Each pen was uniquely numbered and then a random number table was used to select pens to be sampled. These pens were identified on the farm, for example by attaching a card with the pen identity to the door or another convenient place, or by stencilling it onto the front pen wall, according to the wishes of the farm manager. The sampling kits used by VLA or farm staff contained jars that were marked with the unique pen number to facilitate sample collection. This ensured that sequential samples could be linked with specific pens.

Data management

All results from laboratory tests and questionnaires were entered into a custom made Access database held in CERA at VLA Weybridge by experienced data entry staff. All data entries were visually checked and a series of computerised checks were performed for completeness and consistency – errors or omissions were followed up with farmers whenever possible.

Table 7.3. Summary of recruitment activities for an intervention study for *Salmonella* in GB finisher pig farms

Date	Event
Feb 2003	Sent invitations to 107 farms involved in cross sectional study
	Contacted managers of 3 largest integrated companies who agreed to contribute up to 10-20 units each – these numbers were later reduced
	Information sent to all senior vets in VLA Regional Laboratories, and those in the VLA pig group
March 2003	Letter sent to the President of Pig Veterinary Society for distribution to Members
	Letter sent to National Pig Association (NPA) for their group meeting. With the collaboration of the NPA's staffs, details of this work were mentioned at their Producer Group meetings, and copies of the summary page were given to them. Additionally this page was forwarded by e-mail to those not then present, and details of the work placed on their website. Accessible through the PIG WORLD magazine web site.
	Lancashire Pig Discussion Group contacted to inform them of our project. The secretary, agreed to pass on the information during their meeting.
	Meeting with MLC in order to prepare a mailing list, to reach more than 500 pig farmers.
	Attended Pig Group Discussion in Driffield
	Sent (200) more reply cards and envelopes to MLC for sending on the letter to Scottish producers
	Sent letters and reply cards to Norfolk Pig Discussion Group for their last meeting at the beginning of April.
	Letter sent by email to secretary after having talked on the phone (Rodbaston Pig Club meeting)
	Letter sent by email to secretary after having talked on the phone (Fosseway Pig Discussion group)
Various	Discussed study with breeding company asking for more integrated producer farms; sent an update to NPA (by email)
April 2003	Sent further information to Lancashire Pig Discussion group
	Text for a "TechTalk" – published by MLC / BPEX
May 2003	Text for the VLA Pig Group Quarterly Report
	Leaflet sent to Pig Veterinary Society meeting for display
June 2003	Update published in Pig World and on NPA website
July 2003	Leaflets distributed at Royal Agricultural Show
	Leaflets distributed at Yorkshire Agricultural Show
All summer 2003	Contact various producers, integrated companies, and organisations directly by phone – at least 32 were contacted directly of whom 9 were eligible and interested

Data analysis

Unique identifiers were allocated to each farm, to each pen within each farm, and to each sample collected during the study. After data cleaning, a descriptive analysis of farm characteristics was performed. The primary unit of interest for the study outcome was the pen and after each sample had been cultured, the result was recorded as negative if no growth of *Salmonella* was observed. For positive samples the serovar name was recorded with a separate code used for *Salmonella* that could not be identified as a particular serovar. Initially, the proportion of *Salmonella* positive pens on each farm at each sampling time point was calculated. The impact of the intervention was estimated by fitting a Poisson regression model to pen-level data, with robust estimates of standard error used to account for clustering of pens within farms.

At the end of the study period, meat juice (MJ) samples were collected from a single batch of pigs at an abattoir and these were subjected to the MJ ELISA test. The impact of the intervention, using MJ ELISA as the outcome of interest, was investigated using multivariable logistic regression with within farm clustering taken into account through a GEE model. All analyses were conducted using Stata releases 8 and 9 (www.stata.com).

Results

We received 257 reply cards amongst which were17 positive answers without contact details. There were 78 replies declining the invitation to participate; the most frequent reason was lack of time. Many interested farms were not eligible because they did not operate on an all in/all out basis. Forty-eight producers were interested and eligible and of these, 16 farms came from 3 integrated producers, the remainder were smaller companies or independently owned farms. The farms were randomly assigned to either the intervention (22 farms) or comparison group (26 farms) after the pre-trial visit had been completed. Two of the 48 recruited farms dropped out before the study batch of pigs was delivered, one because the owner became seriously ill and the second because they judged that participation would have a detrimental impact on their business due to the extra time required. Data from these farms were excluded from the analysis of the study. Thus 46 of the recruited farms (22 intervention and 24 control farms)

completed the initial stages of the study, up to the collection of the first sample set approximately three days after re-stocking. As shown in table 7.8 and discussed again later, there was a progressive loss of pens and farms to follow-up after the first visit. During the study, a total of 362 sets of pooled faecal samples were submitted of which 103 were collected by VLA staff. The first samples were collected on 3/3/05, with farms continuing to join throughout the year, as they reached appropriate points in their production cycles. The final farm entered the study on 18/10/05.

The study farms had a mean of 2637 finisher places (range 210 to 14,000; median 1,650 and interquartile range 1800 – 3,894). The mean number of finisher places in the comparison farms was 2115 (95% CI 1219 – 3011) and in the intervention farms was 3207 (95% CI 1698 – 4717).

Three intervention farms and 4 comparison farms reported that *Salmonella* had previously been diagnosed on the farm. One intervention farm reported a previous case of salmonellosis in a human contact.

The first component of the intervention protocol comprised a series of measures to be conducted after the building had been emptied and before it was re-stocked. The intervention group were instructed to remove all solid waste from pig accommodation and then to power wash the housing. They were further required to leave the accommodation to dry for a minimum of 12 hours before applying a disinfectant and to allow a further 48 hours or more before the building was re-stocked with the study batch of pigs. Farms were classified according to whether or not they complied with the cleaning and disinfection regime. Table 7.4 shows the number of farms that completed each element of the between-batch protocol. There was no evidence of any important change in behaviour between the two groups expect for pressure-washing the partitions within the buildings ($p=0.08$) and time for which the accommodation was left empty ($p=0.03$), where adherence was greater amongst the intervention farms.

Table 7.4: Farm level adherence with intervention protocols for between-batch cleaning and disinfection during a study on GB finisher pig farms.

		Comparison (N=24)	Intervention (N=22)	P (Chi^2)
Were the hoppers completely emptied after the previous pigs left?	No	2	4	0.29
	Yes	22	17	
Was all solid waste cleaned out of hoppers?	No	2	3	0.53
	Yes	22	18	
Were the hoppers pressure washed?	No	7	4	0.43
	Yes	17	17	
If applicable were the pits under the slats drained?	No	0	1	0.34
	Yes	4	4	
Was the muck heap moved away from pig housing?	No	8	5	0.56
	Yes	14	13	
Was the muck heap area cleaned?	No	6	2	0.42
	Yes	16	11	
Was the water system cleaned and drained?	No	12	9	0.74
	Yes	12	11	
Was the water system flushed with a disinfectant?	No	17	11	0.28
	Yes	7	9	
Were pig living areas pressure washed?	No	6	3	0.37
	Yes	18	18	
Were all pig living areas disinfected?	No	4	3	0.83
	Yes	20	18	
Were all areas pigs move through cleaned of muck and puddles?	No	6	6	0.71
	Yes	18	14	
Were all areas pigs move through disinfected	No	9	11	0.39
	Yes	15	11	
Were all partitions in pig buildings pressure washed?	No	7	3	0.23
	Yes	17	18	
Were all partitions in pig buildings pressure washed?	No	9	3	0.08
	Yes	15	18	
Was all large equipment pressure washed?	No	10	8	0.53
	Yes	11	13	
Was all large equipment disinfected?	No	16	14	0.67
	Yes	6	7	
Was all small equipment cleaned and disinfected?	No	12	8	0.62
	Yes	11	10	
Compared to normal, how long was the pig accommodation left empty?	Less	9	2	0.03
	Same	9	8	
	More	4	10	

Table 7.5: Adherence to biosecurity measures at a farm level during an intervention study to control *Salmonella* infection on GB finisher pig farms

		Comparison	Intervention	P-value
Are there bootdips set up on the farm?	No	6	4	0.69
	Yes	18	16	
Do staff have boots and overalls only for the pig unit?	No	6	2	0.20
	Yes	18	18	
Is protective clothing provided for visitors?	No	1	2	0.47
	Yes	22	18	
Will buildings contain pigs from more than one source?	No	13	15	0.37
	Yes	8	5	
Are there dedicated sick pens?	No	0	2	0.11
	Yes	24	18	
Are sick pens in a separate building?	No	12	9	0.86
	Yes	12	10	
Will sick pen be C&D before use?	No	7	3	0.40
	Yes	17	14	
Measures taken against entry of wild birds?	No	11	12	0.26
	Yes	13	7	
Do any other animals have access to pig buildings?	No	10	9	0.92
	Yes	13	11	

Table 7.5 lists the biosecurity measures required in the protocol and details the number of farms in the comparison and intervention groups that performed them. There were no important differences between the groups. Some practices e.g. use of dedicated boots and protective clothing or use of sick pens are already widely accepted. Other measures, such as excluding wild birds or changing rules concerning dogs or cats entering pig accommodation may not have been seen as necessary or feasible.

A total of 472 weekly reports were returned during the study. The number of reports received per farm ranged from 2 to 21. There was no important difference (p=0.275) in the mean number of weekly reports between the comparison group (mean = 11.5, 95% CI 9.5 – 13.6) and the intervention group (mean = 9.8, 95% CI 7.1 – 12.4).

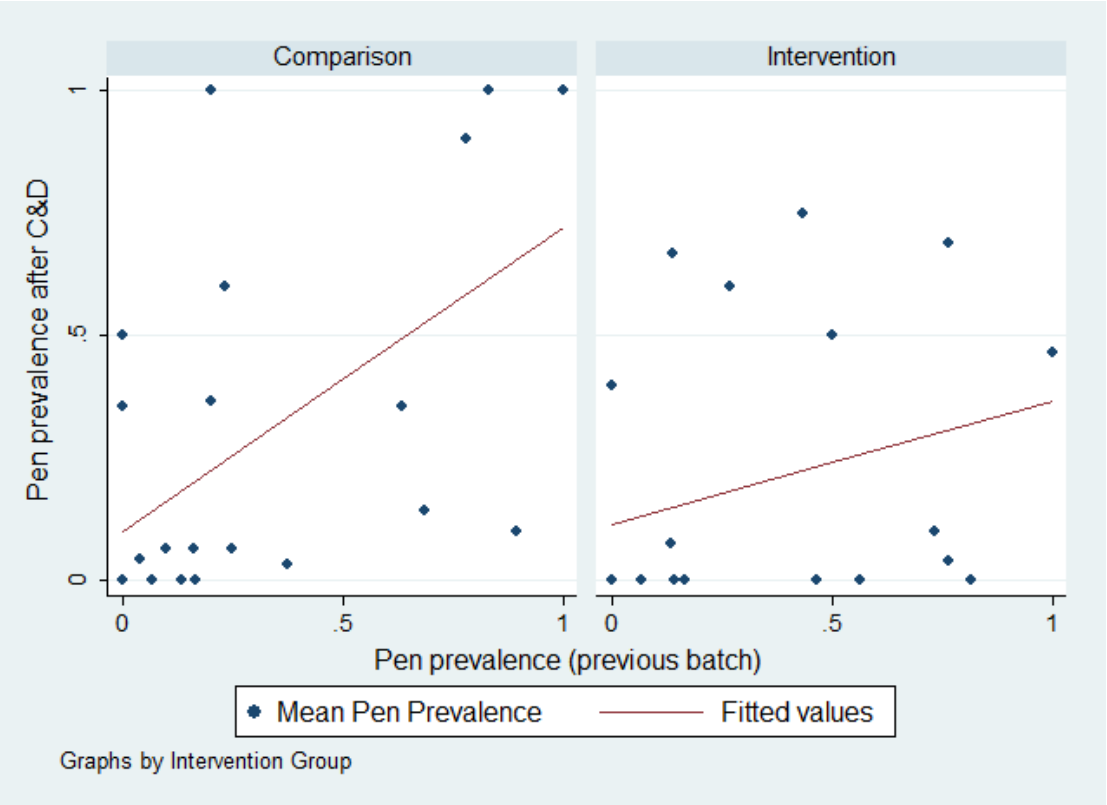
Overall, this descriptive analysis indicates that allocation to the intervention group had little impact on the adoption of the intervention measures.

A full description of the culture results is provided in Chapter 6.

At the pre-trial visit, 149/596 (25%) of pens on comparison farms were positive for *Salmonella* compared to 174/477 pens on intervention farms (36.5%); evidence that by chance, the preceding batch pen prevalence on intervention farms was greater than on comparison farms.

There is evidence that the prevalence of contaminated pens in the comparison group is associated with the prevalence of infected pens in the pre-trial batch of pigs (p=0.020) and this association is also observed amongst the intervention group farms (p=0.058) (see fig 7.2).

Figure 7.2. The association between the prevalence of *Salmonella* positive pens in the pre-trial batch of pigs and the prevalence of *Salmonella* contaminated pens after Cleaning & Disinfection during a study on GB finisher pig farms.



Post-cleaning visit

Participating farms were visited after the pre-study batch of pigs had been sent for slaughter and before the study batch of pigs entered. Farms which were in the intervention group were asked to follow the rigorous cleaning and disinfection protocol summarised earlier and detailed in the appendix. In each farm, a random sample of pens was tested for presence of *Salmonella* after cleaning and disinfection and before the study batch of pigs was introduced. Within each pen, 4 separate samples were collected and if any one of these was positive, then the pen was classified as positive. Intervention farms were expected to adhere to the study protocol whilst comparison farms followed their usual procedures. Sampling was planned to take place as close as possible to the date on which the pens would be re-populated. Between 8 and 50 pens were sampled per farm, with a median of 30 pens per farm. Within farms the prevalence of contaminated pens varied from 0% to 100%. The mean prevalence amongst intervention farms was 19.5% (95% ci 14.0% - 27.4%) and amongst the comparison farms was 27.5% (95% ci 12.2% - 42.9%) (p=0.41), which is compatible with a reduction in prevalence in intervention farms although this result could clearly have arisen by chance. Overall, there was a longer period (p=0.04) from the departure of the preceding batch of pigs to re-stocking with the study batch on intervention farms (20.7 days; 95% ci 10.9 – 30.6 days) than on the comparison farms (10.7 days; 95% ci 6.9 - 14.5 days). This difference was less marked when the number of hours from reported completion of cleaning and disinfection to entry of the first pigs was considered; this was 136.6 hours (95% ci 84.7 – 188.5 hours) for the comparison group and 203.0 hours (95% ci 102.2 – 227.4 hours) for the intervention group (p=0.29). There was considerable variation between farms in both groups with respect to the number of hours between completion of cleaning and disinfection and entry of pigs – the range was 10 – 720 hours for the intervention group (inter-quartile range 24-168 hours) and 4 – 336 hours for the comparison group (inter-quartile range 48 – 240 hours). Farmers were also asked about usual between-batch practice, to investigate whether any difference in the duration was associated with the adoption of the intervention protocol or whether by chance, the intervention farms usually had a longer between-batch interval. It proved difficult for them to recall the duration of the previous between-batch

period accurately and only a qualitative response of “less, same or more” was elicited. As shown in table 7.4, intervention farms were more likely to report a longer period than usual, which may indicate that the increase was due to a change in behaviour associated with the intervention. However, since these data are self-reported, it is possible that some reporting bias occurred, with farmers in the intervention group wanting to show that they had followed the study protocol.

Transport

Participating farms were provided with sample kits to collect faecal material from the vehicles that delivered the study batch of pigs and 151 samples were collected. *Salmonella* was isolated from 13/24 (54.2%) vehicles delivering pigs to the comparison farms and 12/22 (54.6%) of vehicles delivering to intervention farms (p=0.98). There was some evidence that pens on farms that had received pigs from contaminated transport were more likely to be *Salmonella* positive on Day 3 after re-stocking (OR 2.03; 95% ci 0.94 – 4.40; p=0.071); see table 7.6. However, on some farms, more than one batch of pigs was delivered and only one vehicle was sampled, so some bias may have occurred.

Table 7.6. The association between *Salmonella* contamination of transport and pen contamination after re-stocking (day 3 approx) during a study on GB finisher pig farms.

Pen *Salmonella* status (visit 1)	Comparison farms (number of pens)		Intervention farms (number of pens)	
	Transport status		Transport status	
	Negative	Positive	Negative	Positive
Negative	333	170	244	166
Positive	89	89	57	92

Re-stocking samples

On the farms, a total of 1,240 pens were followed, ranging from 8-50 pens per farm. On Day 3 after restocking, *Salmonella* was isolated from 178/681 (26.1%) pens on the comparison farms and 149/559 pens (26.7%) on the intervention farms. All pens followed the same sampling schedule; farmers were not informed of the sample results until the trial had ended and the study team were also unaware of the pen level results. There were 327 positive pens on visit 1 and these had a mean of 2.60 (95% ci 2.46 – 2.74) subsequent samples collected compared to 913 negative pens on visit 1 which had a mean of 2.41 (95% ci 2.32 – 2.49) further samples submitted (p=0.02).

All pens on 3 farms in the comparison group and 4 farms in the intervention group were negative throughout the study. Of these, 1 farm in the intervention group and another farm in the comparison group had no positive pigs in the MJ ELISA test, supporting the conclusion that these batches of pigs were probably *Salmonella*–free. One farm in each group did not submit MJ samples. One farm in the intervention group where all pen samples were negative had 35 MJ samples which were all positive, suggesting that either active infection was missed, that there was some systematic failure in sample collection or handling or that the pigs had all been exposed to *Salmonella* prior to delivery and had maintained sufficient antibodies to remain positive when tested at slaughter. In any event, these are surprising results. The final farm in the intervention group that had no positive pen samples, had 1/40 positive MJ samples and the final farm in the comparison group had 3/40 positive MJ samples. These results are compatible with a very low incidence of infection.

Salmonella was isolated at the first visit but at no subsequent visit from 2 farms in the comparison group and one farm in the intervention group. One farm in the intervention group was only sampled at visit 1 and therefore, does not contribute to the incidence analysis. Two farms in the comparison group had positive pens on visit 1 that were positive on further visits but no new positive pens were detected. Consequently, these 14 farms have an estimated incidence rate of 0.0 pen infections/ 100 pen-days at risk – as no new infections were detected. As shown in table 7.8, pens that were *Salmonella* positive at the first sample date

were at a greater risk of yielding a subsequent *Salmonella* positive sample than pens which were negative at the first sample date (OR 1.54; 95% ci 1.02 – 2.33; adjusted for farm and intervention group).

Table 7.7. The association between pen *Salmonella* status at first visit and any subsequent visit during a study on GB finisher pig farms.

		Pen status at any subsequent visit	
		Negative	Positive
Pen salmonella visit 1	No	593 (75.6%)	191 (24.4%)
	Yes	146 (49.8%)	147 (50.2%)

Monthly monitoring

Samples were requested at approximately 4 week intervals until pigs were sent to slaughter. However, the number of pens from which samples were submitted declined during the study (see table 7.8).

Table 7.8. Number of pen samples by visit during a longitudinal study on GB finisher pig farms.

Group	Total no. pens	Number of pens sampled					
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Comparison	681	681 (100%)	598 (88%)	553 (81%)	439 (64%)	98 (14%)	39 (6%)
Intervention	559	559 (100%)	479 (86%)	425 76%)	267 (48%)	124 (22%)	24 (4%)

Only one sample was actually submitted from 163 pens (13%) and whilst the median number of samples per pen was 4, a further 371 pens (30% approx.) had only 2 or 3 samples collected. The mean number of samples submitted per pen was 3.53 (95% ci 3.44 – 3.63) on the comparison farms and 3.36 (95% ci 3.25 – 3.47) on the intervention farms (p=0.02). The proportion of positive samples was greatest after re-stocking (day 3 samples - see table 7.7) and was lowest on the

final collection day. Overall, 303/681 (44.5%) pens in the comparison group and 215/559 (38.5%) pens in the intervention group yielded a culture of *Salmonella* on at least one occasion. Although this difference is consistent with a reduction in the intervention group, the result could also have arisen by chance (OR= 0.68; 95% ci = 0.32 – 1.46; p=0.33 after adjustment for farm-level clustering).

Table 7.9. Results by visit from pooled pen floor samples cultured for *Salmonella* during a longitudinal study on GB finisher pig farms

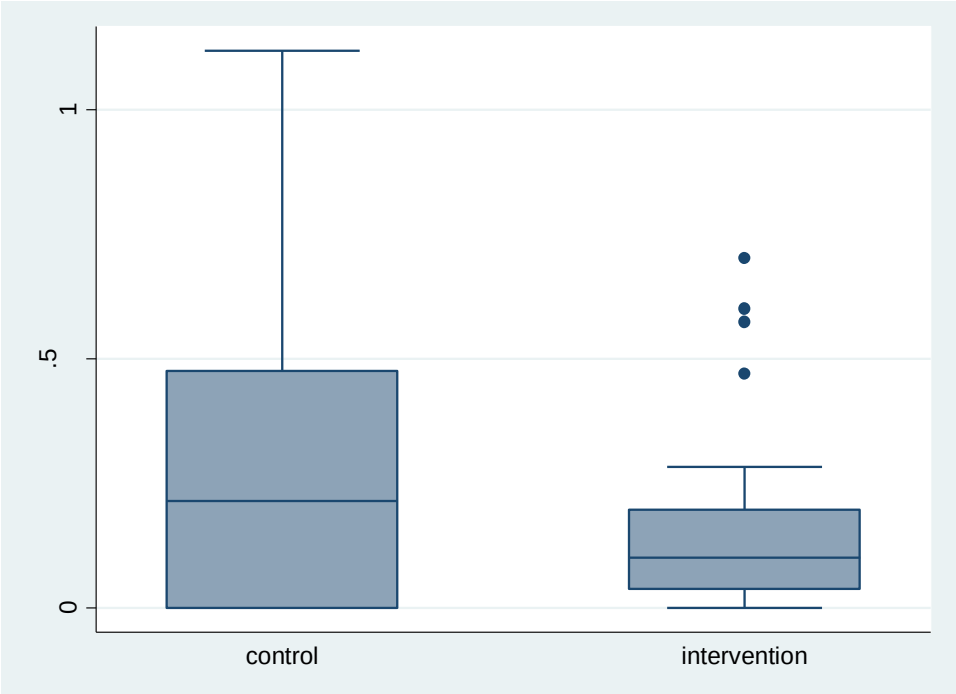
Sample visit		Comparison			Intervention	
	Number of farms	Total pens	Positive (%)	Number of farms	Total Pens	Positive (%)
Visit 1 (day3)	24	681	178 (26.1%)	22	559	149 (26.7%)
Visit 2 (week 4)	24	598	101 (16.9%)	21	479	65 (13.6%)
Visit 3 (week 8)	23	553	117 (21.2%)	20	425	52 (12.2%)
Visit 4 (week 12)	18	439	60 (13.7%)	14	267	40 (15.0%)
Visit 5 (week 16)	6	98	9 (9.2%)	8	124	23 (18.6%)
Visit 6 (week 20)	2	39	2 (5.1%)	1	24	0 (0%)
Total pens sampled		2408	467 (19.4%)		1878	329 (17.5%)

Univariable analysis of pen incidence

Analysis of these data was carried out by using a Poisson regression model, which estimates the rate at which previously *Salmonella* negative pens of pigs become infected. The impact of putative risk factors is assessed by calculation of the incidence rate ratio and the estimated confidence intervals were adjusted for the cluster effect exhibited at farm level. Data were also analysed using a random effects model based on farm. However, the model did not provide a useful explanation of the data; the estimated incidence rate ratio was 0.56 (95% ci 0.21 – 1.51) whereas the crude data showed the value was approximately 1.00. This may be due to the observed data departing importantly from a gamma-distribution, which underlies the random effects model. There was considerable variation in incidence rates amongst the 45 farms as shown in figure 7.3 below and as indicated by the between-farm variance, which was 2.28. Figure 7.4

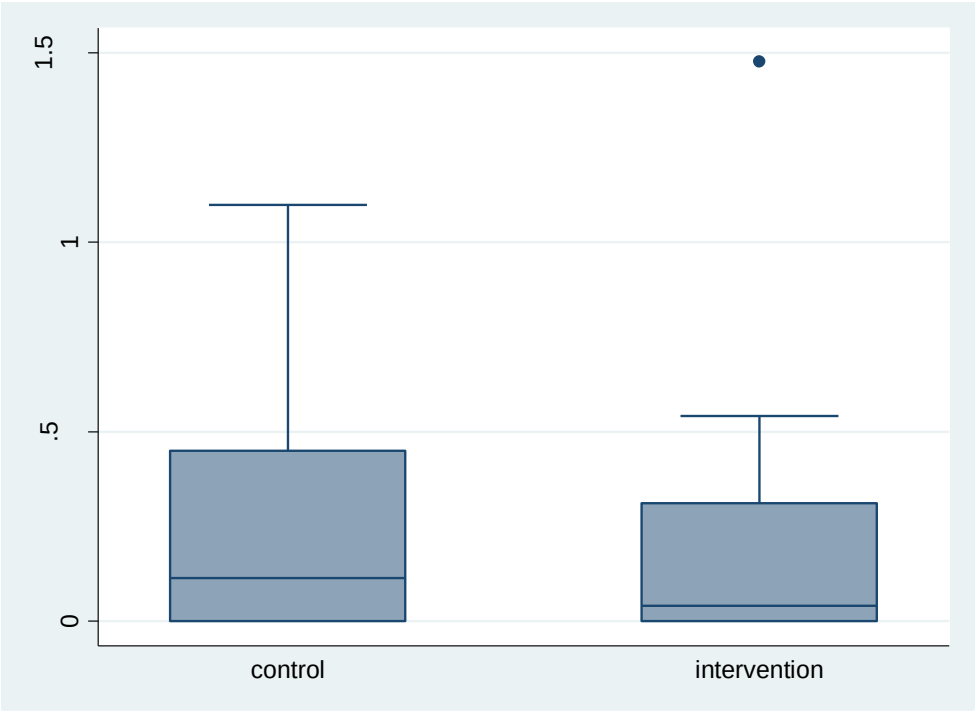
shows the pen incidence rate when *S*. Typhimurium was the outcome of interest. These figures are suggestive of an intervention effect.

Figure 7.3. Incidence of pen-level *Salmonella* infection amongst intervention and comparison farms (any *Salmonella*) during a study on GB finisher pig farms [1]



[1] In the figure, the box indicates the inter-quartile range of values and the line that segments this box represents the median value. The whiskers represent the limits within which 95% of observations lie and the single dots above the whiskers represent any outliers

Figure 7.4. Incidence of pen-level *Salmonella* infection amongst intervention and comparison farms (*Salmonella* Typhimurium) during a study on GB finisher pig farms [1]



[1] In the figure, the box indicates the inter-quartile range of values and the line that segments this box represents the median value. The whiskers represent the limits within which 95% of observations lie and the single dots above the whiskers represent any outliers

Two outcomes were considered: the pen incidence rate for *S*. Typhimurium and the pen incidence rate for any *Salmonella*, including *S*. Typhimurium.

The pen incidence rate for *S*. Typhimurium was calculated by estimating the number of pens which were negative for this serovar at visit one and became positive thereafter. The date at which the pen first became positive was noted and the number of pen-days at risk was estimated as the mid-point between the first positive date and the last negative date, plus the number of days between the first visit date and the last negative visit date. If a pen was positive for a serovar other than *S*. Typhimurium, then the pen could contribute an event and pen-days at risk to the incidence rate. In the comparison group, there were 23 pens which were positive for a serovar other than *S*. Typhimurium at visit one and which were positive for *S*. Typhimurium at a subsequent visit. In the intervention

group, there were 31 pens which were positive for a serovar other than *S*. Typhimurium at visit 1 and that later became positive for *S*. Typhimurium. Overall, 98 comparison group pens became positive for *S*. Typhimurium and there were 39457 pen-days at risk whilst in the intervention farms, there were 78 pens that became positive for *S*. Typhimurium and there were 31239.5 pen-days at risk. There was no evidence of a difference in the pen incidence rate with respect to *S*. Typhimurium (p=0.168; Wilcoxon rank sum test); the rate amongst comparison farms was 0.25 per 100 pen-days at risk (95% ci 0.21 – 0.31) and was also 0.25 per 100 pen-days at risk in the intervention group (95% ci 0.20 - 32).

For any *Salmonella*, pens that were positive for *S*. Typhimurium or any other serovar on visit 1 did not contribute events or pen-days at risk. In the comparison group, there were 125 new pen infections with any serovar, including *S*. Typhimurium and there were 30785 pen-days at risk. In the intervention group, there were 66 new pen infections with any serovar and there were 24341.5 pen-days at risk. The apparently paradoxical observation that the number of new pen infections in the any *Salmonella* group is lower than in the *S*. Typhimurium group is accounted for by the number of pens with any *Salmonella* aside from *S.* Typhimurium on visit one and the number of these that became positive for *S*. Typhimurium during the study.

The incidence rate in the comparison group for any *Salmonella* was 0.41 per 100 pen-days at risk (95% ci 0.34 – 0.49) whilst in the intervention group it was 0.27 per 100 pen-days at risk (95% ci 0.22 – 0.35), which provides only weak evidence for an impact of the intervention (p=0.105 Wilcoxon Rank Sum test). After taking into account within farm clustering, the incidence rate ratio for the intervention with respect to Typhimurium was 1.02 (95% ci 0.41 – 2.54; p=0.957) whilst for any *Salmonella* it was 0.64 (95% ci 0.32 – 1.31; p=0.225).

MJ samples were collected at the abattoir from a single batch of pigs from each of 37 farms, 19 in the comparison group and 18 in the intervention group. The pigs could not be linked to individual pens. These samples were not collected from other farms for various reasons, including – dropped out of the study; lack of available staff at abattoir; failure to notify the study team of despatch to slaughter

(amongst contracted farms, as little as 24-48 hours notice may be given to the farm manager). In the comparison group using the MJ25 cut-off, 231 out of 772 MJ ELISA results were positive (29.7%) and in the intervention group, 274 of 780 MJ ELISA samples were positive (34.3%). In the GB "ZAP" scheme, a cut-off for a positive MJ result is defined as a signal:positive (SP) ratio of 0.25 whereas it is accepted that a SP of 0.10 or greater is evidence of prior infection with a Group B or C_1 *Salmonella*. Participation in the intervention group was not associated with the MJ results at either cut-off – for the SP 0.25 cut off, OR=1.54 $ci_{95\%}$ 0.61-3.90 and for SP 0.1, OR=1.24; $ci_{95\%}$ 0.48-3.18.

Discussion

The study design and planned sample size for this intervention study were informed by the pilot study that was conducted previously. An important factor was that pen-level *Salmonella* infection on pig farms showed very strong within farm clustering and a pilot study indicated a design effect of approximately 4 based on 30 pens per farm. Recruitment was limited to farms that operated their finishing accommodation on an all-in/all out basis since this is a necessary first step in any *Salmonella* control programme, and unless it was in operation, the intervention would be unlikely to have any important impact. The recruitment rate was disappointing and only 48 farms were enrolled rather than a planned 200. Restricting eligibility to farms that were operating strict all-in/all-out management resulted in the loss of many interested potential participants. However, it would have been impossible to implement the between-batch components of the intervention protocol on continuously-occupied accommodation. Another issue that became apparent was that there are many finishing units that are contracted to large integrated companies. On these units, the physical facilities are under the ownership of one person but the pigs remain the property of the company. These companies agreed to limited participation amongst their contractors. Finally, the low recruitment may reflect the relatively low priority given to *Salmonella* by producers amongst the many other factors influencing pig production in GB (23). The poor recruitment was discussed with the funding body Defra and it was agreed that the study should proceed, despite the reduced sample size, in full cognisance that this would jeopardise the chances of delivering unequivocal evidence. It was considered that there was nevertheless a good opportunity to collect valuable data, to meet the expectations of both the farmers who had agreed to participate and of the industry in general that new information would be forthcoming. Many reasons were given for declining to participate. Chief amongst these was that the study would interfere with the normal management of the farm and that, despite the compensation being offered, there was insufficient time to collect samples and complete records. A study of the attitudes of pig farmers to the control of *Salmonella* showed that whilst they understood the potential risks to human health, they had little belief in the efficacy of control measures and also considered that there should be greater financial compensation for the cost

involved in participation (23). These views may also have reduced willingness to participate.

Two of the recruited farms failed to participate in the longitudinal component of the project and one farm did not submit samples or information after the first post-restocking visit. As described earlier, there was also a loss of pens to follow-up due to management factors, including mixing pigs and emptying pens particularly towards the end of the finishing period. On occasions, pens were missed or the wrong pens were sampled due to human error.

A number of the farms also received visits at the end of the study in order to follow pigs from the farm to the abattoir. Using variable number tandem repeat (VNTR) analysis of *Salmonella* isolates, this demonstrated that some *Salmonella* present in farms immediately prior to slaughter could be isolated from pigs and or carcass swabs at the abattoir, whilst in other cases strains were isolated from the abattoir environment and from carcasses that had not been found on farms, suggesting contamination at the abattoir (24).

Two outcome measures were used to judge the impact of the intervention. The primary goal of control of *Salmonella* in pigs is to reduce human salmonellosis and *S.* Typhimurium is recognised as the most frequent serovar amongst pigs that may be implicated in some human cases. The primary outcome measure was incidence of infection with *S.* Typhimurium. The second outcome measure was incidence of infection with any *Salmonella* serovar. A strength of the project design was that samples were collected from the same pens throughout the follow up period, from introduction to the finisher accommodation to despatch for slaughter. This allowed the pen incidence rate to be estimated, which measured the rate at which pens that were initially free of infection changed their status to become infected. A pen of pigs was chosen as the unit of interest rather than individual pigs. There were several reasons for this. Firstly, from a practical point of view, non-invasive pooled sampling reduces stress to individual animals. Secondly, it was practical to train farmers to collect these samples and reduce study costs; this also allowed the farmers to be an active part of the study team. Thirdly, individual infected pigs only excrete *Salmonella* bacteria intermittently

and therefore, a negative sample does not imply that the animal is free of *Salmonella*. A within and between pen transmission model (25) predicts that infection is transmitted readily amongst pen mates and therefore, it is probable that at least one pig within an infected pen will be excreting *Salmonella* at the time of sampling. Work at VLA (26, 27) demonstrated that pooled pen faecal sampling is a sensitive detection method. Finally, the proposed interventions were applied at a group level – e.g. not mixing pigs from different sources, not returning pigs to the main herd if they had been isolated in sick pens.

The intervention was of necessity limited to measures that could be adopted by individual farms. The risk of introduction of infection when the farm was re-stocked could not be controlled directly, though randomisation provides some reassurance that risk was equally distributed between intervention and control arms and the prevalence of *Salmonella* was very similar in the comparison and intervention groups at the first sampling visit. There is very weak evidence that the incidence rate of new pen-level infections with any *Salmonella* was lower in the intervention group. The study did not show any evidence of benefit with respect to *S.* Typhimurium. This may reflect the small sample size, the amount of *Salmonella* infection introduced to the farms on re-stocking, difficulty in effectively implementing the intervention protocol and the "Hawthorne effect", in which comparison group farms improved their hygiene and biosecurity measures compared to their normal management. The recording sheets could have acted as a stimulus for such a change. It may also reflect the more invasive nature of Typhimurium and its particular affinity for pigs. Analysis of the recording sheets showed that there was little difference between the compliance with the intervention protocol amongst farmers in either group with respect to most components of the intervention programme except for the period of time for which accommodation was left empty between batches, which can have an important effect on survival of *Salmonella* in the environment.

Thus, a principal cause is likely to have been the lack of change in behaviour in the intervention group, with the exception of the increased duration of the period between the despatch of the last pig of the previous group and the arrival of the first pigs of the study batch. This underlines the importance of an "intent to treat" design, since policy-makers may be tempted to conclude that the benefits seen

from limited proof of principle studies or from assuming that protective effects observed in observational studies would result from the widespread promotion of such practices. The study may under-estimate the efficacy of the measures if applied thoroughly and consistently on a farm; however, the results provide striking evidence of the lack of impact from promotion of an acceptable intervention and the enormous challenge that policy-makers and the industry faces if *Salmonella* control is promoted as a single issue. Evidence that the generic hygiene and biosecurity measures that were promoted could also yield meaningful benefits in terms of a reduced incidence of endemic diseases and thus offer the producer a financial incentive affords a more realistic approach (28).

Assessment of pen *Salmonella* infection in the pre-trial batch of pigs confirmed that 2/3rd of the farms had at least one infected pen and on two farms, all tested pens were infected. Thus, there was likely to have been a substantial burden of infection amongst the previous batch of pigs and in the general farm environment which could act as a source of *Salmonella* to the next batch of pigs, as shown by the association between pre-trial and post-cleaning pen infection. Farms in the intervention group were more likely to have no infected pens after cleaning, although *Salmonella* was isolated from more than half of all studied pens after cleaning and disinfection had been carried out. *Salmonella* was also isolated from 16/46 of the vehicles delivering the study batches of pigs to the farms. This result supports the opinion of many farmers that investment in control of *Salmonella* at the finisher stage is not worthwhile unless *Salmonella*-free weaners can be produced (29). The EU baseline survey of *Salmonella* in breeding pig units showed that more than 50% were infected, showing that these farms are potent sources of infection for the finisher herd (30).

The fact that the mean prevalence amongst all study farms was reduced compared to the prevalence in the pre-trial samples may be because all studied farms consciously or unconsciously improved their standards of hygiene and biosecurity. Alternatively, this may simply have been by chance. The value of results from a prior, untreated batch compared to an intervention batch without a

contemporaneous control is dubious, as shown in other field studies conducted by VLA (10, 31).

Conclusions

In summary, this study demonstrated that promotion of an enhanced biosecurity and hygiene protocol did not result in any measurable reduction in the incidence of infection in pens of pigs in the intervention group, which is likely to be due at least in part to the failure of the intervention group to change behaviour to any great degree during the study. The data suggested that there may have been two changed behaviours in the intervention group. Firstly, improved between-batch cleaning and disinfection practice was reported, which reduced the risk of contamination in pens before re-stocking. Secondly, the accommodation was left empty for longer in the intervention farms, which will have reduced the survival of *Salmonella* in the environment. However, the farmers were unable to influence the *Salmonella* status of their study batch of pigs at arrival on the farm, many of which were infected on delivery, as shown by the transport status and the prevalence of infected pens on visit 1, approximately three days after arrival. It is clear that unless attention is focused upon the provision of "*Salmonella* free" growers or weaners (29) investment in hygiene and biosecurity on finisher units may be prejudiced by introducing pigs that are already infected with *Salmonella*. These results suggest that there are unlikely to be any industry level benefits from promotion of "best practices" for cleaning and disinfection, such as those promoted via the Defra Code of Practice unless the problem of introducing infection on re-stocking is addressed.

The results were disseminated to the industry through meetings, the first of which took place at the Royal Agricultural Society showground on March 20th 2007 as a part of a meeting entitled "Serious about *Salmonella*". This led to the production of an FSA Guide with the same title:

http://www.food.gov.uk/multimedia/pdfs/publication/*Salmonella*pig1207.pdf

The knowledge gained from the study was used by ADAS in the development of a "roadshow" that was implemented across GB in 2007-2008.

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Chapter 8. An investigation into the association between reported biosecurity and hygiene practices and the incidence of *Salmonella*

Introduction

Salmonella infection in pigs represents a potential origin for some cases of human salmonellosis, especially with respect to *Salmonella* Typhimurium. In GB, this is the predominant serovar in pigs and the second most frequently isolated serovar in human disease (1). As discussed in Chapter 7, a randomised intervention trial was conducted to test whether farms that were allocated to an enhanced hygiene and biosecurity protocol had a reduced risk of *Salmonella* infection. The impact of the intervention was measured through repeated collection of pooled pen floor faecal samples and collection of meat juice (MJ) samples at slaughter. The former were cultured for *Salmonella* as described in Chapter 2. A single colony from each culture plate was selected these isolates were identified to the serovar level. The meat juice samples were tested using the MJ ELISA test for evidence of antibodies against *Salmonella* using two cut-off values (s:p ratio = 0.10 [MJ10] or s:p ratio = 0.25 [MJ25]). The impact of the intervention was assessed by estimating the pen incidence rate ratio for any *Salmonella* and for *S.* Typhimurium and by estimating the odds ratio for MJ ELISA positive pigs at slaughter using either the MJ10 or MJ25 cut-off. The trial did not detect convincing evidence of an effect of the intervention on any of these outcome measures. However, the relatively small sample size and the strong within-farm clustering limited the power of the study and the results were not incompatible with an important impact at a population level.

Importantly, the trial had very limited impact on most reported behaviours relating to hygiene and biosecurity practices except that farms in the intervention group reported that accommodation was left empty for a longer period between batches. This was in part due to a longer period for the accommodation to dry after cleaning and disinfection had been accomplished. However, there was a considerable range of behaviour within each group. There are few longitudinal studies of *Salmonella* infection in which intensive sampling has been conducted in more than a handful of farms and this dataset relating to 46 all-in/ all-out finishing batches provides a valuable opportunity to investigate whether there is

any association between reported practices and *Salmonella* incidence. Each farm was scored according to the number of the recommended practices that were reported firstly, in the period leading up to re-stocking and secondly, during the finishing period of the study group of pigs. The association between composite hygiene scores and other related variables and *Salmonella* infection was investigated, as described below.

Materials and methods

Full details on farm recruitment are provided in Chapter 7. Briefly, 46 recruited farms participated in the study, which began after the preceding batch of pigs had been sent for slaughter and the finisher accommodation was completely emptied. One farm completed the between-batch stage but did not complete the longitudinal part of the study.

Environmental pen floor samples were collected after cleaning and disinfection had been completed but before the accommodation was re-populated with the study batch of pigs. Samples were also collected from the transport that delivered the study batch of pigs. Pooled pen floor samples were collected from up to 30 randomly-selected pens on each farm approximately 3 days after re-population and at approximately 4-weekly intervals thereafter. These were sent for culture for *Salmonella* at the National Reference Laboratory at AHVLA Weybridge using the methods described in Chapter 2.

At the end of the finishing period, a random sample of up to 40 pigs were selected from one slaughter batch on each farm. Meat juice samples were collected from each pig and tested using the MJ ELISA test; full details are provided in Chapter 2. Data on hygiene and biosecurity measures were recorded every week by the participants and sent to CERA, AHVLA-Weybridge for entry onto a dedicated project database. The recording sheets are provided as an appendix.

A hygiene and biosecurity score (HBS) was calculated as the sum of each recommended measure that had been reported during the cleaning and disinfection phase and before the accommodation was re-stocked with the study

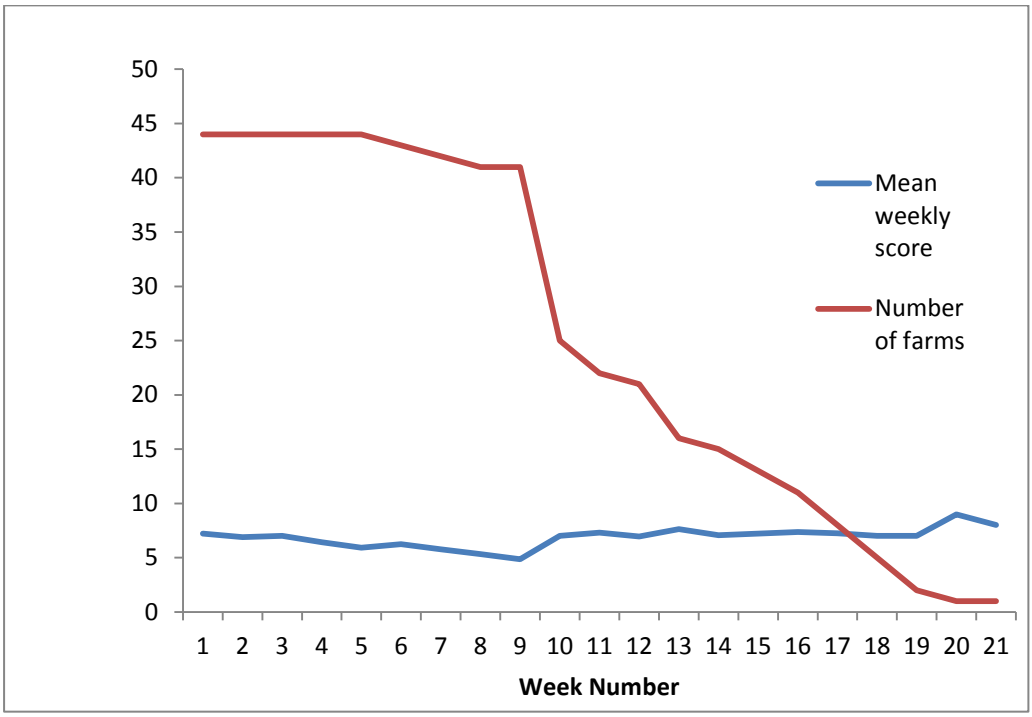
batch of pigs.. One “point” was awarded for each measure taken, so that if cleaning and disinfection were required, this resulted in 2 points whilst cleaning without disinfection was rewarded with one point; no points were awarded if the measure had not been implemented. A maximum of 19 points was possible, as shown in table 8.1 below.

Table 8.1. Practices contributing to the between-batch hygiene and biosecurity score (HBS) in a study on GB finisher pig farms

Variable	Category	Score
Feeders completely emptied after pigs left	No	0
	Yes	1
Solid waste cleaned out of feeders	No	0
	Yes	1
Feeders pressure washed	No	0
	Yes	1
Slurry/ manure	Pits beneath slatted floors emptied (slurry system)	1
	Muck heap removed (solid system)	1
	Slurry/ muck not removed	0
Water system	No treatment	0
	Drained	1
	Drained & flushed with disinfectant	2
Pig accommodation	No treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Pig transit areas	No treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Partitions in pig accommodation	No treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Equipment for moving pigs	No treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Large equipment	No Treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Small equipment	No Treatment	0
	Cleaned only	1
	Cleaned & disinfected	2
Protective clothing	No Treatment	0
	Cleaned	1
MAXIMUM POSSIBLE SCORE		**19**

Forty-four farms submitted 5 weekly reports; thereafter, the number of reports declined and the maximum number of reports submitted was 21, by one farm (see figure 8.1 below)

Figure 8.1 Number of weekly reports and mean weekly hygiene and biosecurity score during a study on GB finisher pig farms.



Data from the weekly reports were summarised into a mean hygiene score (MHS). To account for varying numbers of reports and duration of the study between farms, the total number of points was divided by the number of weekly reports that were received.

Table 8.2 Variables contributing to mean weekly hygiene score (MHS) during a study on GB finisher pig farms

Variable	Category	Score
Visitors	None	2
	Yes – but did not enter pig accommodation	1
	Yes – used protective clothing	1
	Yes; no protective clothing & entered pig accommodation	0
Staff visited other livestock farm	No	1
	Yes	0
Muck scraper cleaned & disinfected (C&D)	No	0
	Cleaned only	1
	Cleaned & disinfected	2
Tractor tyres C&D	No	0
	Cleaned only	1
	Cleaned & disinfected	2
Large equipment C&D	No	0
	Cleaned only	1
	Cleaned & disinfected	2
Small equipment C&D	No	0
	Cleaned only	1
	Cleaned & disinfected	2
Equipment taken off farm	No	2
	Yes – C&D on return	1
	Yes – no C&D on return	0
Rodent bait checked & replenished	No	0
	Checked only	1
	Replenished	2
Boot dips replaced	No	0
	Yes	1
Overalls washed	No	0
	Yes	1
Protective clothing washed	No	0
	Yes	1
Hands always washed on entering unit	No	0
	Yes	1
MAXIMUM POSSIBLE SCORE		**19**

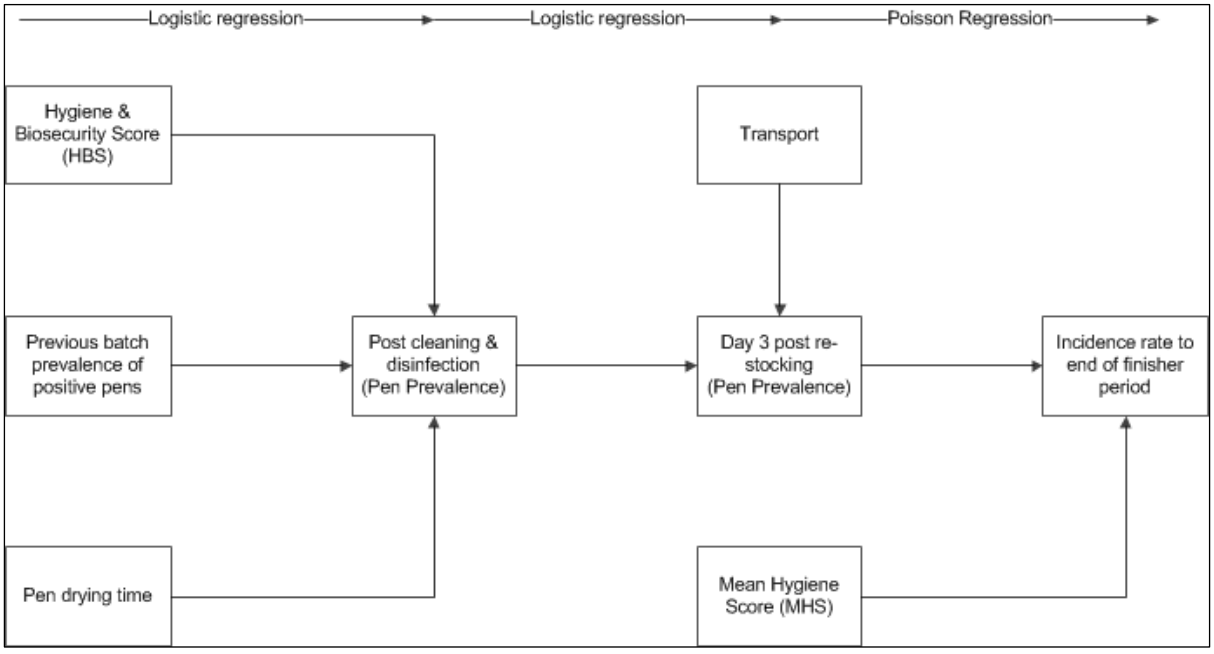
As illustrated in figure 8.1, there was no evidence of any important variation in average MHS by week (ANOVA; p=0.55). Figure 8.2 below shows the framework used to analyse the data from this study. Three outcome points for the isolation of *Salmonella* from pens were considered:

1. after de-stocking, cleaning and disinfection and drying
2. on day 3 after re-stocking

3. between Day 3 and the end of the finisher period.

Figure 8.2 shows a causal framework linking exposures to outcomes at different time points. Explanatory variables for each outcome are shown; the post-cleaning and disinfection status was considered as a potential causal factor for the pen status on day 3 after re-stocking and this in turn was considered as a potential causal factor for the incidence of new pen infections during the finisher period. Variables were categorised such that approximately one third of observations were allocated to each group.

Figure 8.2 Framework for investigation of factors associated with *Salmonella* status of finisher farms in a longitudinal study in GB



Post-cleaning and disinfection pen prevalence

Each pen was categorised as *Salmonella*-positive or negative according to the results of culture of pen floor samples. Individual pen results were not available at the serovar level. Three explanatory variables were defined: HBS (see table 8.1), prevalence of *Salmonella*-positive pens in the previous batch of pigs and the time for which pens were allowed to dry after cleaning and disinfection. Results were analysed at the pen level using multiple logistic regression and GEE was used to account for clustering by farm.

Day 3 post re-stocking pen prevalence

Results from pen floor samples on Day 3 after re-stocking were used to classify pens as *Salmonella*-negative, positive for any *Salmonella* and positive for *S*. Typhimurium. The latter were included within the any *Salmonella* category. Two explanatory variables were considered: prevalence of *Salmonella*-positive pens after cleaning and disinfection and presence of *Salmonella* on the transport that delivered the study batch of pigs. Data were analysed by multiple logistic regression using GEE to account for clustering by farm.

Finisher period incidence rate

As described, pen samples were collected at approximately monthly intervals during the finisher period. For pens that were *Salmonella*-negative at day 3, pen-days at risk were calculated as the days to the latest negative sample plus half of the number of days between the last negative test and the first positive test. The incidence rate was calculated separately for any *Salmonella* and for *S*. Typhimurium. For pens that did not become positive, pen-days at risk were calculated as the number of days between the first and the last sampling dates. A Poisson regression model was used to estimate the strength of association between the prevalence of positive pens at day 3 post re-stocking, the mean hygiene score (MHS – see table 8.2) and the incidence of new pen infections. Robust standard errors were used to take account of clustering at the farm level. Finally, the impact of the prevalence of *Salmonella*-positive pens at day 3 post re-stocking and of MHS on the MJ ELISA test results was considered using the MJ10 cut-off in a multiple logistic regression model using GEE to adjust the standard error of the odds ratio to account for farm-level clustering.

All data were analysed using Stata release 12 (www.stata.com).

Results

Post-cleaning and disinfection

Table 8.3 below summarises the results of the analysis of the association between reported explanatory variables and pen status after cleaning and disinfection. Crude odds ratios and adjusted odds ratios have been estimated using GEE to estimate robust standard errors.

Table 8.3 Risk factors for *Salmonella*-contaminated pens after cleaning and disinfection in a longitudinal study on GB finisher pig farms

Variable	Class	Number of farms	Number of Pens (%)		Crude Odds Ratio (95% ci)	Adjusted Odds Ratio (95% ci)
			Salmonella negative	*Salmonella* positive		
Prevalence previous batch (ppb)	0%	14	230 (89%)	27 (11%)	1.00	1.00
	0%<ppb<= 50%	19	358 (78%)	101 (22%)	3.34 (0.86 – 12.95)	1.24 (0.19 – 7.93)
	50%<ppb< =100%	13	239 (69%)	107 (31%)	5.64 (1.34 – 23.84)	9.63 (1.50 – 61.70)
Hygiene & Biosecurity Score (HBS)	0 – 9	13	253 (73%)	95 (27%)	1.00	1.00
	10 – 14	14	225 (75%)	74 (25%)	0.55 (0.15-2.02)	0.28 (0.06-1.28)
	15 – 19	19	349 (84%)	66 (16%)	0.36 (0.11-1.21)	0.11 (0.01-0.92)
Dry days	<1 day	8	80 (50%)	79 (50%)	1.00	1.00
	1-3 days	5	68 (76%)	22 (24%)	0.35 (0.04-2.78)	0.10 (0.01-0.96)
	>3 days	20	392 (88%)	53 (12%)	0.10 (0.02-0.48)	0.27 (0.04-1.85)

A total of 1062 pens were tested on 46 farms in the pre-study batch, prior to de-stocking. The crude odds ratio shows strong evidence (p= 0.02) for an increasing risk of *Salmonella* contamination as the prior batch prevalence increases. HBS varied from 1 to 19 across the 46 farms, with a median value of 13 and an inter-quartile range of 9 -16. The mean value was 12.1 and the 95% confidence interval was 10.5 – 13.6. The crude odds ratios suggest an important trend (p=0.08) of a reducing risk of pen contamination as HBS increases although this could be achance finding (p=0.24). Thirty-three of the 46 farms reported the number of days that pens were left to dry after cleaning and disinfection and a total of 694 pens were tested, as shown in table 8.3. The number of hours that pens were left to dry varied markedly, from 4 hours to 720 hours. A period of 30 days between batches is very unusual but may occur if, for example, extensive

repairs to the accommodation are required that cannot be done whilst pigs are present. The median period was 120 hours and the inter-quartile range was 48 – 240 hours; the mean was 165 hours. The crude odds ratio provides some evidence that increased dry days reduces the risk of pen contamination (p=0.02) and there is strong evidence of a trend (p=0.004).

The confidence intervals from the multivariable analysis are very wide and point estimates must be interpreted with caution. This may have arisen because data on the number of dry days was not provided by 13 of the 46 farms (28%) and thus, pen data from these could not contribute to the multivariable analysis. However, the results are consistent with the consideration that all 3 factors play a role in determining the prevalence of *Salmonella*-contaminated pens after cleaning and disinfection. Conservatively, a high prevalence of infected pens in the previous batch increases the risk of pen contamination by a factor of approximately 2 or more. Greater attention to hygiene and biosecurity may reduce the risk of pen contamination by at least 10% approximately whilst increased dry days may reduce this risk by 5% or more.

Day 3 post re-stocking

Data from 46 farms were analysed for the between-batch period, up to day 3 after re-stocking and results are shown in table 8.4 below. Amongst these farms, the prevalence of *Salmonella*-contaminated pens after cleaning and disinfection but prior to re-stocking varied from 0% - 100%, with a median of 6% and an inter-quartile range of 0% - 47%; the mean was 24%. This skewed distribution is explained by the observation that 20 of the 46 farms (43%) had no contaminated pens (prevalence of 0%). *Salmonella* was isolated from 19 vehicles delivering pigs and 7 of these isolates were *S*. Typhimurium. Only the first vehicle delivering pigs was sampled on each farm; it is possible that other vehicles also delivered pigs but this information was not recorded. Pen status at day 3 was classified as: negative for *Salmonella*, positive for any *Salmonella* serovar or positive for *S*. Typhimurium. The prevalence of contaminated pens after cleaning and disinfection was only estimated for any serovar but the status of transport was also differentiated between any serovar and *S*. Typhimurium.

Univariable analysis did not show any evidence of an association between the prevalence of contaminated pens after cleaning and disinfection and pen status on day 3 (p = 0.4) although the results are consistent with a weak association. In contrast, univariable analysis showed some evidence that detection of any serovar on transport increased the risk of a positive pen (any serovar) on day 3 (OR = 1.99; 95% ci 0.92 – 4.30; p = 0.07). Both putative risk factors were included in a multivariable model, with robust standard errors estimated by GEE as shown in table 8.4.

Table 8.4 Risk factors for *Salmonella*-infected pens on day 3 after re-stocking in a longitudinal study on GB finisher pig farms

Variable	Class	Number of farms	Number of pens (%)		Crude odds ratio (95% ci)	Adjusted odds ratio (95% ci)
			Salmonella negative	*Salmonella* positive		
Transport status	Negative	27	577 (80%)	146 (20%)	1.00	1.00
	Positive	19	336 (65%)	181 (35%)	1.99 (0.92-4.30)	1.96 (0.94-4.11)
Pen prevalence after C&D	0%	20	364 (78%)	101 (22%)	1.00	1.00
	0%<pcd<15%	10	206 (66%)	105 (34%)	1.93 (0.71-5.30)	1.71 (0.67-4.36)
	15%<=pcd	16	343 (74%)	121 (26%)	1.51 (0.62-3.67)	1.61 (0.66-3.94)

There is no indication of any important confounding between these variables and the association between transport status and pen status remains important, with odds of isolation of any *Salmonella* serovar increasing approximately twofold if the transport was positive (p=0.075). Table 8.5 shows the results when pen status at day 3 is restricted to *S*. Typhimurium.

Table 8.5 Risk factors for *S*. Typhimurium-infected pens on day 3 after re-stocking during a study on GB finisher pig farms

Variable	Class	Number of pens (%)		Crude odds ratio (95% ci)	Adjusted odds ratio (95% ci)
		S. Typhimurium negative	*S.* Typhimurium positive		
Transport status	Negative	636 (88%)	87 (12%)	1.00	1.00
	Positive	449 (87%)	68 (13%)	0.89 (0.29-2.74)	0.93 (0.32-2.72)
Pen prevalence after C&D (pcd)	0%	433 (93%)	32 (7%)	1.00	1.00
	0%<pcd<15%	267 (86%)	44 (14%)	2.28 (0.43-12.21)	2.32 (0.44-12.16)
	15%<=pcd	385 (83%)	79 (17%)	3.52 (0.82-15.08)	3.49 (0.83-14.80)

The crude odds ratio for transport status shows no evidence of an association with pen status for *S*. Typhimurium (p=0.83). There is some evidence of an association with prevalence of contaminated pens post cleaning and disinfection and the test for trend shows the risk of *S*. Typhimurium pen infection increasing as the prevalence of pen contamination increases (p=0.08). There was no evidence of any association between pen status for *S*. Typhimurium and detection of this serovar from transport (p=0.52; results not shown).

Finisher period pen incidence rate

Forty-four farms were included in the longitudinal component of the study, from day 3 post re-stocking until the end of the finisher period. The mean weekly hygiene and biosecurity score (MHS) amongst these farms varied from 3.2 – 12.8 with a median of 7.5 and an interquartile range of 5.4 – 9.7; the mean was 7.6. There was no evidence of any correlation between HBS and MHS (p=0.48), which is perhaps surprising since it might have been expected that those farmers who were more diligent in the between-batch phase might also have made greater effort in the finisher period. Table 8.6 shows the pen incidence rate for each risk factor and the estimated crude and adjusted incidence rate ratio (IRR). A Poisson regression model using robust estimates of standard error to adjust for the farm-level cluster effect was fitted to the data.

Table 8.6 Risk factors for new pen *Salmonella* infections during the finishing period during a study on GB finisher pig farms

Variable	Class	Pen-days at risk (pdar)	New pen infections	Pen incidence rate (per 100 pdar)	Crude incidence rate ratio (95% ci)	Adjusted incidence rate ratio (95% ci)
MHS	0<=MHS<6	14634	45	0.31	1.00	1.00
	6<=MHS<9	19907	21	0.11	0.54 (0.19-1.48)	0.51 (0.23-1.12)
	9<=MHS<=19	20810.5	52	0.25	0.78 (0.31-1.93)	0.72 (0.35-1.48)
Pen prevalence day 3 (pp3)	0%	17071.5	1	0.01	1.00	1.00
	0%<pp3<=27%	28613.5	60	0.21	16.64 (4.71 – 58.88)	18.00 (5.12-63.25)
	27%<pp3<=100%	12502.5	61	0.49	30.58 (9.02-103.70)	30.62 (9.33-100.59)

The crude IRR shows no evidence of any important association between MHS and pen incidence rate for any *Salmonella* (p=0.48). Strong evidence of an association between the prevalence of infected pens on day 3 and pen incidence was observed (p<0.001). However, the confidence intervals are extremely wide, so point estimates must be interpreted with caution. The multivariable model shows no important evidence of confounding between the two explanatory variables. A conservative interpretation of these results is that the risk of new pen *Salmonella* infections is increased by a factor of 4 if the prevalence of infected pens on day 3 was up to 27% and could be even greater if this prevalence were above 27%.

Table 8.7 shows the results when the pen incidence rate is estimated for new infections with *S.* Typhimurium.

Table 8.7 Risk factors for new pen *S*. Typhimurium infections during the finishing period during a study on GB pig farms

Variable	Class	Pen-days at risk (pdar)	New pen infections	Pen incidence rate (per 100 pdar)	Crude incidence rate ratio (95% ci)	Adjusted incidence rate ratio (95% ci)
MHS	0<=MHS<6	14634	45	0.31	1.00	1.00
	6<=MHS<9	19907	21	0.11	0.40 (0.15-1.08)	0.46 (0.19 – 1.11)
	9<=MHS<=19	20810.5	52	0.25	0.86 (0.31-2.38)	0.95 (0.35-2.66)
Pen prevalence day 3 – any serovar (pp3)	0%	17071.5	1	0.01	1.00	1.00
	0%<pp3<=27%	28613.5	60	0.21	33.74 (4.16-273.88)	36.58 (4.54-294.85)
	27%<pp3<=100%	12502.5	61	0.49	87.83 (11.12-693.54)	85.58 (11.00-665.65)

The crude IRR shows very weak evidence of an association between MHS and *S*. Typhimurium pen incidence rate (p=0.17) and there is no consistent trend, as the intermediate IRR is lower than that estimated for MHS of 9 or more. However, there is evidence that the incidence rate increases in association with an increased prevalence of infected pens on Day 3, although the confidence intervals are very wide. This analysis was repeated, using the prevalence of pens infected with *S*. Typhimurium on Day 3 as an explanatory variable. No trend of increasing incidence associated with increased day 3 prevalence was observed (p=0.32) nor was there any important evidence of a trend with MHS (p=0.21).

All explanatory variables (HBS, previous batch prevalence, drying time, transport, MHS and pen prevalence – Day 3) were also analysed to investigate any association with the MJ ELISA results, using the MJ10 cut-off value. A multivariable logistic regression GEE model was used with robust standard errors to account for clustering by farm. Data from 1008 tests on pigs from 24 farms were included. Only two variables showed evidence of any important association (see table 8.8). The first of these was the number of days accommodation was allowed to dry after between-batch cleaning and disinfection, which was associated with a reduced risk of a positive MJ ELISA result (OR for > 3days compared to <1 day 0.10; 95% ci 0.04 – 0.25). Secondly, the prevalence of *Salmonella*-contaminated pens after cleaning & disinfection was associated with

an increased risk of a positive MJ ELISA result although these estimates were associated with a very wide 95% confidence interval (OR for prevalence Day 3 >15% compared to 0% 27.36; 95% ci 4.91 – 152.33). .

Table 8.8 Variables associated with MJ ELISA *Salmonella* results in a multivariable logistic regression model adjusted for clustering by farm, using a 0.10 s:p ratio cut-off (other variables omitted) during a study on GB finisher pig farms

Variable	Category	Odds Ratio	95% confidence interval
Days dry	<1	1.00	
	1-3	0.38	0.13-1.14
	>3	0.10	0.04-0.25
Prevalence contaminated pens after C&D	0%	1.00	
	0%-15%	30.97	7.22-132.73
	>15%	27.36	4.91-152.33

Discussion

These data were obtained from 46 all-in/ all-out finisher units, which were followed from the end of one production cycle through to the despatch to the abattoir of the study group of pigs. The participating farmers submitted more than 500 weekly reports and collected or assisted in the collection of more than 7400 samples that were cultured for *Salmonella*. At the end of the study, more than 1500 MJ ELISA tests were conducted. There are relatively few longitudinal studies of *Salmonella* infection in pigs reported up to the present date and many of these are limited to fewer than ten farms (2-12) or consist of visits to farms at the same stage of production on repeated occasions (13, 14), when the population of pigs that are present has changed. One study conducted in Spain and Denmark included 13 farms but only used serology to investigate seroconversion to *Salmonella* and a range of other infectious agents (15). Other studies have included more farms and have gathered data over a number of years but have not employed consistent sampling strategies (16-20). One study in Alberta, Canada included 90 farms each of which was visited 2 or 3 times over a 5-month period and 5 pen samples were collected at each visit. However, each set of 5 pen samples was selected randomly so pen incidence could not be estimated. Furthermore, no explanatory variable data were collected (21). Therefore, this is the largest longitudinal study of *Salmonella* infection in finishing pigs using a systematic and intensive sampling regime and collecting exposure data that has been reported. It is also the only study to have estimated the incidence rate and incidence rate ratio from the data; most other studies have relied on examining the prevalence at different points during the study although Beloeil *et al* (9) used survival analysis to measure the rate of seroconversion amongst a cohort of finisher pigs.

Despite the size and cost of this study, the power to detect important associations between potential risk factors and *Salmonella* incidence is limited by the small sample size and by the strong within-farm clustering. As discussed in Chapter 7, a larger study was proposed but it proved to be impossible to recruit more farms. This was largely due to the lack of time that farmers had to undertake the data and sample collection. The option of abandoning the study was discussed with the funding body (Defra) and it was decided to proceed in the knowledge that the

number of farms was less than had been intended. Inevitably, there was some loss to follow up as the study progressed, as shown in figure 8.1.

A framework for data analysis was defined (figure 8.2) that accounted for the potential impact of reported exposure variables upon three outcomes – the prevalence of *Salmonella*-contaminated pens after cleaning and disinfection but prior to re-stocking with the study batch of pigs, the prevalence of infected pens at day 3 after re-stocking and the incidence of new pen infections during the finisher period. Each outcome was also considered as an explanatory variable for the subsequent event. Thus, the prevalence of contaminated pens was included in the model to consider day 3 prevalence, which in turn was investigated as an explanatory variable for the pen incidence rate.

A large number of individual biosecurity and hygiene practices were reported during the between-batch period and on a weekly basis after re-stocking. These were summarised as two composite measures – HBS, which addressed the between-batch period and MHS, which summarised weekly reports (see tables 8.1 and 8.2). Each reported action was given a score of one "point" and these were summed to give a farm-level score. This was estimated as a mean weekly score for MHS to account for variation in the number of reports each farm submitted. It is not intended to suggest that each of these individual actions would have an equal effect on the incidence of *Salmonella* infection nor that each action required equal effort to implement. Instead, it is intended that the scores reflect to some extent the total effort expended by each farmer on hygiene and biosecurity practices. The wide range of values for MHS and HBS suggest that this approach has succeeded in discriminating amongst the participants. It might have been expected that farms which made greater efforts with hygiene and biosecurity prior to re-stocking would have acted in a similar manner afterwards. Surprisingly, there was no evidence of any association between HBS and MHS. Speculatively this may have been due to a number of factors. Firstly, the motivation for more rigorous attention to hygiene and biosecurity between batches (HBS) may have been promoted amongst all farms by their inclusion in the study. These measures were followed over a relatively short period of time and many farmers may have wanted to make extra effort, whether or not they

were recruited into the intervention group. However, once this period ended, many farmers may have been more inclined to revert to their usual practices. It is also possible that some random reporting bias arose during the longer finisher period and that MHS was less accurately reported. The reported mean weekly hygiene and biosecurity score (MHS) varied greatly amongst farms although it was fairly consistent within farms. As shown in figure 8.1, there was a considerable reduction in the number of weekly reports received after week 9 of the follow-up period. Some of this may be due to those pigs that were heaviest at delivery and that grew fastest being sent for slaughter. However, it is also a result of a loss of motivation amongst some farmers to continue with completion of the weekly reports.

Exposures were measured at the farm level whilst outcomes were considered at pen level. Multivariable logistic regression was used to estimate the association for the first two models and the point estimates and standard errors of the odds ratios were adjusted by GEE to account for clustering at the farm level. Poisson regression was used to estimate the incidence rate ratio for association between explanatory variables and pen incidence – robust standard errors were calculated to account for clustering.

Results showed that the prevalence of infected pens in the previous batch of pigs was strongly associated with the prevalence of contaminated pens after cleaning and disinfection even after taking into account the reported hygiene and biosecurity measures and the number of days for which pens were left to dry (Table 8.3). *Salmonella* infection may lead to widespread contamination of the farm environment, including areas outside pig buildings and it may be brought back into the accommodation on clothing or equipment. Rodents are also susceptible to infection and can act as a local reservoir for recontamination of cleaned surfaces, especially if feed is not completely removed. Therefore, it is unsurprising that a greater prevalence of infected pens in one finisher batch increases the risk of pen contamination. The results also indicate that improved hygiene and biosecurity practices and a longer period for drying after disinfection may mitigate this risk to some degree although the effect may be modest. Whilst it may seem obvious that better cleaning and disinfection should reduce the risk

of *Salmonella* contamination, previous work has shown that cleaning procedures may in fact increase the contamination within pig pens by re-distributing bacteria from e.g. floors onto clean surfaces (22). In addition, some floor surfaces may be damaged enabling *Salmonella* to be harboured with cracks and crevices and finally, there may be inaccessible corners and crannies where contaminated material may be trapped. Previous experience has also shown that farmers may not perform optimal disinfection practices. For example, many are unaware that some common disinfectants are inactivated by organic material, they may be uncertain of correct dilution rates and where administration is via a lance attached to a pressure-washer, they may be unable to estimate the flow rate or to check that any calibration system is functioning correctly. It is important to recollect that all of these finisher units operated on an all-in/ all-out basis; it is intuitively reasonable to suppose that the effect of persisting environmental contamination and the prevalence of infected pigs in continuously-occupied accommodation would be as great or even greater.

The results in table 8.4 and 8.5 show that isolation of *Salmonella* from transport delivering the study batch of pigs was associated with a two-fold increased risk of pen infection on day 3 after re-stocking. Since animal transport must be cleaned and disinfected after every trip and pigs are only on the transport for a few hours, presence of *Salmonella* is more likely to arise from active excretion by the pigs or by accidental contamination of the vehicle during loading, unloading or during the sampling process. It is easier to accomplish effective cleaning and disinfection of a vehicle than of pig accommodation, as surfaces tend to be impervious and are less prone to damage or areas that may harbour contaminated material, provided that sufficient downtime is allowed for drying (23). Movement and mixing of pigs is an inevitable cause of stress, which may both stimulate re-activation of latent infection in carrier animals and also increase susceptibility to infection (24, 25). The evidence for an association between the prevalence of contaminated pens and day 3 pen infection was very weak, although compatible with an increased risk. The number of *Salmonella* bacteria that survive in a dry empty pen is likely to be very much less than the number that are excreted by actively infected pigs. Table 8.5 shows *S*. Typhimurium as the outcome rather than any serovar. These results do not demonstrate any important association but are compatible with an

increased risk of pen infection if transport was contaminated or if the post-cleaning and disinfection prevalence of contaminated pens was high. The elevated risk of pen infection as a consequence of introducing infected pigs is of considerable practical significance. As a farm manager, it is not possible to influence the status of pigs that are delivered for finishing. Pigs that are reared under contract usually remain the property of the company concerned and are selected and despatched according to commercial considerations that do not generally include the *Salmonella* status of either the recipient or supplying herd or herds – frequently, pigs from more than one herd are combined for finishing. The potential for the investment of money, time and physical effort in cleaning and disinfection to be overwhelmed by the introduction of *Salmonella* infection is liable to be very de-motivating.

The incidence rate of new pen infections with any *Salmonella* serovar was strongly associated with the prevalence of infected pens on day 3 after re-stocking, although the estimated confidence intervals of the incidence rate ratios were very wide (Table 8.6 and 8.7). A conservative interpretation is that a day 3 pen prevalence of up to 27% could be associated with a fourfold increased incidence and that this might be even greater if the day 3 prevalence were higher. Where pen incidence of *S*. Typhimurium was specifically investigated as an outcome, the prevalence of any serovar was also a strong risk factor (Table 8.7). Where the prevalence on day 3 was restricted to pen infection with *S*. Typhimurium, the evidence for an association with serovar-specific incidence was weak but the results were compatible with an increasing risk. There was little or no evidence that MHS was associated with the pen incidence rate in any of these models. Each of the finisher pens in the study farms typically held around 30 pigs and where the day 3 pen prevalence is high, it implies a very large number of pigs were excreting *Salmonella* bacteria into the pen, which is likely to lead to substantial environmental contamination, far greater than that attributable to residual contamination or reservoirs in e.g. rats or mice. In these circumstances, dissemination of infection through the whole accommodation is likely so that many new pen infections occur. The absence of any convincing evidence that greater efforts in within-batch hygiene and biosecurity measures has any effect on incidence may be because these measures are overwhelmed by the weight of

environmental contamination. MHS is a composite score, so it is likely that the particular measures adopted by farms with similar MHS scores varied. Possibly the component parts of MHS varied in efficacy, so evidence for any beneficial effect from some actions might be swamped by the lack of effect from other actions. The possibility that some respondents failed to implement recommended measures or failed to correctly record those actions which were taken cannot be discounted. However, periodic visits were made to the study farms and no evidence for this was detected. Furthermore, pen samples were submitted as requested and there is no reason to doubt the good intentions of participants to record correct information to the best of their abilities. The results of this study support farmer opinion that investment in hygiene and biosecurity in the finisher period is of dubious value with respect to *Salmonella* control if they are unable to re-stock with *Salmonella*-free weaners (26, 27).

The final outcome variable that was considered was the prevalence of MJ ELISA positive pigs at slaughter. This indicates the lifetime exposure of pigs to *Salmonella* infection tempered by the rate of loss of circulating antibody to the extent that the test becomes negative. Pens of pigs that were positive at Day 3 post introduction were excluded from the estimation of incidence rates, since they were positive at first sampling. However, the individual pigs within those pens were eligible for selection for MJ ELISA testing at slaughter and it is likely that infected pigs from these pens would yield a positive result, since the duration of a detectable titre has been estimated to exceed the length of the finishing period for many pigs (28). All exposure variables were included in this multiple logistic regression analysis and the prevalence of infected pens on day 3 was associated with the outcome (table 8.8). Evidence for an association between duration of the post-cleaning and disinfection drying period and the MJ ELISA prevalence was weak but compatible with a reduced risk as drying period increased. Until the cessation of the BPEx MJ ELISA monitoring scheme in 2012, the MJ ELISA was the only routine measure of *Salmonella* status available to farm managers and their veterinary advisers. If *Salmonella* status is only measured at the end of production through MJ ELISA tests at the abattoir, it is not possible to determine when infection occurred so farmers may receive no useful feedback on whether any interventions they have put in place actually yield a benefit. However, since

2012 even this poor indicator of herd status (29) has been lost. A key message for producers, for veterinary surgeons and for policy makers in Government and industry is that firstly, any intervention programme must include robust measures for evaluation and secondly, these must measure impact at multiple points in the production cycle, not just at the point of slaughter.

The hygiene and biosecurity measures employed in the present study and summarised in HBS and MHS (see tables 8.1 and 8.2) included actions to eliminate or reduce the weight of *Salmonella* contamination of the farm environment. Survival of *Salmonella* will be affected by factors including the pH, the ambient temperature, the presence of organic material, biofilms and availability of water or dessication. Many publications report on prolonged survival times of weeks or even months (30-33). However, other studies have shown that effective cleaning and disinfection coupled with drying, e.g. over a weekend, can significantly reduce the number of organisms that are present (34-38). This study provides evidence that corroborates reports that increasing the period that accommodation is left to dry is likely to be an effective measure for reducing the incidence of *Salmonella* during production.

In another study, isolates of *Salmonella* Typhimurium from the series of samples from 3 of the farms participating in this study were subjected to variable number tandem repeat (VNTR) analysis (39). On one farm, the same VNTR profile was observed on the transport, in Day 3 samples and up to 12 weeks after re-stocking. This provides corroborative evidence that infection may be transferred from transport to the finisher herd and persists thereafter. A study that used a PCR to identify DNA from *Salmonella* on transport delivering pigs to finishing farms stated that this was an important means of transmission between farms (40), although data were not subject to quantitative epidemiological analysis. Other authors have reported that infection may occur during the transport from farms to abattoirs or during the period that they are held in lairage prior to slaughter (41-45). These findings show that transmission of *Salmonella* between pigs during transport and in pens in the hours after unloading is a recognised problem.

As shown in table 8.2, many different activities contributed to MHS and those implemented by different farms with the same MHS varied. Therefore, this measure is a crude indicator of impact of any specific measures and is better regarded as a proxy for "reported effort". Farmers have reported that implementing rigorous hygiene and biosecurity practices is difficult (26, 46, 47) and this demotivates them, especially since benefits are hard to detect (48).

In conclusion, this study showed that the introduction of *Salmonella* infection onto all-in/ all-out units during re-stocking had a profound effect on the incidence of new infections. Although the results are compatible with a modest benefit from the rigorous implementation of hygiene and biosecurity measures, these could be overwhelmed by infection that was introduced with incoming pigs. Vaccination has been proposed as a useful tool in the control of Salmonella infection in pigs, as it was very successful in controlling *Salmonella* in poultry. There are technical issues to be overcome, including the need for a multivalent killed vaccine that does not interfere with serological tests. From a feasibility point of view, vaccine cost and the effort of delivery by injection, which is time-consuming and stressful for pigs, would also impact adoption. However, if there were a market advantage from employment of an effective vaccine then this could have a double benefit by reducing the burden of introduced infection, which in turn could deliver greater benefits from hygiene measures. A virtuous circle might emerge, in which sequential reduction in the burden of *Salmonella* within a herd leads to a reduction in the environmental load to which each batch of pigs is subjected. Vaccination might also be a useful tool to employ following establishment of a new, *Salmonella*-free herd on a new site and this approach has some favour amongst farmers (49).

The outcome measures that were defined were only concerned with *Salmonella* infection, which is seldom a cause of clinical disease in pigs in GB or linked with any important impact on productivity. It would have been interesting to have evaluated the impact of these measures on other infectious agents that are associated with clinical disease, since evidence of a benefit in this respect would be more influential with farmers if they could anticipate a financial return for their efforts. Although this is currently the largest longitudinal study on *Salmonella* in

pigs to be reported, it nevertheless did not have sufficient power to deliver evidence that is likely to persuade farmers to change their behaviour with respect to introducing interventions aimed solely at control of *Salmonella*.

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Chapter 9. Summary and Conclusions

The research reported in this thesis aimed to design and test a feasible intervention strategy to reduce the incidence of *Salmonella* infection in all-in/ all-out finisher pig units in Great Britain. Although *Salmonella* infection can cause clinical disease in pigs, it is frequently subclinical and the motivation for control lies in the zoonotic threat to public health. An enhanced hygiene and biosecurity intervention was devised, based on existing literature and additional research as described below.

Background

Human salmonellosis is the second most frequent cause of bacterial foodborne gastro-intestinal disease and has a significant public health impact (1). In 2011, there were 9455 reported cases in the UK, suggesting a total community burden of approximately 54,000 cases. The most common serovar isolated from human cases was *S*. Enteritidis, which is usually from a poultry source and was isolated from 31% of the reported cases. *S.* Typhimurium was the second most frequent isolate, accounting for 26% of cases. This serovar has a wide host range and is commonly associated with pigs. In recent years, the incidence of laboratory reports of *S.* Typhimurium has increased whilst that of *S*. Enteritidis has decreased (2). There is convincing evidence that some human infections can be attributed to pigs but the burden of human disease from this origin remains uncertain.

In 2003 the European Union (EU) decreed that *Salmonella* infection in domestic livestock including pigs and poultry should be controlled (3). This stimulated surveys amongst several EU Member States, including Denmark, Netherlands, UK, Spain, Belgium, Greece and Germany. In Great Britain, two abattoir surveys were conducted (4, 5). A European baseline survey of finisher pigs at slaughter was also conducted (6). These national and European observational studies also collected data on possible risk factors. However, these studies varied considerably in their design and in the variables that were recorded, precluding formal meta-analysis (7). As summarised in Chapter 1, risk factors could be considered in groups:

1. Biosecurity, hygiene and disinfection – as might be expected, higher standards were associated with a lower risk of *Salmonella* infection, although some equivocal studies were reported. Residual environmental contamination was particularly important as a source of infection to pigs entering finisher accommodation.
2. Herd characteristics – there was greater variation in reported results amongst studies. For example, although 5 studies reported that increased herd size was associated with an increased risk of *Salmonella* infection, one study reported a reduced risk. There were 3 studies of outdoor herds – one reported an increased risk, a second reported a reduced risk and the final study did not show any evidence of an important association with *Salmonella* infection. Importantly, all studies showed an increased risk of infection where pigs were fed on a pelleted diet compared to a home mill and mix or liquid diet. The meta-analysis of 12 studies reported in Chapter 1 indicates that pigs on pelleted feed were at least 2.3 times more likely to be *Salmonella* positive than pigs on home mill and mix or liquid diets (see figure 1.2).
3. Pig and health-related factors – concurrent infection with PRRS or PMWS, viral infections that are immune-suppressive, were associated with an increased risk of infection. Mixing pigs during the finisher phase, restocking with pigs from many sources and pen to pen nose contact were also associated with an increased risk of *Salmonella* infection. Unsurprisingly, the introduction of pigs that were already infected with *Salmonella* was also associated with an increased risk of infection during finishing.
4. Environmental factors – rodents, wild birds and flies were all associated with an increased risk of infection.

In summary, risks comprise those that may be associated with the survival of *Salmonella* in the farm environment, factors associated with the introduction of *Salmonella* into a previously uncontaminated environment and factors that predispose to transmission within the accommodation after re-stocking, including feed-related variables that impact the susceptibility of the pigs to infection.

These results are discussed in detail in Chapters 1, 4 and 5.

Approaches to testing pigs for Salmonella

National and European surveys used isolation of *Salmonella* by culture (see Chapter 2). In common with some other EU countries the UK pig industry introduced a *Salmonella* monitoring scheme based on ELISA tests on meat juice samples collected at abattoirs (8). These relatively cheap tests offered an alternative to isolation and culture for establishing the status of pigs and holdings with respect to *Salmonella*. However, as discussed in Chapter 3, there are important differences between these approaches. MJ ELISA tests are indicators of lifetime exposure to *Salmonella* infection whilst isolation demonstrates the presence of active infection or passive transient passage through the gut at the time of sampling. Chapter 3 also considered the use of carcass swabs to test for contamination after slaughter, at a point closer to human consumption. The results show that the prevalence of contaminated carcass swabs within a batch of pigs is not closely associated with the presence of *Salmonella* in lymph nodes or in the gut contents. This is as a consequence of abattoir effects, which on the one hand may act to de-contaminate carcasses e.g. during singeing or may increase contamination e.g. through poor technique in removal of guts or through cross-contamination from other carcasses or equipment. As a result of this research, it was judged that pooled pen floor samples were the more informative measure of *Salmonella* infection for the intervention study. However, since farmers only had ready access to MJ ELISA results, it was decided that these should also be monitored in the intervention study.

Cross-sectional studies

The abattoir surveys described above were supplemented by a farm-based survey, as described in Chapter 4. This survey utilised pooled pen floor faecal samples to measure farm-level prevalence, since this approach reduced costs and stress to pigs while retaining a satisfactory sensitivity (9, 10). This farm-based survey was a valuable addition to the results from previous abattoir-based studies, showing that on almost half of the sampled farms fewer than 10% of pens were infected with *Salmonella*. Thus, it is reasonable for those farms with a higher prevalence to aspire to achieve a similar level of infection with an effective intervention. The results were subjected to a multivariable analysis (see table

4.5), which showed that use of home-mix rations and use of a contractor to conduct rodent control were associated with a reduced risk of infection whilst concurrent PCV2 infection and an increased finisher mortality were associated with an increased risk of infection. The latter result is especially interesting, since it suggests that adoption of measures to control *Salmonella* might also benefit animal welfare and reduce mortality. This could motivate famers to adopt control measures and might offset the costs that might be incurred.

Chapter 5 describes the analysis of a dataset that combined MJ ELISA results with farm-level risk factors that had been recorded for Quality Assurance (QA) purposes. This study succeeded in linking two sets of data that had been collected for different purposes. It had the advantage that a large sample size of more 1600 farms was achieved for little cost. Multivariable analysis showed that pigs from breeder-finisher units were less likely to be MJ ELISA positive than pigs from specialist finisher units. In common with other surveys, home mix rations were associated with a reduced risk of infection, as measured by MJ ELISA. Pigs derived from outdoor units and pigs finished in solid-floored accommodation were at an increased risk of a positive MJ ELISA test (see table 5.6).

The literature review and the research reported in Chapters 4 and 5 informed the intervention that was devised and tested in a randomised controlled study as described in Chapter 7.

Intervention study

The intervention required farmers to implement an enhanced hygiene and biosecurity protocol (see appendix for details). The intervention began once the preceding batch of finisher pigs had been sent for slaughter and the accommodation was empty. A rigorous cleaning and disinfection regime was stipulated, including an increased time from completion of this process to re-stocking with the study batch of pigs. During the finisher period, further hygiene procedures were required, including the regular cleaning and disinfection of equipment such as scrapers used to move faecal material. After recruitment, farms were randomly assigned to either the intervention or the comparison groups. All farms were monitored by periodic pooled faecal sample collection and participants reported their actions through weekly reporting sheets (see

Appendix). After initial training, farmers also collected the pooled pen floor faecal samples and sent these to VLA for testing.

The study was designed to be analysed on an intent-to-treat basis. The longitudinal study enabled the incidence rate of new pen infections to be estimated. The introduction of infected pigs was known to be an important source of *Salmonella* and lies beyond the control of the finisher unit manager. The incidence rate indicates the success of the intervention at reducing further infections; the prevalence at the end of the finisher period is a combination of introduced and new infections and thus, may under-estimate the efficacy of the measures implemented on farm (11).

There was a poor participation rate in the intervention study and the target sample size was not achieved. More than 900 invitations were distributed and 257 replies were received but only 46 farms actually joined in the study. The main reason provided was that there was insufficient time to complete the extra work that would be needed for sample collection and completion of recording sheets. The study was funded by Defra and they were consulted on whether to go ahead or to abort the study. It was decided that the study should proceed. However, the poor response rate may be interpreted as showing relatively little interest amongst farmers concerning *Salmonella* in pigs. This may also be due to their belief that it is not amenable to control (12, 13).

There was no evidence that farms in the intervention group had any discernible reduction in the incidence of new *Salmonella* infections. This may be because there was no important change in farmer behaviour during the intervention period apart from an increased period between the despatch of the preceding batch of pigs to slaughter and restocking with the study batch. It may also be due to a “Hawthorne effect” in which all farmers, whether in the intervention or control group, improved their hygiene and biosecurity practices as a consequence of participating in the study. Finally, the results suggest that the burden of infection introduced with infected pigs on re-stocking may have overwhelmed the control

measures that were taken. It was impossible to conduct a blinded study, as the farmers had to be aware of the outcome of interest in order to give informed consent.

As described in Chapter 8, since there was no important difference between the intervention and comparison groups of farms, a further analysis was conducted to estimate the association between recorded variables and different outcomes during the study (see figure 8.2). The risk that an empty pen was contaminated with *Salmonella* after cleaning and disinfection but before re-stocking was reduced if the days left empty increased and if the between-batch hygiene and biosecurity practices were more rigorous. A greater prevalence of infection in the preceding batch was associated with an increased risk of *Salmonella* contamination (see table 8.3). The *Salmonella* status of pens 3 days after restocking was associated with the prevalence of contaminated pens prior to re-stocking and with the status of the transport in which the pigs were delivered (see table 8.4). As shown in table 8.6, there was little evidence that the pen incidence rate during the finisher period was associated with the rigour with which hygiene and biosecurity measures were applied. However, there was a very strong association with the prevalence of infected pens on day 3 after re-stocking. The results suggest that there may be modest benefits from rigorous hygiene and biosecurity but that the time and effort invested in these activities may be overwhelmed by the introduction of infected pigs to the finisher accommodation.

Key findings from this thesis

- Evidence that the prevalence of *Salmonella* infection on finisher farms in GB varies markedly (see Chapters 4 and 5). Thus, it is reasonable to conclude that farms with a higher than median prevalence might feasibly aspire to achieve a similar prevalence to the majority of farms.
- New evidence on the use of microbiological and serological tests and their comparability, which will inform test selection for research and surveillance purposes (see Chapter 3)
- Confirmation of the reduced risk of *Salmonella* infection in finisher pigs associated with home mill and mix or liquid feed compared to pelleted feed

(see meta-analysis in Chapter 1, survey results in Chapter 4 and analysis of routine data in Chapter 5)

- Confirmation that PCV2 infection is associated with an increased risk of *salmonella* infection (see Chapter 4)
- Further evidence that outdoor production may be associated with an increased risk of *Salmonella* infection (see Chapter 4)
- Further evidence that fully slatted floors are associated with a reduced risk of *salmonella* infection (see Chapter 4)
- Further evidence that poor rodent control is associated with an nceased risk of *Salmonella* infection (see Chapter 4)
- New evidence that pigs from breeder-finisher farms are at a reduced risk of *Salmonella* infection compared to pigs moved to specialist finisher units (see Chapter 5)
- New evidence that increased finisher herd mortality is associated with an increased risk of *Salmonella* infection (see Chapter 5)
- Implicit evidence that farmers have limited motivation to engage with interventions to control *Salmonella* in finisher pigs (see Chapter 7)
- Demonstration that little change behaviour with respect to biosecurity and hygiene in finisher pig farms occurred during an intent-to-treat intervention study (see Chapter 7)
- Evidence that a prolonged between-batch empty period and improved hygiene reduces the risk of *Salmonella* – contaminated pens before re-stocking (see Chapter 8)
- Evidence that between-batch cleaning and disinfection processes may not eliminate *Salmonella* contamination and that such contamination increases the risk of detecting *Salmonella* infection in pigs three days after re-stocking (see Chapter 8)
- Evidence that *Salmonella* contamination can be found in transport bringing pigs into finisher accommodation and this may be associated with infection three days after re-stocking (see Chapter 8)
- Evidence that improved hygiene and biosecurity practices during the finisher cycle may reduce the incidence of new pen-level *Salmonella* infections

- Strong evidence that the introduction of *Salmonella* infection with pigs at restocking has a profound effect on the incidence of new pen-level *Salmonella* infections, to the extent that the modest impact from other remedial measures may be completely overwhelmed.

Conclusions and recommendations

The research reported in this thesis has contributed to the formulation of policy on the control of *Salmonella* in pigs by the industry and Government in Great Britain. There is now a substantial body of evidence on this subject from GB and elsewhere but this is mainly based on either small-scale experimental studies or on cross-sectional studies. The work reported in Chapters 7 and 8 represents one of a very few longitudinal intervention studies conducted on more than a small (<10) number of farms.

The focus of the work reported here was on the control of infection on finisher units, since these represent the most immediate on-farm source of risk to the human population. There is little incentive for farmers to adopt measures to control *Salmonella* since to do so will certainly incur costs but has not been shown to deliver any benefits either at the individual farm level or at a societal level. It is unlikely that there will be any direct financial benefits for producers from delivering *Salmonella*-free pigs, since consumers do not pay a dividend for food safety as they regard it as an intrinsic quality of the food they buy. Equally, major retailers do not offer any dividend but may impose quality assurance processes to safeguard the meat that they buy. This tension between private cost and public benefit has been reported previously (14) amongst dairy farmers and there is a general lack of appreciation of the public benefits that may accrue from farm biosecurity (15). In order to adopt measures to control foodborne disease, farmers must not only perceive that they have a responsibility but also believe such measures will be effective (13, 16, 17). Therefore, if the widespread adoption of *Salmonella* control measures is to occur, it will require firstly, the availability of *Salmonella* free weaners to supply finishing units (18). Secondly, it must be financially advantageous either by delivering other benefits e.g. improved pig welfare, reduced mortality and thus a gain in performance or by obviating consequential losses e.g. if a penalty system were to be introduced.

Unfortunately, the perilous state of the GB pig industry does not seem likely to enable farmers to raise the capital that would be needed to improve the standards of accommodation that is available. From the public health perspective, it has been argued that intervention in the abattoir is more likely to bring cost-effective benefits (19).

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Appendix Chapter 3: Winbugs code

Model mj10 3 test no conditional dependence between test

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*Seln*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*Secc*Seln*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spmj
p1 [i, 3] <- pi [ i ]*Secc*(1-Seln)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spmj
p1 [i, 4] <- pi [ i ]*Secc*(1-Seln)*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spmj)
p1 [i, 5] <- pi [ i ]*(1-Secc)*Seln*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*(1-Secc)*Seln*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spmj
p1 [i, 7] <- pi [ i ]*(1-Secc)*(1-Seln)*Semj + (1-pi[i])*Spcc*Spln*(1-Spmj)
p1 [i, 8] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spmj
pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}
Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model MJ10 3 tests and non-informative priors

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*Seln*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*Secc*Seln*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spmj
p1 [i, 3] <- pi [ i ]*Secc*(1-Seln)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spmj
p1 [i, 4] <- pi [ i ]*Secc*(1-Seln)*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spmj)
p1 [i, 5] <- pi [ i ]*(1-Secc)*Seln*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*(1-Secc)*Seln*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spmj
p1 [i, 7] <- pi [ i ]*(1-Secc)*(1-Seln)*Semj + (1-pi[i])*Spcc*Spln*(1-Spmj)
p1 [i, 8] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spmj
pi[i] ~dbeta(1,1) # prior of pig prevalence at abattoir
}
Secc ~ dbeta(1,1) # caecal culture sensitivity non-informative prior
Seln ~ dbeta(1,1) # lymph node sensitivity non-informative prior
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
#pi2 ~ dbeta (1,1) ## non-informative prior
#mj sens and spec
Semj ~ dbeta(1, 1) ## non-informative prior
Spmj ~ dbeta(1, 1) ## non-informative prior
}
```

Model mj10 3 tests conditional dependence between caecal content and lymph node

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc*Seln+covDp)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*(Secc*Seln+covDp)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spmj
p1 [i, 3] <- pi [ i ]*(Secc*(1-Seln)-covDp)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spmj
p1 [i, 4] <- pi [ i ]*(Secc*(1-Seln)-covDp)*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spmj)
p1 [i, 5] <- pi [ i ]*((1-Secc)*Seln-covDp)*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*((1-Secc)*Seln-covDp)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spmj
p1 [i, 7] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*Semj + (1-pi[i])*Spcc*Spln*(1-Spmj)
p1 [i, 8] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spmj
pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}

# terms for codependence cc ln
ls <- (Secc-1)*(1-Seln)
us <- min(Secc, Seln) - Secc*Seln
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc*(1-Secc)*Seln*(1-Seln))

Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure >
0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure
>0.30
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj10 4 tests and no conditional dependence

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*Seln*Secs*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*Secc*Seln*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 3] <- pi [ i ]*Secc*Seln*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 4] <- pi [ i ]*Secc*Seln*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*Spmj
p1 [i, 5] <- pi [ i ]*Secc*(1-Seln)*Secs*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*Secc*(1-Seln)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*Spmj
p1 [i, 7] <- pi [ i ]*Secc*(1-Seln)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*Spln*Spcs*(1-Spmj)
p1 [i, 8] <- pi [ i ]*Secc*(1-Seln)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spcs*Spmj
p1 [i, 9] <- pi [ i ]*(1-Secc)*Seln*Secs*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 10] <- pi [ i ]*(1-Secc)*Seln*Secs*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 11] <- pi [ i ]*(1-Secc)*Seln*(1-Secs)*Semj + (1-pi[i])*Spcc*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 12] <- pi [ i ]*(1-Secc)*Seln*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spcs*Spmj
p1 [i, 13] <- pi [ i ]*(1-Secc)*(1-Seln)*Secs*Semj + (1-pi[i])*Spcc*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 14] <- pi [ i ]*(1-Secc)*(1-Seln)*Secs*(1-Semj) + (1-pi[i])*Spcc*Spln*(1-Spcs)*Spmj
p1 [i, 15] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Secs)*Semj + (1-pi[i])*Spcc*Spln*Spcs*(1-Spmj)
p1 [i, 16] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spcs*Spmj
pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}
Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj10 4 tests and non-informative priors

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*Seln*Secs*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*Secc*Seln*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 3] <- pi [ i ]*Secc*Seln*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 4] <- pi [ i ]*Secc*Seln*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*Spmj
p1 [i, 5] <- pi [ i ]*Secc*(1-Seln)*Secs*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*Secc*(1-Seln)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*Spmj
p1 [i, 7] <- pi [ i ]*Secc*(1-Seln)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*Spln*Spcs*(1-Spmj)
p1 [i, 8] <- pi [ i ]*Secc*(1-Seln)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spcs*Spmj
p1 [i, 9] <- pi [ i ]*(1-Secc)*Seln*Secs*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 10] <- pi [ i ]*(1-Secc)*Seln*Secs*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 11] <- pi [ i ]*(1-Secc)*Seln*(1-Secs)*Semj + (1-pi[i])*Spcc*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 12] <- pi [ i ]*(1-Secc)*Seln*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spcs*Spmj
p1 [i, 13] <- pi [ i ]*(1-Secc)*(1-Seln)*Secs*Semj + (1-pi[i])*Spcc*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 14] <- pi [ i ]*(1-Secc)*(1-Seln)*Secs*(1-Semj) + (1-pi[i])*Spcc*Spln*(1-Spcs)*Spmj
p1 [i, 15] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Secs)*Semj + (1-pi[i])*Spcc*Spln*Spcs*(1-Spmj)
p1 [i, 16] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spcs*Spmj
pi[i] ~dbeta(1,1) # prior of pig prevalence at abattoir
}
Secc ~ dbeta(1,1) # caecal culture sensitivity
Seln ~ dbeta(1,1) # lymph node sensitivity
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1,1)
#mj sens and spec
Semj ~ dbeta(1,1)
Spmj ~ dbeta(1,1)
}
```

Model mj10 four tests, with conditional dependence between lymph node and carcass swab

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*(Seln*Secs+covDp)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*Secc*(Seln*Secs+covDp)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-
Spcs)*Spmj
p1 [i, 3] <- pi [ i ]*Secc*(Seln*(1-Secs)-covDp)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 4] <- pi [ i ]*Secc*(Seln*(1-Secs)-covDp)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*Spmj
p1 [i, 5] <- pi [ i ]*Secc*((1-Seln)*Secs-covDp)*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*Secc*((1-Seln)*Secs-covDp)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*Spmj
p1 [i, 7] <- pi [ i ]*Secc*((1-Seln)*(1-Secs)+covDp)*Semj + (1-pi[i])*(1-Spcc)*Spln*Spcs*(1-
Spmj)
p1 [i, 8] <- pi [ i ]*Secc*((1-Seln)*(1-Secs)+covDp)*(1-Semj) + (1-pi[i])*(1-
Spcc)*Spln*Spcs*Spmj
p1 [i, 9] <- pi [ i ]*(1-Secc)*(Seln*Secs+covDp)*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*(1-
Spmj)
p1 [i, 10] <- pi [ i ]*(1-Secc)*(Seln*Secs+covDp)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*(1-
Spcs)*Spmj
p1 [i, 11] <- pi [ i ]*(1-Secc)*(Seln*(1-Secs)-covDp)*Semj + (1-pi[i])*Spcc*(1-Spln)*Spcs*(1-
Spmj)
p1 [i, 12] <- pi [ i ]*(1-Secc)*(Seln*(1-Secs)-covDp)*(1-Semj) + (1-pi[i])*Spcc*(1-
Spln)*Spcs*Spmj
p1 [i, 13] <- pi [ i ]*(1-Secc)*((1-Seln)*Secs-covDp)*Semj + (1-pi[i])*Spcc*Spln*(1-Spcs)*(1-
Spmj)
p1 [i, 14] <- pi [ i ]*(1-Secc)*((1-Seln)*Secs-covDp)*(1-Semj) + (1-pi[i])*Spcc*Spln*(1-
Spcs)*Spmj
p1 [i, 15] <- pi [ i ]*(1-Secc)*((1-Seln)*(1-Secs)+covDp)*Semj + (1-pi[i])*Spcc*Spln*Spcs*(1-
Spmj)
p1 [i, 16] <- pi [ i ]*(1-Secc)*((1-Seln)*(1-Secs)+covDp)*(1-Semj) + (1-
pi[i])*Spcc*Spln*Spcs*Spmj

pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}

# terms for codependence ln cs
ls <- (Seln-1)*(1-Secs)
us <- min(Seln, Secs) - Seln*Secs
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Seln*(1-Seln)*Secs*(1-Secs))

Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj10 four tests, with conditional dependence between caecal content and carcass swab

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc*Seln+covDp)*Secs*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*(Secc*Seln+covDp)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 3] <- pi [ i ]*(Secc*Seln+covDp)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 4] <- pi [ i ]*(Secc*Seln+covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*Spmj
p1 [i, 5] <- pi [ i ]*(Secc*(1-Seln)-covDp)*Secs*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*(Secc*(1-Seln)-covDp)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*Spmj
p1 [i, 7] <- pi [ i ]*(Secc*(1-Seln)-covDp)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*Spln*Spcs*(1-Spmj)
p1 [i, 8] <- pi [ i ]*(Secc*(1-Seln)-covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spcs*Spmj
p1 [i, 9] <- pi [ i ]*((1-Secc)*Seln-covDp)*Secs*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 10] <- pi [ i ]*((1-Secc)*Seln-covDp)*Secs*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 11] <- pi [ i ]*((1-Secc)*Seln-covDp)*(1-Secs)*Semj + (1-pi[i])*Spcc*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 12] <- pi [ i ]*((1-Secc)*Seln-covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spcs*Spmj
p1 [i, 13] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*Secs*Semj + (1-pi[i])*Spcc*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 14] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*Secs*(1-Semj) + (1-pi[i])*Spcc*Spln*(1-Spcs)*Spmj
p1 [i, 15] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*(1-Secs)*Semj + (1-pi[i])*Spcc*Spln*Spcs*(1-Spmj)
p1 [i, 16] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spcs*Spmj
pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}

# terms for codependence cc ln
ls <- (Secc-1)*(1-Seln)
us <- min(Secc, Seln) - Secc*Seln
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc*(1-Secc)*Seln*(1-Seln))

Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj10 4 tests with conditional dependence between caecal content and lymph node

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc*Seln+covDp)*Secs*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 2] <- pi [ i ]*(Secc*Seln+covDp)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 3] <- pi [ i ]*(Secc*Seln+covDp)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 4] <- pi [ i ]*(Secc*Seln+covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*(1-Spln)*Spcs*Spmj
p1 [i, 5] <- pi [ i ]*(Secc*(1-Seln)-covDp)*Secs*Semj + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 6] <- pi [ i ]*(Secc*(1-Seln)-covDp)*Secs*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*(1-Spcs)*Spmj
p1 [i, 7] <- pi [ i ]*(Secc*(1-Seln)-covDp)*(1-Secs)*Semj + (1-pi[i])*(1-Spcc)*Spln*Spcs*(1-Spmj)
p1 [i, 8] <- pi [ i ]*(Secc*(1-Seln)-covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*(1-Spcc)*Spln*Spcs*Spmj
p1 [i, 9] <- pi [ i ]*((1-Secc)*Seln-covDp)*Secs*Semj + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*(1-Spmj)
p1 [i, 10] <- pi [ i ]*((1-Secc)*Seln-covDp)*Secs*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*(1-Spcs)*Spmj
p1 [i, 11] <- pi [ i ]*((1-Secc)*Seln-covDp)*(1-Secs)*Semj + (1-pi[i])*Spcc*(1-Spln)*Spcs*(1-Spmj)
p1 [i, 12] <- pi [ i ]*((1-Secc)*Seln-covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*(1-Spln)*Spcs*Spmj
p1 [i, 13] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*Secs*Semj + (1-pi[i])*Spcc*Spln*(1-Spcs)*(1-Spmj)
p1 [i, 14] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*Secs*(1-Semj) + (1-pi[i])*Spcc*Spln*(1-Spcs)*Spmj
p1 [i, 15] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*(1-Secs)*Semj + (1-pi[i])*Spcc*Spln*Spcs*(1-Spmj)
p1 [i, 16] <- pi [ i ]*((1-Secc)*(1-Seln)+covDp)*(1-Secs)*(1-Semj) + (1-pi[i])*Spcc*Spln*Spcs*Spmj
pi[i] ~dbeta(18.937, 16.2797) # prior of pig prevalence at abattoir
}

# terms for codependence cc ln
ls <- (Secc-1)*(1-Seln)
us <- min(Secc, Seln) - Secc*Seln
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc*(1-Secc)*Seln*(1-Seln))

Secc ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 3 tests no conditional dependence

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc25*Seln25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*Secc25*Seln25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spmj25
p1 [i, 3] <- pi [ i ]*Secc25*(1-Seln25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spmj25
p1 [i, 4] <- pi [ i ]*Secc25*(1-Seln25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spmj25)
p1 [i, 5] <- pi [ i ]*(1-Secc25)*Seln25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*(1-Secc25)*Seln25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spmj25
p1 [i, 7] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spmj25
pi[i] ~dbeta(43.1003, 127.3008) # prior of pig prevalence at abattoir
}
Secc25 ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 3 tests non-informative priors

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc25*Seln25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*Secc25*Seln25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spmj25
p1 [i, 3] <- pi [ i ]*Secc25*(1-Seln25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spmj25
p1 [i, 4] <- pi [ i ]*Secc25*(1-Seln25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spmj25)
p1 [i, 5] <- pi [ i ]*(1-Secc25)*Seln25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*(1-Secc25)*Seln25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spmj25
p1 [i, 7] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spmj25
pi[i] ~dbeta(1, 1) # prior of pig prevalence at abattoir
}
Secc25 ~ dbeta(1,1) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln25 ~ dbeta(1,1) # lymph node sensitivity mode=0.45, 95% sure >0.30
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1, 1) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(1, 1) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(1, 1) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 3 tests conditional dependence between caecal content and lymph node

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc25*Seln25+covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*(Secc25*Seln25+covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spmj25
p1 [i, 3] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spmj25
p1 [i, 4] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spmj25)
p1 [i, 5] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spmj25
p1 [i, 7] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spmj25
pi[i] ~dbeta(43.1003, 127.3008) # prior of pig prevalence at abattoir
}

# terms for codependence cc ln
ls <- (Secc25-1)*(1-Seln25)
us <- min(Secc25, Seln25) - Secc25*Seln25
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc25*(1-Secc25)*Seln25*(1-Seln25))

Secc25 ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 4 tests no conditional dependence

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc25*Seln25*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*Secc25*Seln25*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 3] <- pi [ i ]*Secc25*Seln25*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 4] <- pi [ i ]*Secc25*Seln25*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*Spmj25
p1 [i, 5] <- pi [ i ]*Secc25*(1-Seln25)*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*Secc25*(1-Seln25)*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*Spmj25
p1 [i, 7] <- pi [ i ]*Secc25*(1-Seln25)*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*Secc25*(1-Seln25)*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*Spmj25
p1 [i, 9] <- pi [ i ]*(1-Secc25)*Seln25*Secs25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 10] <- pi [ i ]*(1-Secc25)*Seln25*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 11] <- pi [ i ]*(1-Secc25)*Seln25*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 12] <- pi [ i ]*(1-Secc25)*Seln25*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*Spmj25
p1 [i, 13] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Secs25*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 14] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*Spmj25
p1 [i, 15] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*Spln25*Spcs25*(1-Spmj25)
p1 [i, 16] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spcs25*Spmj25
pi[i] ~dbeta(43.1003, 127.3008) # prior of pig prevalence at abattoir mode=0.25 95% sure >0.20
}
Secc25 ~ dbeta(13.3494,29.8154) # caecal culture sensitivity Mode=0.30, 95% sure > 0.2
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity mode=0.45, 95% sure >0.30
Secs25 ~ dbeta(1,1) # carcass swab sensitivity
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
Spcs25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 4 tests non-informative priors and no conditional dependence

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc25*Seln25*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*Secc25*Seln25*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 3] <- pi [ i ]*Secc25*Seln25*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 4] <- pi [ i ]*Secc25*Seln25*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*Spmj25
p1 [i, 5] <- pi [ i ]*Secc25*(1-Seln25)*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*Secc25*(1-Seln25)*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*Spmj25
p1 [i, 7] <- pi [ i ]*Secc25*(1-Seln25)*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*Secc25*(1-Seln25)*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*Spmj25
p1 [i, 9] <- pi [ i ]*(1-Secc25)*Seln25*Secs25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 10] <- pi [ i ]*(1-Secc25)*Seln25*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 11] <- pi [ i ]*(1-Secc25)*Seln25*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 12] <- pi [ i ]*(1-Secc25)*Seln25*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*Spmj25
p1 [i, 13] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Secs25*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 14] <- pi [ i ]*(1-Secc25)*(1-Seln25)*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*Spmj25
p1 [i, 15] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*Spln25*Spcs25*(1-Spmj25)
p1 [i, 16] <- pi [ i ]*(1-Secc25)*(1-Seln25)*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spcs25*Spmj25
pi[i] ~dbeta(1,1) # prior of pig prevalence at abattoir
}
Secc25 ~ dbeta(1,1) # caecal culture sensitivity
Seln25 ~ dbeta(1,1) # lymph node sensitivity
Secs25 ~ dbeta(1,1) # carcass swab sensitivity
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
Spcs25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1, 1) ##
#mj sens and spec
Semj25 ~ dbeta(1, 1)
Spmj25 ~ dbeta(1, 1)
}
```

Model mj25 4 tests with conditional dependence between caecal content and lymph node

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc25*Seln25+covDp)*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*(Secc25*Seln25+covDp)*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 3] <- pi [ i ]*(Secc25*Seln25+covDp)*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 4] <- pi [ i ]*(Secc25*Seln25+covDp)*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*Spmj25
p1 [i, 5] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*Secs25*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*Secs25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*Spmj25
p1 [i, 7] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*(1-Secs25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*(Secc25*(1-Seln25)-covDp)*(1-Secs25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*Spmj25
p1 [i, 9] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*Secs25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 10] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 11] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 12] <- pi [ i ]*((1-Secc25)*Seln25-covDp)*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*Spmj25
p1 [i, 13] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*Secs25*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 14] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*Secs25*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*Spmj25
p1 [i, 15] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*(1-Secs25)*Semj25 + (1-pi[i])*Spcc25*Spln25*Spcs25*(1-Spmj25)
p1 [i, 16] <- pi [ i ]*((1-Secc25)*(1-Seln25)+covDp)*(1-Secs25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spcs25*Spmj25
pi[i] ~dbeta(43.1005, 127.3008) # prior of pig prevalence at abattoir
}

# terms for codependence cc ln
ls <- (Secc25-1)*(1-Seln25)
us <- min(Secc25, Seln25) - Secc25*Seln25
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc25*(1-Secc25)*Seln25*(1-Seln25))

Secc25 ~ dbeta(26.453,52.677) # caecal culture sensitivity 95% sure >0.25 mode=0.33
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity 95% sure >0.30 mode=0.45
Secs25 ~ dbeta(1,1) # carcass swab sensitivity non-informative prior
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
Spcs25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model mj25 4 tests with conditional dependence between lymph node and carcass swab

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc25*(Seln25*Secs25+covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*Secc25*(Seln25*Secs25+covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 3] <- pi [ i ]*Secc25*(Seln25*(1-Secs25)-covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 4] <- pi [ i ]*Secc25*(Seln25*(1-Secs25)-covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*Spmj25
p1 [i, 5] <- pi [ i ]*Secc25*((1-Seln25)*Secs25-covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*Secc25*((1-Seln25)*Secs25-covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*Spmj25
p1 [i, 7] <- pi [ i ]*Secc25*((1-Seln25)*(1-Secs25)+covDp)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*Secc25*((1-Seln25)*(1-Secs25)+covDp)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*Spmj25
p1 [i, 9] <- pi [ i ]*(1-Secc25)*(Seln25*Secs25+covDp)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 10] <- pi [ i ]*(1-Secc25)*(Seln25*Secs25+covDp)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 11] <- pi [ i ]*(1-Secc25)*(Seln25*(1-Secs25)-covDp)*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 12] <- pi [ i ]*(1-Secc25)*(Seln25*(1-Secs25)-covDp)*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*Spmj25
p1 [i, 13] <- pi [ i ]*(1-Secc25)*((1-Seln25)*Secs25-covDp)*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 14] <- pi [ i ]*(1-Secc25)*((1-Seln25)*Secs25-covDp)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*Spmj25
p1 [i, 15] <- pi [ i ]*(1-Secc25)*((1-Seln25)*(1-Secs25)+covDp)*Semj25 + (1-pi[i])*Spcc25*Spln25*Spcs25*(1-Spmj25)
p1 [i, 16] <- pi [ i ]*(1-Secc25)*((1-Seln25)*(1-Secs25)+covDp)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spcs25*Spmj25

pi[i] ~dbeta(18.2007,32.9441) # prior of pig prevalence at abattoir
}
# terms for codependence ln cs
ls <- (Seln25-1)*(1-Secs25)
us <- min(Seln25, Secs25) - Seln25*Secs25
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Seln25*(1-Seln25)*Secs25*(1-Secs25))

Secc25 ~ dbeta(7.3057, 12.7106) # caecal culture sensitivity 95% sure >0.2 mode 0.35
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity 95% sure > 0.30 mode 0.45
Secs25 ~ dbeta(1,1) # carcass swab sensitivity
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
Spcs25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

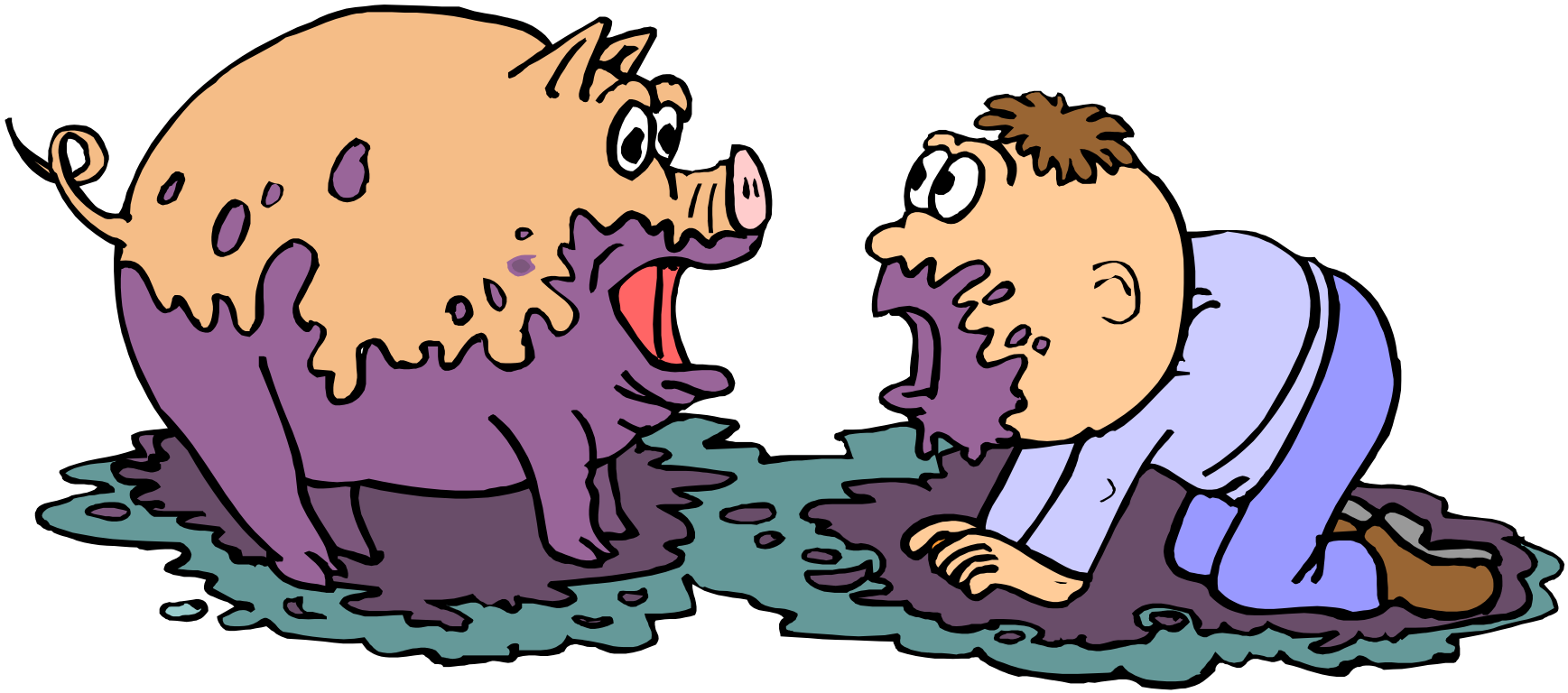
Model mj25 4 tests conditional dependence between caecal content and carcass swab

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:16] ~ dmulti (p1 [i, 1:16], n[ i ])
p1 [i, 1]<- pi [ i ]*(Secc25*Secs25+covDp)*Seln25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 2] <- pi [ i ]*(Secc25*Secs25+covDp)*Seln25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 3] <- pi [ i ]*(Secc25*(1-Secs25)-covDp)*Seln25*Semj25 + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 4] <- pi [ i ]*(Secc25*(1-Secs25)-covDp)*Seln25*(1-Semj25) + (1-pi[i])*(1-Spcc25)*(1-Spln25)*Spcs25*Spmj25
p1 [i, 5] <- pi [ i ]*(Secc25*Secs25+covDp)*(1-Seln25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 6] <- pi [ i ]*(Secc25*Secs25+covDp)*(1-Seln25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*(1-Spcs25)*Spmj25
p1 [i, 7] <- pi [ i ]*(Secc25*(1-Secs25)-covDp)*(1-Seln25)*Semj25 + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*(1-Spmj25)
p1 [i, 8] <- pi [ i ]*(Secc25*(1-Secs25)-covDp)*(1-Seln25)*(1-Semj25) + (1-pi[i])*(1-Spcc25)*Spln25*Spcs25*Spmj25
p1 [i, 9] <- pi [ i ]*((1-Secc25)*Secs25-covDp)*Seln25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*(1-Spmj25)
p1 [i, 10] <- pi [ i ]*((1-Secc25)*Secs25-covDp)*Seln25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*(1-Spcs25)*Spmj25
p1 [i, 11] <- pi [ i ]*((1-Secc25)*(1-Secs25)+covDp)*Seln25*Semj25 + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*(1-Spmj25)
p1 [i, 12] <- pi [ i ]*((1-Secc25)*(1-Secs25)+covDp)*Seln25*(1-Semj25) + (1-pi[i])*Spcc25*(1-Spln25)*Spcs25*Spmj25
p1 [i, 13] <- pi [ i ]*((1-Secc25)*Secs25-covDp)*(1-Seln25)*Semj25 + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*(1-Spmj25)
p1 [i, 14] <- pi [ i ]*((1-Secc25)*Secs25-covDp)*(1-Seln25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*(1-Spcs25)*Spmj25
p1 [i, 15] <- pi [ i ]*((1-Secc25)*(1-Secs25)+covDp)*(1-Seln25)*Semj25 + (1-pi[i])*Spcc25*Spln25*Spcs25*(1-Spmj25)
p1 [i, 16] <- pi [ i ]*((1-Secc25)*(1-Secs25)+covDp)*(1-Seln25)*(1-Semj25) + (1-pi[i])*Spcc25*Spln25*Spcs25*Spmj25

pi[i] ~dbeta(18.2007, 32.9441) # prior of pig prevalence at abattoir 95% sure >0.25 mode 0.35
}
# terms for codependence cc cs
ls <- (Secc25-1)*(1-Secs25)
us <- min(Secc25, Secs25) - Secc25*Secs25
covDp ~ dunif (ls, us)
rhoD <- covDp/ sqrt(Secc25*(1-Secc25)*Secs25*(1-Secs25))

Secc25 ~ dbeta(7.3057, 12.7106) # caecal culture sensitivity 95% sure >0.2 mode 0.35
Seln25 ~ dbeta(12.1391,14.6145) # lymph node sensitivity 95% sure > 0.30 mode 0.45
Secs25 ~ dbeta(1,1) # carcass swab sensitivity
Spcc25 <- 1.0 # culture specificity
Spln25 <- 1.0 # culture specificity
Spcs25 <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
#mj sens and spec
Semj25 ~ dbeta(4.8416, 3.5611) ## Mode=0.60, 95% sure >0.30
Spmj25 ~ dbeta(26.8438, 5.9226) ## Mode 0.84, 95% sure >0.70
}
```

Model 3-test no conditional dependence for culture of caecal content (cc), carcass swab (cs and lymph node (ln).

```
Model
{
for (i in 1:NoOfAbs) {
z[i, 1:8] ~ dmulti (p1 [i, 1:8], n[ i ])
p1 [i, 1]<- pi [ i ]*Secc*Seln*Secs + (1-pi[i])*(1-Spcc)*(1-Spln)*(1-
Spcs)
p1 [i, 2] <- pi [ i ]*Secc*Seln*(1-Secs) + (1-pi[i])*(1-Spcc)*(1-
Spln)*Spcs
p1 [i, 3] <- pi [ i ]*Secc*(1-Seln)*(1-Secs) + (1-pi[i])*(1-
Spcc)*Spln*Spcs
p1 [i, 4] <- pi [ i ]*Secc*(1-Seln)*Secs + (1-pi[i])*(1-Spcc)*Spln*(1-
Spcs)
p1 [i, 5] <- pi [ i ]*(1-Secc)*Seln*Secs + (1-pi[i])*Spcc*(1-Spln)*(1-
Spcs)
p1 [i, 6] <- pi [ i ]*(1-Secc)*Seln*(1-Secs) + (1-pi[i])*Spcc*(1-
Spln)*Spcs
p1 [i, 7] <- pi [ i ]*(1-Secc)*(1-Seln)*Secs + (1-pi[i])*Spcc*Spln*(1-
Spcs)
p1 [i, 8] <- pi [ i ]*(1-Secc)*(1-Seln)*(1-Secs) + (1-
pi[i])*Spcc*Spln*Spcs
pi[i] ~dbeta(115, 385) # prior of pig prevalence at abattoir
}
Secc ~ dbeta(40,11) # caecal culture sensitivity
Seln ~ dbeta(1,1) # lymph node sensitivity
Secs ~ dbeta(1,1) # carcass swab sensitivity
Spcc <- 1.0 # culture specificity
Spln <- 1.0 # culture specificity
Spcs <- 1.0 # culture specificity
#pi2 ~ dbeta (1.73, 2.71) ## Mode=0.30, 95% sure pi2 > 0.08
}
```

PIG SALMONELLA – FARMER QUESTIONNAIRE



IN CONFIDENCE

Please read the following notes before you answer the questions:

- Most questions can be answered by ticking a box ☑ or writing down a number
- Where a question relates to past events, this period is measured from the date on which you complete the questionnaire e.g. 'In the last 12 months' refers to 12 months from today
- There is only ONE answer for most questions, unless you are asked to tick every applicable box
- Please write any comments on the notes page provided at the back
- If you have any questions, please get in touch with: Alasdair Cook ☎ 01932 357977; 💻 a.j.cook@vla.defra.gsi.gov.uk
  or Sandy Miller ☎ 01932 357623; 💻 a.miller@vla.defra.gsi.gov.uk
- Please look through your questionnaire to check that you have not missed any questions, and return it to us in the reply-paid envelope provided
- Please DO NOT send any samples with this questionnaire

➢ **Are all of the pigs in your enterprise kept at one site?** YES ☐ NO ☐

➢ **If NO, then please give the number of different sites at which pigs are kept?** ☐

➢ **And if NO, do you hold either a Defra 'Sole Occupancy Licence' (SOL)?** YES ☐ NO ☐

or 'Sole Occupancy Authority' (SOA)? YES ☐ NO ☐

If you have several sites, but don't hold an SOL or SOA then please complete the questionnaire for **only the main site** in your enterprise.

If you do hold an SOL or SOA then please answer the questions for your **whole enterprise**, treating every site together as one unit.

Farm ID:

CPH Number: / /

Farm Owner

Name of Farm Manager/Foreman

Farm Address
(including County and Postcode)

If your pig enterprise operates from more than one site, then you should use the main postal address here

County:

Postcode:

Address where pigs are kept
(if different from the main postal address)

County:

Postcode:

OS Map Reference of pig unit *(if known)*

Name of person completing questionnaire

Please give your daytime telephone number

Position of person completing this questionnaire

- **Owner/ manager** ☐
- **Owner** ☐
- **Manager** ☐
- **Stockperson** ☐
- **Other (please specify)** _____ ☐

Date of completion of questionnaire:

Are you or your farm part of: **NPA** ☐ **ABPigs** ☐ **QMS** ☐ **Others**……☐

PTO

SECTION 1: STAFF & VISITORS

1.1 Staff. In answering the following, include yourself as appropriate. If staff divide their time between the pig enterprise and other work, then they are regarded as **part time** for the purpose of this questionnaire:

Please Write Number

a) How many people are employed **full time** to work with pigs? ☐

b) How many of these people have received or are currently receiving **formal** training (e.g. NVQ, OND, BSc etc.) ☐

c) How many people are employed **part time** to work with pigs? ☐

d) How many of these people have received or are currently receiving **formal** training (e.g. NVQ, OND, BSc etc.) ☐

e) Do any of the **part time** staff also work on other enterprises **on <u>this</u> farm**? YES ☐ NO ☐ Not Known ☐ Not Applicable ☐

f) Do any of the **part time** staff also work on other enterprises **on <u>other</u> farms**? YES ☐ NO ☐ Not Known ☐ Not Applicable ☐

If you answered **YES** to either **e) or f)**, then please list the enterprises on which staff work below:

Type of Enterprise	Your Farm	Other Farm
e.g. Beef cattle	☑	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐
	☐	☐

1.2 How many times has your vet visited the farm during the **past 12 months?**

- 12 or more times ☐
- 4-11 times ☐
- 2-3 times ☐
- Once ☐
- Never ☐
- Not Known ☐

1.3 Visitors.

List everyone who has visited your pig unit during the **past four weeks**. Please state 1) how often they usually visit the farm 2) whether they entered pig houses, 3) whether they entered pig pens, 4) to the best of your knowledge, if they had contact with livestock on other farms within 24 hours prior to their visit.

Visitor Occupation	1) How often do they usually visit?			2) Entered pig houses		3) Entered pig pens		4) Contact with livestock on other farms in previous 24 hours					
	At Least Once/ month	Once/ month to Once/ 3 months	Less than once/3 months	Yes	No	Yes	No	Yes	No	Not Known	If YES, what type(s) of livestock		
eg Ventilation engineer			✓	✓			✓	✓			*pigs*	*cattle*	

Please use the sheets at the end for any further responses

SECTION 2: FARM LOCALITY AND SURROUNDINGS

2.1 Does a continuous perimeter fence secure the farm? YES ☐ NO ☐

2.2 Can the public go up to perimeter fences? YES ☐ NO ☐ Not Applicable ☐

2.3 How many entry/exit points are there to the pig unit:

a) for vehicles? 1 ☐ 2 ☐ 3 ☐ 4 or more ☐

b) on foot? 1 ☐ 2 ☐ 3 ☐ 4 or more ☐

2.4 Does a footpath used by the public cross the site or run around the periphery? Across site ☐ Around periphery ☐ Both ☐ No ☐

2.5 Are there any open watercourses **within one mile** of the farm? YES ☐ NO ☐ Not Known ☐

If YES, are these: *(tick all that apply)*

River ☐ Canal ☐ Stream ☐

Pond ☐ Lake ☐

Other *(specify)* _____

If YES, give the distance of the nearest one:

Runs through the farm ☐

Less than ½ mile from farm boundary ☐

½ - 1 mile ☐

PLEASE ANSWER CAREFULLY!

2.5 Continued…

Please indicate whether any of the following lie within 3 miles on this watercourse, and if they are *upstream and/*or *downstream* of your farm.

	Lies on watercourse (within 3 miles)			If YES, is it upstream or downstream of your farm?:			
	Yes	No	Not Known	Upstream	Downstream	Not Known	Not Applicable
Pig farm	☐	☐	☐	☐	☐	☐	☐
Poultry farm	☐	☐	☐	☐	☐	☐	☐
Cattle farm	☐	☐	☐	☐	☐	☐	☐
Sheep farm	☐	☐	☐	☐	☐	☐	☐
Sewage plant	☐	☐	☐	☐	☐	☐	☐
Landfill site	☐	☐	☐	☐	☐	☐	☐
Hospital	☐	☐	☐	☐	☐	☐	☐
Pharmaceutical or Chemical plant	☐	☐	☐	☐	☐	☐	☐
Abattoir	☐	☐	☐	☐	☐	☐	☐

2.6 Are any of the field boundaries of your farm formed by water-filled ditches? YES ☐ NO ☐ Not Known ☐

2.7 Are there any **pig** farms within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, enter the number of farms (if known) ☐

Is the nearest farm: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.8 Are there any **poultry** farms within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, enter the number of farms (if known) ☐

Is the nearest farm: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

PTO

2.9 Are there any **cattle** farms within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, enter the number of farms (if known) ☐

Is the nearest farm: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.10 Are there any **sheep** farms within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, enter the number of farms (if known) ☐

Is the nearest farm: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.11 Is there a **sewage plant** within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, is it: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.12 Is there a **landfill site** within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, is it: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.13 Is there a **hospital** within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, is it: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.14 Is there a **pharmaceutical or chemical plant** within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, is it: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.15 Is there an **abattoir** within 3 miles of the farm? YES ☐ NO ☐ Not Known ☐

If YES, is it: Adjacent ☐ Less than 1 mile ☐ 1-2 miles ☐ More than 2 miles ☐

2.16 Has any animal waste or sewage been **spread** on land adjacent to your pig unit in the past 12 months? YES ☐ NO ☐ Not Known ☐

If YES, was it:

(please tick all that apply)

Cattle ☐ Pig ☐ Poultry ☐ Human ☐

Other *(specify)* _____ ☐

2.17 Has any animal waste been **stored** on land adjacent to your pig unit in the past 12 months? YES ☐ NO ☐ Not Known ☐

If YES, was it:

(please tick all that apply)

Cattle ☐ Pig ☐ Poultry ☐ Human ☐

Other *(specify)* _____ ☐

2.18 Are pigs **only** loaded **and** unloaded at the perimeter of the site?

Loaded: YES ☐ NO ☐

Unloaded: YES ☐ NO ☐

2.19 Are feed lorries **only** unloaded at the perimeter of the site? YES ☐ NO ☐

2.20 What is the source of **drinking** water for the pigs? Mains ☐ Borehole ☐ Other *(specify)* _____ ☐

2.21 Is your pig unit: *(tick one box only)*

a) conventional? ☐

b) organic? ☐

c) in conversion to organic status? ☐

d) status not known ☐

SECTION 3: HYGIENE

3.1 Is any equipment (e.g. tractor) shared between the pig unit and other farm enterprises? YES ☐ NO ☐ Not Known ☐

If YES, then please list the equipment and the other enterprises that share it below:

Equipment	Enterprise	Is this enterprise under the same ownership?
e.g. Tractor	*arable*	YES ☑ NO ☐
		YES ☐ NO ☐
		YES ☐ NO ☐
		YES ☐ NO ☐
		YES ☐ NO ☐

3.2 Please list all disinfectants that are currently used (e.g. for cleaning, bootdips etc) on your pig unit and note any dilution rate used?

Disinfectant (*Name and Manufacturer)*	Dilution Rates (*e.g. 1 part disinfectant : 160 parts water = 1:160*)	
1.		Not Known ☐
2.		Not Known ☐
3.		Not Known ☐
4.		Not Known ☐
5.		Not Known ☐
6.		Not Known ☐

Please use the sheets at the end for any further responses

3.3 Are disinfectant wheeldips or sprays used? YES ☐ NO ☐

If YES, where they are used: *(please tick all that apply)*
At main entrance ☐ At all entrances ☐
Other *(specify)* _____ ☐

Which of the disinfectants in **Q3.2** is used? *(only enter the number)* ☐

How often is the disinfectant in the wheeldips topped up?
Daily ☐ 2-6 times/week ☐ Weekly ☐
1-3 times/month ☐ Less than once/month ☐ NK ☐

How often is the disinfectant in the wheeldips changed?
Daily ☐ 2-6 times/week ☐ Weekly ☐
1-3 times/month ☐ Less than once/month ☐ NK ☐

3.4 Are disinfectant bootdips or sprays available? YES ☐ NO ☐

If YES, where are they used:
At each building ☐ At each pig building ☐
At some buildings ☐
Other *(specify)* _____ ☐

Which of the disinfectants in **Q3.2** are used in the bootdips? *(only enter the number)* ☐

How often are the bootdips topped up?
Daily ☐ 2-6 times/week ☐ Weekly ☐
1-3 times/month ☐ Less than once/month ☐ NK ☐

How often are the bootdips emptied and refilled?
Daily ☐ 2-6 times/week ☐ Weekly ☐
1-3 times/month ☐ Less than once/month ☐ NK ☐

3.5 Is a boot brush present at the entrance to pig buildings?
Yes, all ☐ Yes, some ☐ No ☐ NK ☐

3.6 Do you have access to a pressure washer to clean pig buildings/equipment?
Yes, owned ☐ Yes, hired / shared ☐ No, not used ☐

If YES, do you use: Hot or cold water? Hot ☐ Cold ☐
Detergent? Yes ☐ No ☐

PTO

3.7 What dedicated hygiene facilities are provided for the pig unit? *(please tick all that apply)*

Wash Basin ☐ Toilet ☐ Hand Sanitiser/Bactericidal Soap ☐ Shower ☐ Hand Towel ☐

Warm Air Dryer ☐ Paper Towels ☐ Clean Bucket ☐ Soap ☐ Other *(specify)* _____ ☐

3.8 Are site dedicated boots and protective clothing provided for **and used** by staff and/or visitors to the pig unit?

	Overalls				Boots			
	Staff		Visitors		Staff		Visitors	
	Yes	No	Yes	No	Yes	No	Yes	No
Provided	☐	☐	☐	☐	☐	☐	☐	☐
Used	☐	☐	☐	☐	☐	☐	☐	☐

If YES to any of these: **how** are overalls washed? Machine ☐ Laundry Service ☐ Hand ☐ Other *(specify)* _____ ☐

how often are overalls washed? Every day ☐ Not every day but at least once/week ☐ Not every week but at least once/fortnight ☐ Not every fortnight but at least once/month ☐

Less than once/month ☐ Not known ☐ Other *(specify)* _____ ☐

3.9 Do other members of your family or friends ever enter the pig buildings (e.g. to find you, for a chat etc.) YES ☐ NO ☐

3.10 Are visitors required to take a shower on arrival on the farm? YES ☐ NO ☐

3.11 How many days must people be free from contact with other pigs before visiting the farm? *(if none, write "0")* _____ days

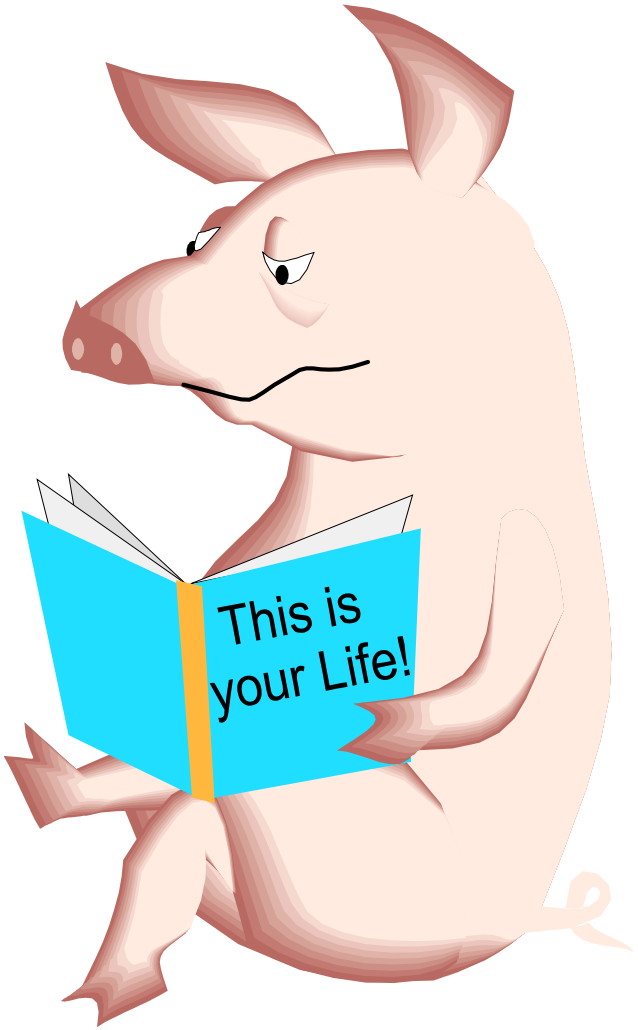
3.12 Is there a **written** biosecurity and /or hygiene plan for the farm? YES ☐ NO ☐ NK ☐

3.13 Do you take any actions on your farm **specifically against *Salmonella*?** YES ☐ NO ☐ NK ☐

If YES, please list these:

1. _____
2. _____
3. _____

Please use the sheets at the end for any further responses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SECTION 4: PIG MOVEMENTS AND TRANSPORT

4.1 Please complete the table below for all pigs that were **moved on to** the farm in the **past 12 months**. Write 0 (zero) in those boxes that do not apply to your farm. NB. There is a separate question for movements off the farm on the next page

- Class of pigs **delivered** (sucking piglets, weaners etc.)
- Approximate **total** number of pigs received of each class
- Approximate **total** number of deliveries of each class
- **Source** of most recent delivery (e.g. Bloggs Pedigree Pig Co)
- **Total** number of sources of each class of pig (e.g. if some pigs were from Bloggs and others from one other source enter "2")
- **Transport** used – i.e. your own transport, commercial haulier or suppliers transport. If more than one transport was used for any class of pig, then tick **all appropriate** boxes

EXAMPLE

Class of pig	Number received	Number of deliveries	Most recent source	Total number of sources	Transport used (tick)		
					Own	Haulier	Supplier
Sucking Piglets	0				☐	☐	☐
Weaner (3 – 10 weeks)	0				☐	☐	☐
Grower (11 – 14 weeks)	0				☐	☐	☐
Finisher (15 + weeks)	0				☐	☐	☐
Gilts	70	4	Bloggs Pedigree Pig Co.	2	☐	☐	☑
Boars	6	3	Bloggs Pedigree Pig Co.	1	☐	☐	☑
Other(specify)_____	0				☐	☐	☐
_____	0				☐	☐	☐

PIG MOVEMENTS ONTO THE FARM IN THE LAST 12 MONTHS

Class of pig	Number received	Number of deliveries	Most recent source	Total number of sources	Transport used (tick)		
					Own	Haulier	Supplier
Sucking Piglets					☐	☐	☐
Weaners (3 – 10 weeks)*					☐	☐	☐
Growers (11 – 14 weeks)*					☐	☐	☐
Finishers (15 + weeks)*					☐	☐	☐
Gilts					☐	☐	☐
Boars					☐	☐	☐
Other(specify)_____					☐	☐	☐
_____					☐	☐	☐

** Or approximately 8-30kg for weaners, 30-50kg for growers and 50-80kg for finishers.*

4.2 Please complete the table below for all pigs that were **moved off** the farm in the **past 12 months**. Write 0 (zero) in those boxes that do not apply to your farm.

- Classes of pigs **moved off** (sucking piglets, weaners etc.)
- Approximate **total** number of pigs moved of each class
- Approximate **total** number of despatches of each class
- **Destination** of most recent batch (e.g. PiggiPackers Abattoir)
- **Total** number of destinations of each class of pig (e.g. if all pigs went to PiggiPackers, enter "**1**")
- **Transport** used – i.e. your own transport, commercial haulier or purchasers transport. If more than one transport was used for any class of pig, then tick **all appropriate** boxes

EXAMPLE

Class of pig	Number moved off	Number of batches	Destination of most recent batch	Total number of destinations	Transport used (tick)		
					Own	Haulier	Purchaser
Sucking Piglets	0				☐	☐	☐
Weaner (3 – 10 weeks)	0				☐	☐	☐
Grower (11 – 14 weeks)	0				☐	☐	☐
Finisher (15 + weeks)	4000	50	PiggiPackers Abattoir	1	☐	☐	☑
Casualty Pigs (any class)	0				☐	☐	☐
Cull sows	0				☐	☐	☐
Cull boars	0				☐	☐	☐
Other(specify)_____	0				☐	☐	☐
_____	0				☐	☐	☐

PIG MOVEMENTS OFF THE FARM IN THE LAST 12 MONTHS

Class of pig	Number moved off	Number of batches	Destination of most recent batch	Total number of destinations	Transport used (tick)		
					Own	Haulier	Purchaser
Sucking Piglets					☐	☐	☐
Weaners (3 – 10 weeks)*					☐	☐	☐
Growers (11 – 14 weeks)*					☐	☐	☐
Finishers (15 + weeks)*					☐	☐	☐
Casualty Pigs (any class)					☐	☐	☐
Cull sows					☐	☐	☐
Cull boars					☐	☐	☐
Other(specify)_____					☐	☐	☐
_____					☐	☐	☐

** Or approximately 8-30kg for weaners, 30-50kg for growers and 50-80kg for finishers.*

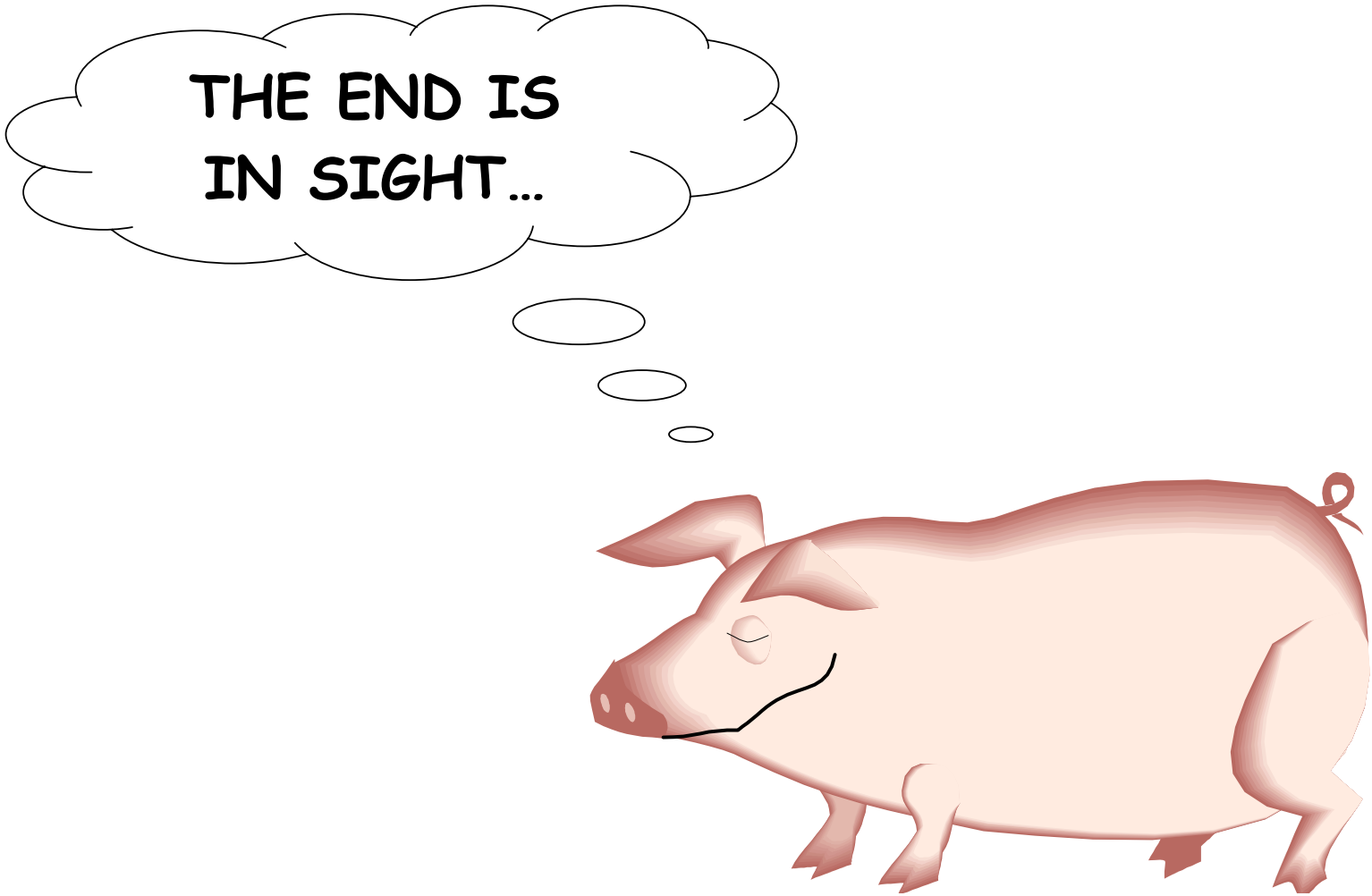
PTO

4.3 PIG MOVEMENTS WITHIN THE FARM

1) How is each class of pig moved from place to place on the farm?	On foot	Barrow or handcart	Trailer	Bucket or crate mounted on a tractor	Other *(specify)* _____	Other *(specify)* _____
Sucking Piglets	☐	☐	☐	☐	☐	☐
Weaners (3 – 10 weeks)*	☐	☐	☐	☐	☐	☐
Growers (11 – 14 weeks)*	☐	☐	☐	☐	☐	☐
Finishers (15 + weeks)*	☐	☐	☐	☐	☐	☐
Boars	☐	☐	☐	☐	☐	☐
Sows / Gilts	☐	☐	☐	☐	☐	☐
Other(specify)_____	☐	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐	☐
2) Is any of the equipment that is used for moving pigs also used for the following purposes?						
Moving feed		☐	☐	☐	☐	☐
Moving bedding		☐	☐	☐	☐	☐
Moving waste		☐	☐	☐	☐	☐
Other(specify)_____		☐	☐	☐	☐	☐
_____		☐	☐	☐	☐	☐

** Or approximately 8-30kg for weaners, 30-50kg for growers and 50-80kg for finishers.*

Please use the sheets at the end for any further responses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SECTION 5: OTHER ANIMALS

5.1 During the past **7 days**, how many live rats have you seen on your farm? ☐

5.2 Which of the following statements best describes the situation with respect to **rats** on your farm **today**? *(tick only one)*

a) Major problem (frequently seen, causing damage and not under control) ☐
b) Minor problem (occasionally seen, causing some nuisance, control has some effect) ☐
c) Under control (seldom seen, minimal nuisance or damage, control is effective) ☐
d) No problem (not seen, no evidence of damage, control completely effective or not required) ☐
e) Not known ☐

5.3 Do you consider that **rats** have been a major problem on your farm at any time in the **past 12 months?** YES ☐ NO ☐ Not Known ☐

5.4 Which of the following statements best describes the situation with respect to **mice** on your farm **today**? *(tick only one)*

a) Major problem (frequently seen, causing damage and not under control) ☐
b) Minor problem (occasionally seen, causing some nuisance, control has some effect) ☐
c) Under control (seldom seen, minimal nuisance or damage, control is effective) ☐
d) No problem (not seen, no evidence of damage, control completely effective or not required) ☐
e) Not known ☐

5.5 Do you consider that **mice** have been a major problem on your farm at any time in the **past 12 months?** YES ☐ NO ☐ Not Known ☐

5.6 Do you conduct your own rodent control programme? YES ☐ NO ☐ Not Known ☐

5.7 Are you currently using a specialist rodent contractor? YES ☐ NO ☐ Not Known ☐

If NO, have you used a specialist rodent contractor at **any time** in the **past 12 months**? YES ☐ NO ☐ Not Known ☐

If YES, how often does the contractor visit the farm?

a) Daily ☐
b) At least once / week ☐
c) Less than once / week but at least once / fortnight ☐
d) Less than once / fortnight but at least once / month ☐
e) Less than once / month ☐
f) Not known ☐

5.8 How many baiting points do you and/or the contractor use? ☐ Not known ☐

5.9 How often is the bait replaced?

a) Daily ☐
b) At least once / week ☐
c) Less than once / week but at least once / fortnight ☐
d) Less than once / fortnight but at least once / month ☐
e) Less than once / month ☐
f) Not known ☐

5.10 How often is the bait removed by rodents?

a) Daily ☐
b) At least once / week ☐
c) Less than once / week but at least once / fortnight ☐
d) Less than once / fortnight but at least once / month ☐
e) Less than once / month ☐
f) Not known ☐

5.11 Please list any other means of rodent control used (e.g. traps, shooting, cats)

5.12 Please tick the boxes below which best describe the presence of wild birds on your farm (*tick all that apply*):

Bird Type	Numbers Seen					Seasons Seen				
	Large Numbers (100+ per day)	Moderate Numbers (20-99 per day)	Low Numbers (<20 per day)	None	Not Known	Winter	Spring	Summer	Autumn	Not Known
Starlings	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gulls	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Crows, Rooks etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pigeons, Doves etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Geese, Ducks etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other (specify) _____	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

5.13 During the **past 7 days**, have wild birds been in any of these areas?

Pig Buildings	YES ☐	NO ☐	Not Known ☐
Feed Stores	YES ☐	NO ☐	Not Known ☐
Bedding Stores	YES ☐	NO ☐	Not Known ☐

5.14 In the table below, please tick whether there have there been any other domestic animals, including pet or working dogs or cats, on the farm? Please tick both whether the species is present **today** and whether it was present during the **past 12 months.**

	Present in last 12 months	Present today
Poultry	☐	☐
Cattle	☐	☐
Horses	☐	☐
Sheep	☐	☐
Dog	☐	☐
Cat	☐	☐
Other (*specify)*_____	☐	☐
_____	☐	☐

5.15 During the **past 7 days,** have dogs or cats been in any of these areas?

Pig Buildings	YES ☐	NO ☐	Not Known ☐
Feed Stores	YES ☐	NO ☐	Not Known ☐
Bedding Stores	YES ☐	NO ☐	Not Known ☐

SECTION 6: FEED STORAGE AND HANDLING

6.1i How many bulk bins are there on your farm? If None, write '0' ☐

ii How many of these bins are open topped, sealed or covered? If None, write '0'

Open ☐ Sealed ☐ Covered ☐ Not Known ☐

6.2 How often do you clean out the bulk bins?

Every batch ☐ Every other batch ☐ Less frequently ☐ Never ☐

Other *(specify frequency)*_____ ☐ Not Applicable ☐

6.3 How do you clean bins? *(Tick all that apply)*

Dry clean *(e.g. hammer & brush)* ☐ Wash ☐ Scrub ☐ Fumigate ☐ Disinfect ☐

Other *(please specify)*_____ ☐ Not Applicable ☐

6.4 Is any bulk feed stored on the floor? YES ☐ NO ☐

If YES, is it in a building protected from the weather and animals? YES ☐ NO ☐

6.5 Do you store any bulk feed in trailers? YES ☐ NO ☐

If YES, are the trailers covered? YES ☐ NO ☐

6.6 Do you purchase any bagged feed for your pigs? YES ☐ NO ☐

If YES, indicate where bags are stored:

a) In a dedicated, closed building ☐

b) In a closed store within pig housing ☐

c) In pig accommodation but not in separate store ☐

d) In open sheds ☐

e) Other (please specify) _____ ☐

6.7 What equipment do you use for **handling feed** on the farm? For each item, please state whether it is used **exclusively** for handling feed and list any other uses, if applicable.

Equipment	Exclusively used for feed?		If NO, then list other uses:
Shovel	YES ☐	NO ☐	
Barrow	YES ☐	NO ☐	
Trailer	YES ☐	NO ☐	
Front loader	YES ☐	NO ☐	
Other equipment *(specify)*_____	YES ☐	NO ☐	
_____	YES ☐	NO ☐	
_____	YES ☐	NO ☐	
_____	YES ☐	NO ☐	

Please use the sheets at the end for any further responses

SECTION 7: PERFORMANCE INDICATORS

7.1 Please state the approximate number of pigs in each class on your unit today, and the number of pigs that have died during the past 4 weeks.

Class of Pig	Approximate number of pigs on the farm today	Number of pigs that died in the past 4 weeks
Sucking Piglets		
Weaners (3 – 10 weeks)*		
Growers (11 – 14 weeks)*		
Finishers (15 + weeks)*		
Boars		
Sows / Gilts		
Other(s) _____		
***(specify)* _____**		

** Or approximately 8-30kg for weaners, 30-50kg for growers and 50-80kg for finishers.*

7.2 Do you use a commercial pig recording scheme? YES ☐ NO ☐

If YES, then please write the name here: _____

and please give the date of the last report: / /

If YES, please provide either a copy of your most recent report or the original (which we will send back by return of post) and we will use that data to answer question **7.3**. Alternatively, please fill in the following question yourself.

7.3 Please complete the following table for the latest performance indicators for the farm

(Enter NOT RECORDED if performance indicator is not recorded)

Performance Indicators		
Pre-weaning mortality (%)		
Post-weaning mortality (%)		
Sow mortality (%)		
Daily Live Weight Gain (g/day)		
Feed Conversion Rate (kg LWG per kg feed)		
Age at slaughter (weeks)		
Weight out (kg) *Give either dead or live weight*	**Dead**	
	Live	

SECTION 8: SICK PENS and FALLEN STOCK

8.1 Do you have any dedicated pens for the exclusive use of animals that are ill, injured or otherwise in poor health, or do you use improvised arrangements when necessary?

Dedicated Pens ☐ Improvised Pens ☐ Not Known ☐ Not Applicable ☐

8.2 How many pigs are there in the sick pens today? ☐

8.3 Sick Pens:

a) Are sick pens in a separate building(s)?		YES ☐	NO ☐
b) Do sick pens drain into other areas holding pigs?		YES ☐	NO ☐
c) Do other areas holding pigs drain into sick pens?		YES ☐	NO ☐
d) Are sick pens:	fully slatted?	YES ☐	NO ☐
	partially slatted?	YES ☐	NO ☐
	solid floored?	YES ☐	NO ☐
e) Are there separate sick pens for different age groups?		YES ☐	NO ☐
f) Are there separate sick pens for each house?		YES ☐	NO ☐
g) Do you use dedicated cleaning out equipment for sick pens?		YES ☐	NO ☐
h) Are sick pens cleaned out and disinfected between batches of pigs?		YES ☐	NO ☐
i) Are sick pens continuously occupied?		YES ☐	NO ☐
j) Are pigs from sick pens mixed with other pigs on recovery?		YES ☐	NO ☐

8.4 How are dead pigs disposed of?

ON SITE: Burial ☐ Muck Heap ☐ Death Pit *(concrete lined fermentation chamber)* ☐ Incineration ☐

Other *(specify)* _____ ☐

OFF SITE: Name of Approved Contractor _____

Other disposal technique *(please specify)* _____

If BURIAL, how soon after death is a pig generally buried? <12 hours ☐ 12-24 hours ☐ 25-48 hours ☐ >48 hours ☐

What depth of earth covers the carcass? ☐ Inches/centimetres *(delete as applicable)*

SECTION 9: ADVICE

9.1 Who do you trust most to give you advice about *Salmonella* and pigs?
Please **rank** them from: 1 (most trusted) to
8 (least trusted)

	Rank
a) BBC Radio (e.g. Farming Today)	
b) Agricultural Press (e.g. Farmers Weekly)	
c) MLC (Meat & Livestock Commision)	
d) ADAS	
e) Your vet	
f) Other pig farmers	
g) Research Scientists (e.g. at universities)	
h) Defra	

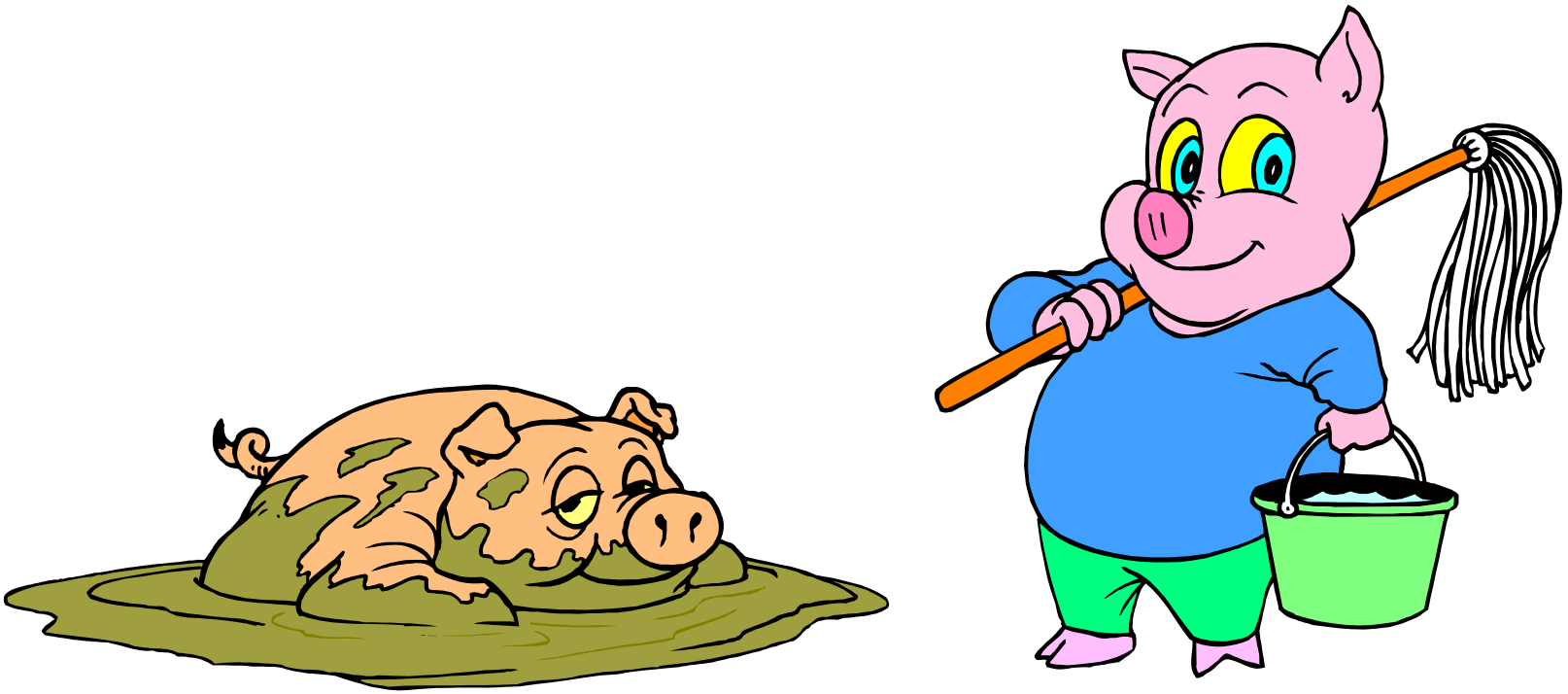
9.2 Please list any other sources of advice on *Salmonella* and pigs which you use

Thank you for taking the time to complete this questionnaire. Your answers will help us to improve our knowledge about *Salmonella* and pigs. We would value any additional opinions or comments that you would like to offer. Please write your remarks here.

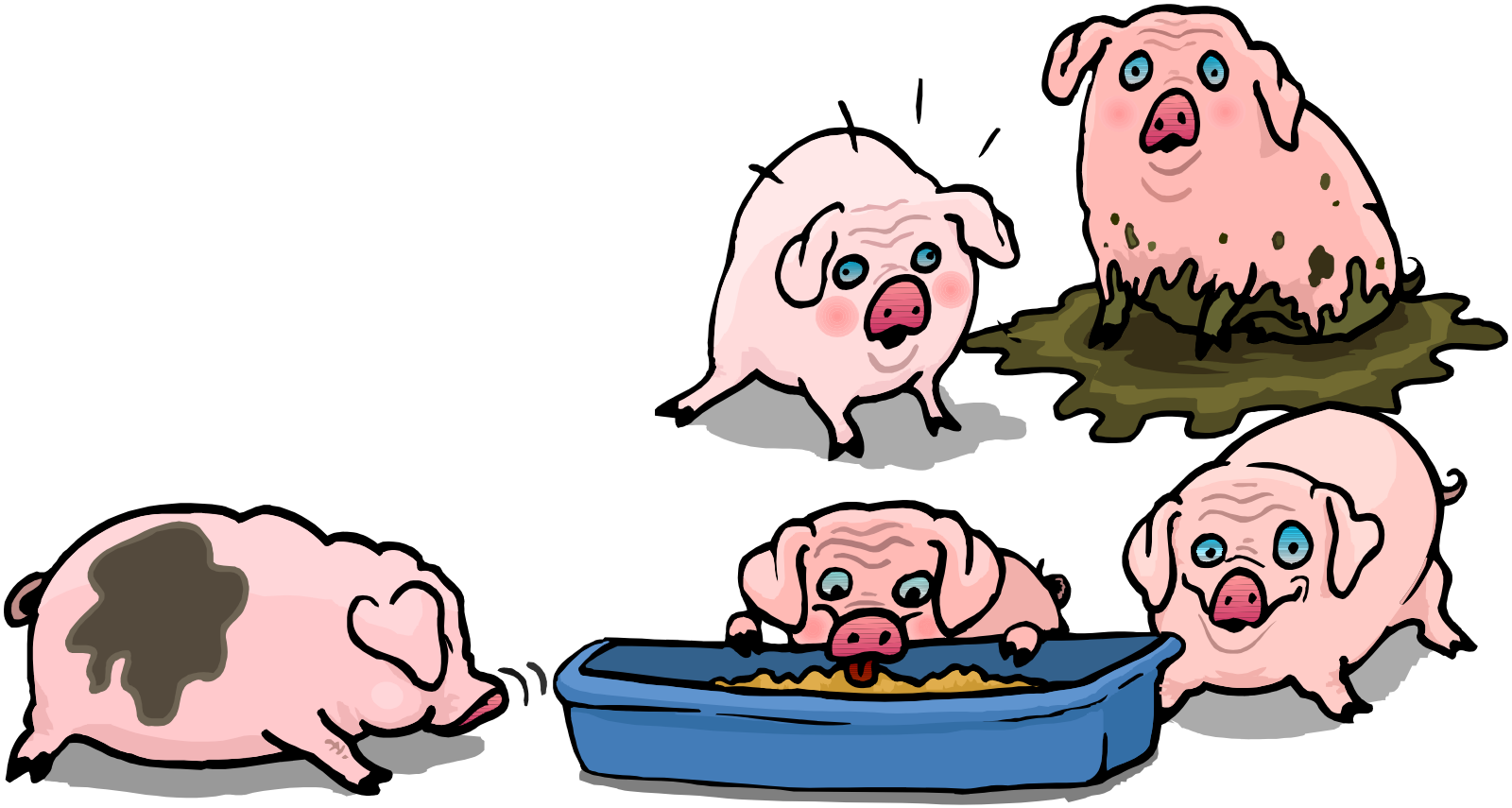
THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

Veterinary Laboratories Agency

Please read through the questionnaire to check for any errors, and to ensure that all questions have been answered.
Once complete, please return the questionnaire as soon as possible in the reply paid envelopes provided.
Please <u>do not</u> send any samples with the questionnaire.



Pig Salmonella - Feed Questions



Farm ID:

CPH Number: / /

Farm Owner

Name of Farm Manager/Foreman
(If NOT the owner)

Farm Address
(including County and Postcode)

If your pig enterprise operates from more than one site, then you should use the main postal address here

County:

Postcode:

Q1. Do you use a wet feeding system for any pigs on your farm?

If **NO**, go to question 2 on the next page

If **YES**: i) Do any pigs receive a fermented liquid feed? YES ☐ NO ☐

If Yes, what pH do you aim for? pH ____ DON'T KNOW ☐

and what methods do you use to achieve this pH?

- Heating liquids ☐
- Inoculations ☐
- Other (*specify*) …….. ☐
- Don't know ☐

ii) What weight range of pigs receive a fermented liquid feed? From ____ kg To ____ kg

iii) What weight range of pigs receive other wet feeds? From ____ kg To ____ kg
(use the back page if more space is needed)

iv) How often is the system completely emptied and cleaned?

	EMPTIED	CLEANED
every day	☐	☐
1-6 times per week	☐	☐
1-3 times per month	☐	☐
once every 2-3 months	☐	☐
once every 4-6 months	☐	☐
once every 7-12 months	☐	☐
less than once every 12 months	☐	☐
never	☐	☐

PTO

v) How do you clean your wet feeding system?

Clean water flu	
Organic acid wash	☐
Disinfectant wash	☐
Other (*specify)* ……..…..	☐
Don't know	☐

Q2. Are any organic acid products administered to pigs in feed or water? YES ☐ NO ☐

If YES: i) Please specify which product(s) are used ……

……

ii) What weight range of pigs receives these products? From ☐ kg To ☐ kg

Q3. i) Does each building have a separate header tank for drinking water? YES ☐ NO ☐

ii) Are all header tanks covered? YES ☐ NO ☐

iii) How often is the drinking water system emptied and cleaned?

	EMPTIED	CLEANED
every day	☐	☐
1-6 times per week	☐	☐
1-3 times per month	☐	☐
once every 2-3 months	☐	☐
once every 4-6 months	☐	☐
once every 7-12 months	☐	☐
less than once every 12 months	☐	☐
never	☐	☐

iv) How do you clean your drinking water system?

Clean water ☐
Organic acid wash ☐
Disinfectant wash ☐
Other (*specify*) ……..….. ☐
Don't know ☐

v) Please list any <u>other</u> products which you add to the drinking water:

……
……
……

Q4. Do you produce any home mill & mix rations for pigs on your farm? YES ☐ NO ☐

If **NO**: skip to question 8 on page 8

If **YES**: i) What is the screen size used for milling your feed? …….. mm DON'T KNOW ☐

ii) Do you use any ingredients grown on your own farm? YES ☐ NO ☐

If YES: Please indicate which

Barley ☐
Wheat ☐
Peas ☐
Other …… ☐

Are the cereals produced under a Quality Assurance Scheme? YES ☐ NO ☐

Please give the name of the scheme ……

iii) Do you purchase any ingredients directly from the farm where they are grown? YES ☐ NO ☐

If YES: Please indicate which

Barley ☐
Wheat ☐
Peas ☐
Other …… ☐

Are the cereals produced under a Quality Assurance Scheme? YES ☐ NO ☐ DON'T KNOW ☐

Please give the name of the scheme …….. DON'T KNOW ☐

PTO

iv) Are cereals that are home grown or brought directly to the farm treated with organic acids? YES ☐ NO ☐

If Yes, what type of product(s) do you use? ……

……

v) Do you purchase any ingredients from a feed merchant? YES ☐ NO ☐

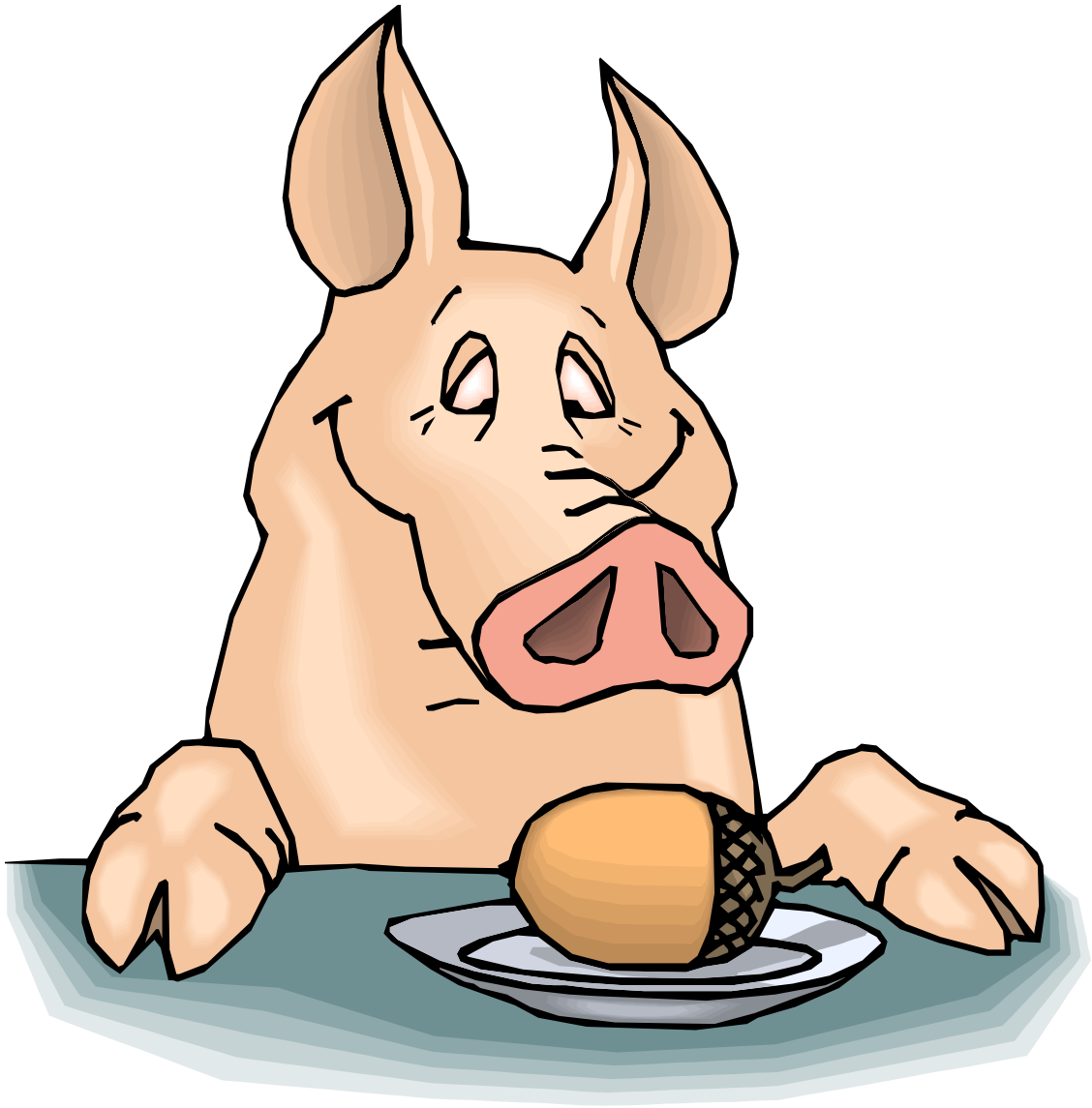
If YES: Please indicate which

Barley	☐
Wheat	☐
Extracted soya	☐
Full fat soya	☐
Vitamin/Mineral Mix	☐
Fishmeal	☐
Extracted rapeseed meal	☐
Crushed whole rape	☐
Peas	☐
Beans	☐
Purchased Protein Concentrates	☐
Other (specify) ……	☐

Table 1: Please TICK the appropriate boxes to show the ingredients used for home mill and mix d during the past 4 weeks

Feed Ingredient	Used? YES	Used? NO	Ingredient used in rations for: Boars	Pregnant sows	Lactating sows	Weaners (approx 8-30kg)*	Growers (approx 30-50kg)*	Finishers (approx 50-80kg)*	Other (*please specify*)
Barley	☐	☐	☐	☐	☐	☐	☐	☐	.....
Wheat	☐	☐	☐	☐	☐	☐	☐	☐	.....
Extracted soya	☐	☐	☐	☐	☐	☐	☐	☐	.....
Full fat soya	☐	☐	☐	☐	☐	☐	☐	☐	.....
Vitamin/ Mineral premix(es)	☐	☐	☐	☐	☐	☐	☐	☐	.....
Fishmeal	☐	☐	☐	☐	☐	☐	☐	☐	.....
Extracted rapeseed meal	☐	☐	☐	☐	☐	☐	☐	☐	.....
Crushed whole rape	☐	☐	☐	☐	☐	☐	☐	☐	.....
Peas	☐	☐	☐	☐	☐	☐	☐	☐	.....
Beans	☐	☐	☐	☐	☐	☐	☐	☐	.....
Biscuit waste	☐	☐	☐	☐	☐	☐	☐	☐	.....
Cereal waste	☐	☐	☐	☐	☐	☐	☐	☐	.....
Purchased Protein concentrates	☐	☐	☐	☐	☐	☐	☐	☐	.....
Dry milk products	☐	☐	☐	☐	☐	☐	☐	☐	.....
Other non-milk liquid co-products	☐	☐	☐	☐	☐	☐	☐	☐	.....
Wet milk co-products	☐	☐	☐	☐	☐	☐	☐	☐	.....
Other *(please list below)*									
.....	☐	☐	☐	☐	☐	☐	☐	☐	.....
.....	☐	☐	☐	☐	☐	☐	☐	☐	.....
.....	☐	☐	☐	☐	☐	☐	☐	☐	.....

* Or approximately 3-10 weeks for weaners, 11-14 weeks for growers, and 15+ weeks for finishers



Q5. If you use non-milk liquid co-products, please LIST all that you have used in the last 4 weeks:

.....

.....

.....

.....

.....

Q6. Do you use a probiotic or other feed additive in any of your home mill and mix feed(s)? YES ☐ NO ☐

If YES: what type do you use?

.....

.....

Q7. Do you hold a prescription for using an antibiotic or other medicine in your home mill & mix feed? YES ☐ NO ☐

If YES: please give the name of the medicine(s)

.....

.....

IF YOU HAVE SKIPPED THE HOME MILL & MIX QUESTIONS, PLEASE START AGAIN FROM HERE:

Q8. Have you used any purchased compound feeds for your pigs in the past 4 weeks? YES ☐ NO ☐

If YES: please complete Table 2 over the page

PTO

APPENDIX CS6

Name of Feed	Company	Mill *(if known)*	Delivered in	Fed	Description
e.g. Rearer 1	PiggiFood	SouthPork	Bulk ☐ Bag ☑	Wet ☐ Dry ☑	Nut/Roll ☐ Pellet ☑ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ....☐

Table 2: Please give details of any purchased compound feeds used in the past 4 weeks.

Method	Growth Promoter/ other feed additives	Prescribed r	from	to
Adlib ☑ Restricted ☐	Copper ☑ Flavomycin ☐ Maxus ☐ Salinomycin ☑ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☑ Name(s) …………………………… ……………………………	20 kg	50 kg
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ………………………☐ Other ……………………………☐ Don't know ☐	Yes ☐ No ☐ Name(s) …………………………… ……………………………		

↑ THIS WAY UP ↑ *(NB. space for more answers over ☞)* PTO

Name of Feed	Company	Mill *(if known)*	Delivered in	Fed	Description
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐
			Bulk ☐ Bag ☐	Wet ☐ Dry ☐	Nut/Roll ☐ Pellet ☐ Meal ☐ Other ………………………………☐

Table 2 *(continued)*:

↑ THIS WAY UP ↑

APPENDIX CS6

Method	Growth Promoter/ other feed additives	Prescribed	from	to
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		
Adlib ☐ Restricted ☐	Copper ☐ Flavomycin ☐ Maxus ☐ Salinomycin ☐ Probiotics ....☐ Other ....☐ Don't know ☐	Yes ☐ No ☐ Name(s)		

↑ THIS WAY UP ↑

PTO

FARMER NOTES: This page is for any comments you may wish

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

Veterinary Laboratories Agency

Please read through the questionnaire to check for any errors and to ensure that all questions have been answered. Once complete, please return the questionnaire as soon as possible in the reply paid envelopes provided. Please do not send the questionnaire in the same envelope as any samples.

If you have any questions, please get in touch with Alasdair Cook or Sandy Miller at VLA Weybridge

Alasdair Cook ☎ **01932 357977;** 💻 **a.j.cook@vla.defra.gsi.gov.uk**

Sandy Miller ☎ **01932 357623;** 💻 **a.miller@vla.defra.gsi.gov.uk**

Farm ID: _____

OZ0316: PIG SALMONELLA – VET QUESTIONNAIRE

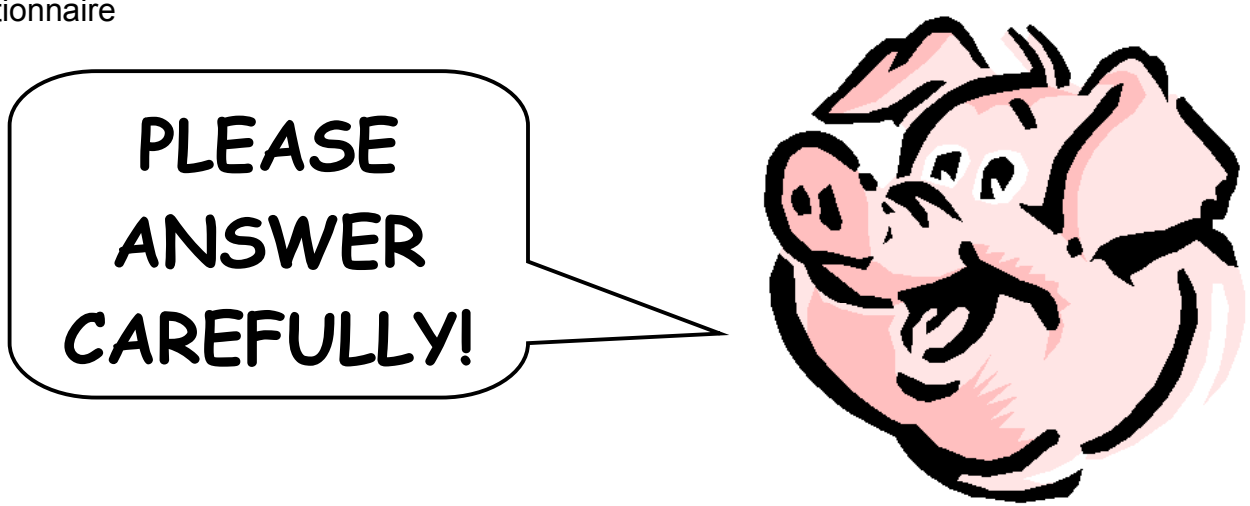
Please read the following notes before you answer the questions:

- Most questions can be answered by ticking a box ☑ or writing down a number
- Where a question relates to past events, this period is measured from the date on which you complete the questionnaire
  e.g. 'In the last 12 months' refers to 12 months from today
- There is only ONE answer for most questions, unless you are asked to tick every applicable box
- Please write any comments on the 'notes page' provided at the back
- If you have any questions, please get in touch with Alasdair Cook or Sandy Miller at VLA Weybridge

Alasdair Cook ☎ 01932 357977; 💻 a.j.cook@vla.defra.gsi.gov.uk

Sandy Miller ☎ 01932 357623; 💻 a.miller@vla.defra.gsi.gov.uk

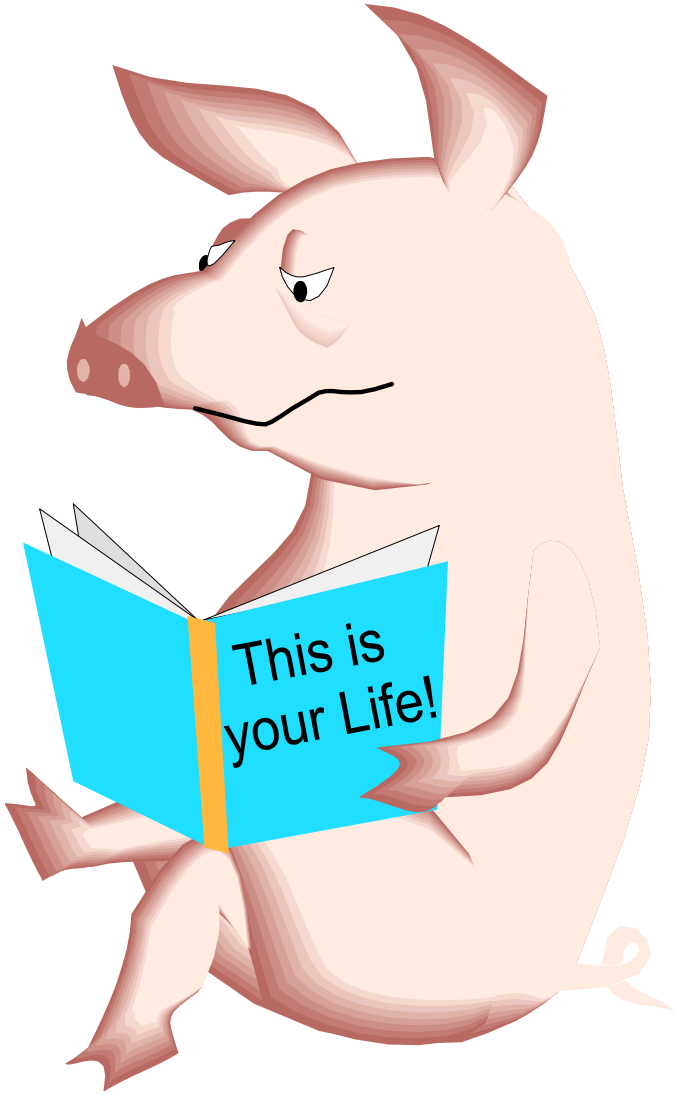
- Please look through your questionnaire to check for any missed questions, and return it in the reply-paid envelope provided.
- Please DO NOT send any samples with this questionnaire



Name of Veterinarian	
Name of Practice	
Practice Address *(including County and Postcode)*	**County:** **Postcode:**
Telephone Number	
Email Address ***(if available)***	

- **To the best of your knowledge, has your client used the services of any other veterinary practice (e.g. consultancy, new client) during the past 12 months?**

YES ☐ NO ☐ NOT KNOWN ☐


This is
your Life!

SECTION 1: DISEASES

Please indicate all of the diseases that have been identified in pigs on the farm during the **past 12 months**. Where signs of a clinical syndrome have been observed without a specific diagnosis (e.g. diarrhoea, cough), then tick the box for the appropriate organ system affected (e.g. for a cough without a specific diagnosis, tick 'Yes' next to 'Respiratory System')

1.1 What diseases have been identified in the herd **during the past 12 months** and how were they confirmed? (*Tick all that apply*)

Disease	Identified		Confirmation	
	Yes	No	Clinical	Lab
1. RESPIRATORY SYSTEM	☐	☐		
1.1 Atrophic rhinitis	☐	☐	☐	☐
1.2 Enzootic pneumonia	☐	☐	☐	☐
1.3 Pleuropneumonia	☐	☐	☐	☐
1.4 PRRS	☐	☐	☐	☐
1.5 Other *(specify)*_____	☐	☐	☐	☐
2. ENTERIC SYSTEM	☐	☐		
2.1 *E.coli* diarrhoea	☐	☐	☐	☐
2.2 Enteric salmonellosis	☐	☐	☐	☐
2.3 Swine dysentery	☐	☐	☐	☐
2.4 Proliferative enteropathy	☐	☐	☐	☐
2.5 Colitis	☐	☐	☐	☐
2.6 Bowel oedema	☐	☐	☐	☐
2.7 Rotavirus	☐	☐	☐	☐
2.8 Gastric ulceration	☐	☐	☐	☐
2.9 Rectal stricture	☐	☐	☐	☐
2.10 Roundworm infestation	☐	☐	☐	☐
2.11 Milkspot liver	☐	☐	☐	☐
2.12 Rectal prolapse	☐	☐	☐	☐
2.13 Other *(specify)*_____	☐	☐	☐	☐

1.1 Continued…

Disease	Identified		Confirmation	
	Yes	No	Clinical	Lab
3. SKIN	☐	☐		
3.1 Mange	☐	☐	☐	☐
3.2 Greasy pig disease	☐	☐	☐	☐
3.3 Swine pox	☐	☐	☐	☐
3.4 Other *(specify)*_____	☐	☐	☐	☐
4. NERVOUS SYSTEM	☐	☐		
4.1 Streptococcal meningitis	☐	☐	☐	☐
4.2 *Haemophilus* meningitis	☐	☐	☐	☐
4.3 Spinal abscess	☐	☐	☐	☐
4.4 Other *(specify)*_____	☐	☐	☐	☐
5. LOCOMOTOR SYSTEM	☐	☐		
5.1 Arthritis	☐	☐	☐	☐
5.2 Leg weakness	☐	☐	☐	☐
5.3 Bush foot/ foot abscess	☐	☐	☐	☐
5.4 Other *(specify)*_____	☐	☐	☐	☐
6. MISCELLANEOUS CONDITIONS	☐	☐		
6.1 “Sudden” death	☐	☐	☐	☐
6.2 PMWS/ PDNS complex	☐	☐	☐	☐
6.3 Sporadic PDNS	☐	☐	☐	☐
6.4 Porcine stress syndrome	☐	☐	☐	☐
6.5 Systemic salmonellosis	☐	☐	☐	☐
6.6 Tail biting	☐	☐	☐	☐
6.7 Other *(specify)*_____	☐	☐	☐	☐

Please use the sheets at the end for any further responses

SECTION 2: TREATMENTS

APPENDIX CS8

2.1 Vaccines: Please indicate all **vaccines and other immunological products** that you have supplied to, prescribed for, or used on pigs on this farm **during the past 12 months**

CODE	VACCINE	Tick
VCOL	Colisorb	☐
VGL6	Gletvax 6 Combined Porcine *E.coli* and *Cl.perfringens* Type B, C and D Vaccine	☐
VHPV	*Haemophilus parasuis* Vaccine	☐
VHEP	Heptavac	☐
VHYP	Hyoresp.	☐
VIPK	Ingelvac PRRS KV	☐
VIPR	Ingelvac PRRS	☐
VIMH	Ingelvac M Hyo	☐
VLBS	Lambisan (Native Lamb Dysentery, Struck and Pulpy Kidney Antiserum)	☐
VLBV	Lambivac	☐
VMOD	Mycoplasma One Dose Vaccine	☐
VMYS	Mypravac Suis	☐
VNCP	Neocolipor	☐
VNPA	Nobi-Porvac Aujeszky Live	☐
VPCV	Pig Coliform Vaccine	☐
VPSV	Pig *Staphylococcus* Vaccine	☐
VPAR	Porcilis AR T	☐
VPAD	Porcilis AR-T DF suspension for injection	☐
VPEY	Porcilis Ery	☐
VPEP	Porcilis Ery+Parvo	☐

CODE	VACCINE	Tick
VPP5	Porcilis Porcol 5	☐
VPPR	Porcilis PRRS	☐
VPGS	Progessis	☐
VSMY	Stellamune Mycoplasma	☐
VSTO	Stellamune Once	☐
VSAP	Suvaxyn APP	☐
VSAJ	Suxaxyn Aujeszky	☐
VSAW	Suvaxyn Aujeszky 783 + O/W	☐
VSEC	Suvaxyn *E.Coli* P4	☐
VSEY	Suvaxyn Erysipelas	☐
VSMP	Suvaxyn M.Hyo – Parasuis	☐
VSMH	Suvaxyn M.Hyo	☐
VSPV	Suvaxyn Parvo	☐
VSPE	Suvaxyn Parvo/E	☐
VSRD	Suvaxyn Respifend	☐
VTAB	Tetanus Antitoxin Behring	☐
VTTC	Tetanus Toxoid Concentrated	☐
	Other (*specify in table*)	
VVC1	_____	☐
VVC2	_____	☐
VVC3	_____	☐

2.2 Anti-Parasite treatments: Please indicate all **anti-parasite** treatments that you have supplied to, prescribed f farm **during the past 12 months**

CODE	ANTI-PARASITE TREATMENT	Tick
PALS	Alstomec	☐
PANI	Animec Injection	☐
PBYP	Bayverm Pellets 1.9%	☐
PBIM	Bimectin Injection	☐
PCUR	Curazole 5% w/w Powder	☐
PDEC	Dectomax Injection for Pigs	☐
PFLI	Flubenol Individual Treatment Pack	☐
PFLP	Flubenol Premix Pack	☐
PGWP	Granofen Wormer for Pigs	☐
PIVI	Ivomec Injection for Pigs	☐
PIVP	Ivomec Premix for Pigs	☐
PORD	Oramec Drench	☐
PP15	Panacur 1.5% Pellets	☐
PP4P	Panacur 4% Powder	☐
PPCS	Panomec Injection for Cattle, Sheep and Pigs	☐
PPRC	Porect	☐
PTKT	Taktic	☐
PTOP	Topline	☐
PVIS	Virbamec Injectible Solution for Cattle and Swine	☐
PZER	Zerofen 4% Powder	☐
PPT1	Other (please specify) _____	☐
PPT2	_____	☐
PPT3	_____	☐

2.3 Antimicrobial Injections: Please indicate all **antimicrobial injections** that you have supplied to, prescribed this farm **during the past 12 months**

APPENDIX CS8

CODE	ANTIMICROBIAL	Tick
AA10	Alamycin 10	☐
AADI	Advocin Injectable Solution	☐
AALL	Alamycin LA	☐
AAL3	Alamycin LA 300	☐
AAM3	Amfipen 30%	☐
AAML	Amfipen LA	☐
AANI	Amoxinsol 150 Injection	☐
AANL	Amoxinsol La	☐
AAXI	Amoxycare Injection	☐
AAXL	Amoxycare LA Injection	☐
AAPI	Amoxypen Injection	☐
AAPL	Amoxypen LA	☐
AAMI	Ampicare 15% Injection	☐
ABY5	Baytril 5% Injection	☐
ABY1	Baytril 10% Injection	☐
ABTX	Betamox	☐
ABTL	Betamox LA	☐
ABL2	Bilosin 200 Injection	☐
ABMI	Bimectin Injection	☐

CODE	ANTIMICROBIAL	Tick
ABXL	Bimoxyl LA	☐
ABG2	Borgal 24% Solution	☐
ACPG	Cephaguard	☐
ACPX	Ceporex Injection	☐
ACLL	Clamoxyl LA Long Acting Injection	☐
ACLR	Clamoxyl Ready to Use Injection	☐
ADLC	Delvoprim Coject	☐
ADPC	Depocillin	☐
ADPM	Depomycin Forte	☐
ADPF	Dipen Forte	☐
ADHC	Duphacillin	☐
ADY1	Duphacycline 100	☐
ADYL	Duphacycline LA	☐
ADYX	Duphacycline XL	☐
ADHX	Duphamox	☐
ADHL	Duphamox LA	☐
ADHF	Duphapen Fort	☐
ADPP	Duphapen	☐
ADPL	Duphapen LA	☐

2.3 Continued…

CODE	ANTIMICROBIAL	Tick
ADPS	Duphapen+Strep	☐
ADIS	Duphatrim IS	☐
ADLA	Duphatrim LA	☐
AECI	Econopen Injection	☐
AEMB	Embacillin	☐
AEML	Embacycline LA	☐
AEG5	Engemycin 5%	☐
AEGD	Engemycin 10% (DD)	☐
AEGF	Engemycin 10% Farm Pack	☐
AEGL	Engemycin LA	☐
AEXR	Excenel RTU	☐
AEXS	Excenel Sterile Powder	☐
AINT	Intradine	☐
ALEI	Lenticillin Injection	☐
ALSS	Lincocin Sterile Solution	☐
ALCJ	Lincoject	☐
AMB2	Marbocyl 2%	☐
AMB1	Marbocyl 10%	☐
AMY1	Mycen 10	☐

CODE	ANTIMICROBIAL	Tick
AMY2	Mycen 20 LA	☐
ANPN	Neopen	☐
ANRB	Norobrittin	☐
ANRC	Norocillin	☐
AND2	Norodine 24	☐
ANTL	Norotyl LA	☐
AOX5	Oxycare 5%	☐
AOX1	Oxycare 10%	☐
AOX2	Oxycare 20/La	☐
AOT1	Oxytetrin 10 DD	☐
AOT2	Oxytetrin 20 LA	☐
AOT5	Oxytetrin 5	☐
APAS	Pen & Strep	☐
APEN	Penacare	☐
AQ15	Qualamox 15	☐
AQLA	Qualamox LA	☐
ASTC	Streptacare	☐
ASTP	Streptopen Injection	☐
ASU3	Sulfoxine 333	☐

2.3 Continued…

CODE	ANTIMICROBIAL	Tick
ASYN	Synulox Ready-to-Use Injection	☐
ATQ1	Terramycin Q-100 Injectable Solution	☐
ATLA	Terramycin/LA Injectable Solution	☐
ATX1	Tetroxy 10% DD Injection	☐
ATX5	Tetroxy 5% Injection	☐
ATXL	Tetroxy LA	☐
ATIA	Tiamutin 200 Injection	☐
ATRI	Tribrissen Injection 48% Sulphadiazine and Trimethoprim Injection Bp(Vet)	☐
ATBI	Trimabac Injection 24%	☐
ATC2	Trimacare 24%	☐
ATCL	Trinacol Injection	☐
ATOL	Trioxyl La	☐
ATYA	Tylan 200 and Tylan 50	☐
ATYV	Tyluvet 20	☐
AULT	Ultrapen LA	☐

CODE	ANTIMICROBIAL	Tick
AVMI	Vidamox Injection	☐
AVML	Vidamox LA Injection	☐
AVCI	Vidocillin Injection	☐
AZ20	Zaquilan 20% Injection	☐
	Other (*specify in table*)	
AAM1	_____	☐
AAM2	_____	☐
AAM3	_____	☐

2.4 Other Antimicrobials: Please indicate all **other antimicrobials** that you have supplied to, prescribed for, or **during the past 12 months**

APPENDIX CS8

CODE	ANTIMICROBIAL	Tick
OALA	Alamycin Aerosol	☐
OAMX	Amoxinsol 50	☐
OAOD	Apralan Oral Doser	☐
AASP	Apralan Soluble Powder	☐
OAUS	Aureomycin Soluble Powder	☐
OATP	Aureomycin Topical Powder	☐
OBPD	Baytril Piglet Doser	☐
OC50	Chlorsol 50	☐
OCOM	Clamoxyl Oral Multidoser	☐
ODPS	Delvoprim Piglet Suspension	☐
ODPA	Duphacycline Aerosol	☐
ODUP	Duphatrim Piglet Suspension	☐
OEMA	Embacycline Aerosol	☐
OEGA	Engemycin Aerosol	☐
OLSP	Lincocin Soluble Powder	☐
ONSP	Neobiotic Soluble Powder 70%	☐
ONOP	Norodine Oral Piglet Suspension	☐
OOXA	Oxycare Aerosol	☐
OPEP	P.E.P. 2% Powder	☐

CODE	ANTIMICROBIAL	Tick
OSSH	Spectam Scour Halt	☐
OTCA	Tectin Aerosol	☐
OTS5	Terramycin Soluble Powder 5%	☐
OTS2	Terramycin Soluble Powder Concentrate 20%	☐
OTT8	Tetsol 800	☐
OTIA	Tiamutin 12.5% Solution	☐
OTYL	Tylan Soluble	☐
OTPS	Tribrissen Piglet Suspension Sulphadiazine and Trimethoprim Mixture Bp(Vet)	☐
OTRP	Trimedoxine Piglet Suspension	☐
	Other (*specify in table*)	
OAM1	_____	☐
OAM2	_____	☐
OAM3	_____	☐

2.5 In-feed medicines: Please indicate all **in-feed medicines** that you have supplied to, prescribed for, or used during the past 12 months

CODE	IN-FEED MEDICINE	Tick
FAG2	Apralan G200 Premix	☐
FA1G	Aurofac 100 Granular	☐
FAUR	Aurogran	☐
FA15	Aurogran 150	☐
FBC1	Bio-Cox 120G	☐
FCFG	Chlortet FG100	☐
FCHS	Cyfac HS Granular	☐
FEP1	Econor Premix 10%	☐
FF40	Flaveco 40	☐
FF80	Flavomycin 80	☐
FLIP	Lincocin Premix	☐
FLSP	Linco-Spectin Premix	☐
FMG2	Maxus G200	☐
FNYP	Neomycin Premix	☐
FPZP	Pigzin Premix	☐
FPOT	Potencil	☐
FPG1	Pulmotil G100 Premix	☐
FPG2	Pulmotil G200 Premix	☐
FSE1	Sal-Eco 120	☐
FSA1	Salocin 120	☐
FS5P	Stabox 5% Premix	☐
FSYF	Synutrim Fortesol	☐

CODE	IN-FEED MEDICINE	Tick
FSYG	Synutrim Granular	☐
FTT1	Tetramin 100 Powder	☐
FTT2	Tetramin 200 Powder	☐
FTS8	Tetsol 800	☐
FT12	Tiamutin 12.5% Solution	☐
FT2P	Tiamutin 2% Premix	☐
FT2I	Tiamutin 200 Injection	☐
FT25	Tiamutin 25% Premix	☐
FT80	Tiamutin 80% Premix	☐
FTD1	Trimediazine 15	☐
FTDB	Trimediazine BMP	☐
FTG1	Tylan G100	☐
FTG2	Tylan G20	☐
FTGP	Tylan G250 Premix	☐
FTG5	Tylan G50 Premix	☐
FTYG	Tylasul G50	☐
FUNP	Uniprim 150 Powder	☐
FUNS	Uniprim 150 S	☐
	Other (*specify in table*)	
FIF1	_____	☐
FIF2	_____	☐
FIF3	_____	☐

2.6 Other treatments: Please indicate all **other treatments** that you have supplied to, prescribed for, or used on **the past 12 months** including anything not already ticked in the previous tables

CODE	OTHER TREATMENTS	Tick
TA4B	Anivit 4BC Injection	☐
TBSI	Bisolvon Injection	☐
TBSP	Bisolvon Powder	☐
TCMV	Combivit	☐
TDLZ	Dalmazin	☐
TDXD	Dexadreson	☐
TDXF	Dexafort	☐
TD4V	Dunlops 4bc Vitamin	☐
TDAF	Duphafral Ade Forte	☐
TDM9	Duphafral Multivitamin 9	☐
TDLY	Duphalyte	☐
TDYS	Dystosel	☐
TEFF	Effydral	☐
TENZ	Enzaprost -t	☐
TFRX	Ferrofax 20%	☐
TFS6	Fostim 6000	☐
TGPS	Gleptosil	☐
THYP	Hyposton	☐
TIFP	Iliren For Pigs	☐
TIMP	Imposil	☐
TINT	Intravit 12	☐
TIOA	Ion Aid	☐
TIOY	Ionalyte	☐
TKET	Ketofen 10%	☐
TLCD	Lectade	☐
TLEO	Leodex 20%	☐
TLFA	Life Aid	☐
TLAP	Life Aid P	☐

CODE	OTHER TREATMENTS	Tick
TLAI	Lignocaine And Adrenaline Injection	☐
TLS1	Linco Spectin 100 Soluble Powder	☐
TLLA	Liquid Life Aid	☐
TLTL	Lutalyse	☐
TMVI	Multivitamin Injection	☐
TMIA	Multivitamin Injection (Arnolds)	☐
TOXS	Oxytocin S	☐
TOXL	Oxytocin Leo	☐
TPSF	Pfizer Scour Formula	☐
TPG6	PG 600	☐
TPLN	Planate	☐
TPMI	PMSG Intervet	☐
TPRV	Prosolvin	☐
TPRP	Prostapar	☐
TRGP	Regumate Porcine	☐
TSDX	Scordex	☐
TSTR	Stresnil	☐
TTOL	Tolfine	☐
TVCI	Vitatrace Injection	☐
TVNI	Vitenium Injection	☐
TVIT	Vitesel	☐
TVOR	Voren Suspension	☐
	Other (*specify in table*)	
TOT1	_____	☐
TOT2	_____	☐
TOT3	_____	☐
TOT4	_____	☐
TOT5	_____	☐

SECTION 3: FLUOROQUINOLONE USE

Aliquots of faecal material will be used to isolate bacteria and test them for sensitivity to fluoroquinolone antibiotics as part of another VLA study. Please complete the table below in addition to Section 2.

3.1 When, if ever, was the **last** time any of the following antibiotics (fluoroquinolones) were used on/supplied for pigs on this farm?
(tick ONE box for each product for the most recent use)

a) Baytril (5% or 10% injection)	Within the last year ☐	1 to 2 years ago ☐	Over 2 years ago ☐	Never used ☐
b) Baytril (piglet doser)	Within the last year ☐	1 to 2 years ago ☐	Over 2 years ago ☐	Never used ☐
c) Marbocyl (2% or 10% injection)	Within the last year ☐	1 to 2 years ago ☐	Over 2 years ago ☐	Never used ☐
d) Advocin (injectable solution)	Within the last year ☐	1 to 2 years ago ☐	Over 2 years ago ☐	Never used ☐

3.2 If any of the above were used/supplied/prescribed **within the last 12 months**, please give details of the disease problems for which they were prescribed, the amount supplied and the type of pigs treated

Name of medicine	Disease problem for which prescribed	Amount supplied (number bottles)	Type of pig treated (tick box if treated)
Baytril (5% injection)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐
Baytril (10% injection)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐
Baytril (piglet doser)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐
Marbocyl (2% injection)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐
Marbocyl (10% injection)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐
Advocin (injectable solution)			piglet ☐ grower ☐ replacement gilt ☐ weaner ☐ finisher ☐ sow/boar ☐

SECTION 4: GENERAL QUESTIONS

4.1 How many times have you visited the farm **during the past 12 months?**

- 12 or more times ☐
- 4-11 times ☐
- 2-3 times ☐
- Once ☐
- Never ☐
- Not Known ☐

4.2 Using the General House Keeping Score descriptions provided on the following page, which category best describes this pig farm?

1	2	3	4
☐	☐	☐	☐

CATEGORY 1	CATEGORY 2
Good, well-maintained, modern buildings/structures. Hard standing perimeter and service roads to most if not all areas. Feed vehicles discharge and services accessible from perimeter. Fenced perimeter. Unit well signed. Weed growth controlled and managed in all areas. Good drainage: no "ponding". Clear access to all areas. Storage areas tidy. No excessive accumulations of muck. No accumulation of scrap equipment, or materials. Good evidence of regular housekeeping action in all areas of the site. Few if any fabric repairs required. Excellent facilities for staff-toilets and canteen area.	Sound buildings or structures – some maintenance may be required to fabric in some area. Some hard standing areas, but may have unlaid roadways and access to certain parts. Perimeter defined but not necessarily fenced entirely. Some weed growth evident around perimeter but controlled around buildings used for feed or pigs. Evidence of management of waste but there may be a need for action in the forthcoming 3 months. Evidence of pest control scheme/system which is effective. Basic staff facilities i.e. toilets and meal arrangements.
CATEGORY 3	**CATEGORY 4**
Older premises where there is a need for essential fabric repairs in several areas. Some buildings (in use) needing structural repairs, e.g. broken doors, windows, roof repairs required. Little definition to perimeter with poorly maintained service roads. Some evidence of pest activity. Control measures agreed, investigated, or in place but in need of improvement. Accumulation of scrap and/or redundant equipment which compromise the ability to control pests. Weed control is required to prevent growth up to and around buildings where pigs are housed or feedstuffs are stored. Very basic staff facilities.	Buildings in poor state of repair. Several items requiring major renovation/repair work to structure. Generally old premises with no obvious investment/maintenance over many years. Perimeter control poor. Accumulation of muck or general equipment in the pig environment or around the pig buildings and feed stores. Evidence of obvious pest activity, e.g. mice, flies, rats or birds. Poor housekeeping in feed stores, evidence of careless feed spillage. Poor pest proofing to areas where pigs are kept. Waste control poor – significant accumulation of waste, dung, muck. Feedstuffs exposed to serious opportunities for contamination. Inadequate facilities for staff.

VET NOTES:

This page is for any comments you may wish to add.

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

Veterinary Laboratories Agency

Please read through the questionnaire to check for any errors and to ensure that all questions have been answered.
Once complete, please return the questionnaire as soon as possible in the reply paid envelope provided.
Please <u>do not</u> send the questionnaire in the same envelope as the faecal samples.

Farm Code XXX

APPENDIX PI3

Farm Name Xxxxxxxxxx Xxxxxxxxx Xxx

Name of Person Completing Sheet……..

OZ0316 : SALMONELLA STUDY RECORDING SHEET

	Was this work done by a contractor or yourself?	Building 1			Building 2			Building 3			Building 4			Building 5		
1. Date last pigs removed from previous batch (e.g.14/04)		/			/			/			/			/		
2. CLEANING PIG HOUSES: (Answer Yes or No and give the **date completed**)		**Yes**	**No**	**Date**	**Yes**	**No**	**Date**	**Yes**	**No**	**Date**	**Yes**	**No**	**Date**	**Yes**	**No**	**Date**
Was the muck heap moved?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
Was the muck heap area disinfected?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
Were the feed hoppers emptied?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
Was the solid waste cleaned out of the feed hoppers?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
Did you pressure wash the: - feed hoppers?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
- walls/partitions/passageways/other surfaces?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
If YES, did you use HOT or COLD water?		HOT ☐ COLD ☐			HOT ☐ COLD ☐			HOT ☐ COLD ☐			HOT ☐ COLD ☐			HOT ☐ COLD ☐		
Was DETERGENT used in the pressure washer?		YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐		
Please give the NAME? (e.g. HD3)																
VOLUME (of concentrate)		*ml*			*ml*			*ml*			*ml*			*ml*		
CONCENTRATION (e.g. 1:160)		:			:			:			:			:		
Were the walls/partitions/passageways/other surfaces disinfected?	Contractor ☐ Self ☐	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/	☐	☐	/
If YES, give the NAME (e.g. Farm Fluid S)																
VOLUME (of concentrate)		*ml*			*ml*			*ml*			*ml*			*ml*		
CONCENTRATION (e.g. 1:50)		:			:			:			:			:		
Was the building left to dry?		YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐			YES ☐ NO ☐		
3. Date first pig from new batch entered building:		/			/			/			/			/		

APPENDIX PI3

4. RODENT CONTROL

- How often do you use a specialist rodent control contractor? Always ☐ Usually ☐ Sometimes ☐ Never
- If contractor used, give their name (……) and send their last report with this form. *We will copy this, and send it back by return of post.*
- When did you last review your rodent control programme? Give date ……/……
- What do you (or the contractor) use? Bait ☐ Traps ☐ Other (please specify)……. ☐
- Please fill in any relevant sections of the following table:

	BAIT	TRAPS
Name (e.g. TOMCAT, ZP Pellets, traditional/electric rat or mouse traps)		
Frequency of Checking and Changing (days)	Checking: …..….days Changing: …..….days	Checking: …..….days Changing: …..….days
How many bait points/traps do you have: i) in pig buildings?		
ii) elsewhere?		

5. EQUIPMENT CLEANING: Please complete the following table concerning the cleaning of equipment on your farm:

Equipment	Was this cleaned with a bucket and brush?			Was this item pressure washed?			Did you use HOT or COLD water?		Did you use Detergent?		Did you use Disinfectant?		Was this work done by a contractor or yourself?	
	Yes	No	Date	Yes	No	Date	Hot	Cold	Yes	No	Yes	No	Contractor	Self
Tractor	☐	☐	/	☐	☐	/	☐	☐	☐	☐	☐	☐	☐	☐
Scraper	☐	☐	/	☐	☐	/	☐	☐	☐	☐	☐	☐	☐	☐
Small equipment (e.g. brushes, shovels, buckets)	☐	☐	/	☐	☐	/	☐	☐	☐	☐	☐	☐	☐	☐
Other (specify) …….	☐	☐	/	☐	☐	/	☐	☐	☐	☐	☐	☐	☐	☐

Please give details of any detergent or disinfectant used for equipment cleaning:

	Bucket & Brush: Detergent	**Bucket & Brush: Disinfectant**	**Power Washer: Detergent**	**Power Washer: Disinfectant**
NAME (e.g. HD3, Farm Fluid S)				
VOLUME (of concentrate)	*ml*	*ml*	*ml*	*ml*
CONCENTRATION (e.g. 1:160, 1:50)	:	:	:	:

6. What hygiene facilities are provided for staff? (*please tick all that apply*) Wash Basin ☐ Toilet ☐ Hand Sanitiser/Bactericidal Soap ☐ Shower/Bath ☐ Hand Towel ☐ Warm air dryer ☐ Paper towels ☐ Clean bucket ☐ Soap ☐ Other (specify)……..……☐

7. Are site-dedicated boots and protective clothing provided for use by staff and/or visitors? Overalls: Staff ☐ Visitors ☐ Boots: Staff ☐ Visitors ☐

- THANK YOU : PLEASE RETURN THIS FORM IMMEDIATELY IN THE PRE-PAID ENVELOPE PROVIDED -

THINK CLEAN – ACT CLEAN

This study is being run by the Veterinary Laboratories Agency (VLA) and is funded by Defra. We have used current expert opinion to develop a hygiene and biosecurity programme that we think will reduce the level of *Salmonella* infection in finisher pigs. We will test this programme by comparing two groups of farms in an intervention study. One group of farms, the **comparison** group, will follow their usual practices. The second group of farms, the **intervention** group, will follow the new programme. Farms will be placed in these groups at random. We will take identical samples from all of the farms in both groups and these will be tested for *Salmonella*. At the end of the study, we will find out how effective the programme has been. We will also collect information about the costs and benefits of the programme.

Veterinary Laboratories Agency

SAMPLES

Up to 30 swab samples will be collected from pen dunging areas as follows:

WHEN	SAMPLES TAKEN BY:
1. Before the last pigs of the current batch are sent to slaughter.	VLA staff
2. After you have carried out the cleaning procedures, and before the study batch arrives.	VLA staff – we will mark the pens to make it easier to remember
3. From the pigs transport as they arrive (2 samples only per lorry).	You
4. Within 3 days of the unit being filled with pigs.	You
5. Every four weeks after this, until the study batch leaves.	You
6. A set of samples, collected within 7 days before the first pigs are sent for slaughter.	You
7. The last set of samples should be taken just before the last pigs are sent to the abattoir	You

A full sampling kit containing swabs, jars, and a reply-paid label will be sent to you each time you are asked to take samples.

HOW TO SAMPLE

1. At the pen side put on two pairs of gloves on top of each other. Change the second pair for a new pair of gloves for each sample.
2. Find a safe clean place to rest the box of jars – it may be helpful to carry a stool with you for this.
3. Tear off the adhesive label and stick it firmly on the side of the sample jar.
4. Enter the pen or yard taking care not to tread on the area that is to be sampled.
5. Unscrew the jar lid, remove the swab and pass the swab through the top 2 inches of the pooled faeces in the main dunging areas of the pen or yard, swabbing over a 2 metre zigzag path so that all sides of the swab except for the point where the swab is held are well coated with faeces.
6. Carefully return the swab to the labelled sample jar so that the outside of the jar remains as clean as possible, and replace the lid securely.
7. Replace the jar in the box. Remove gloves and discard. Proceed to next sample site and follow instructions 1-7.
8. When all samples have been taken seal each tray of jars inside two of the polythene bags provided and replace the trays in the box. The paperwork should also be enclosed in the provided sealed polythene bag.
9. Seal box and take to post office on the day that they are collected. Use reply-paid label to post the box of samples to:

Dr Rob Davies, FES, VLA Weybridge, Addlestone, SURREY KT15 3NB

Meat juice samples

In addition, we will collect forty neck muscle samples when the pigs are in the abattoir to test for *Salmonella* antibodies, as in the ZAP scheme. We will organise the sampling and will ask you to let us know the date when the study pigs are leaving the farm and which abattoir(s) are being supplied.

Information

During the study, we will collect information about your farm and the pigs ourselves during visits and will ask you to fill out short weekly forms. We will keep these to a minimum. All of the information that you provide will be kept confidential – no one else will see it. We will present statistical summaries for all of the farms in each group. For example, we will report the average, maximum and minimum levels of *Salmonella* infection that we find – but we will not identify any farm by name or address.

Reports

No published report that we produce will ever mention your name or your address. When the study is finished we will send a summary of results to everyone who has helped us. We will also present a report to Defra and we expect to publish the results in scientific and agricultural journals. Finally, we will present our findings to suitable scientific and farmers meetings.

Thank you for helping us with this study and don't hesitate to get in touch if you have more questions or need any help.

Elizabeth Marier: 01932 357 618
email: e.marier@vla.defra.gsi.gov.uk

or

Sandy Miller: 01932 357 623
email: a.miller@vla.defra.gsi.gov.uk

INTERVENTION GROUP

Think Clean – Act Clean: Salmonella control for finisher farms

Dear Sir or Madam,

You have been randomly selected to be in the **intervention** group. This means we would like you to do some additional cleaning and follow the hygiene and biosecurity plan outlined in the following pages. We realise that you are already very busy and that we are asking you to do extra work but please follow the programme to the best of your ability. The greatest benefits are expected from following all of the steps in this programme. However we know that this is not possible on every farm , so it is essential that you let us know what you actually do by completing our forms carefully. At the end of the study, we will be able to analyse Salmonella levels according to the number of farmers who completed each part of the programme.

A member of the VLA team will have already visited your farm to take the first set of samples. When we return for the second visit we will take more samples and explain in more detail what we would like you to do. This will also be a good opportunity to ask us any questions you might have about the study. We believe that following these measures will reduce *Salmonella* levels on your farm so please read the following information carefully.

At any time, please do not hesitate to call us if you have any questions or problems carrying out the additional measures we ask.

Elizabeth Marier: 01932 357 618
email: e.marier@vla.defra.gsi.gov.uk

or

Sandy Miller: 01932 357 623
email: a.miller@vla.defra.gsi.gov.uk

Hygiene & Biosecurity Programme for Intervention farms

Here are the steps to follow:

At the end, you will also find information about the use of disinfectants during the programme.

Note that we will give advice about all aspects of this programme when visiting to take samples.

This programme begins when the last pig has left the site

Cleaning pig houses – between batches

A list of suitable disinfectants and their concentrations is given on page 16 - it is very important that enough disinfectant is used at all stages and left to dry on surfaces – not rinsed away. Disinfection will be more effective on clean surface. Make sure that all disinfectant is made up fresh just before use and the highest recommended concentration (e.g. Defra TB order rate) is accurately measured. Do not guess or rely on metering devices.

Cleaning outside the building and the surrounding areas

TASKS	Tick when done
1. All muck heaps should be moved away from pig housing.	
2. Check that waste from muck heaps does not leak into pig, feed or bedding areas; or areas where tractors, people or pigs pass through.	
3. The area should be cleaned and disinfected after the muck heap has been moved.	
4. Empty bins for dead stock and foot dip and clean them	

Cleaning inside the building

TASKS	Tick when done
1. Remove all manure, bedding and waste from the building	
2. Remove portable equipment from the building for cleaning and disinfecting	
3. Remove feed residues, drain water bowls and clean behind flap, float	
4. Power wash the building. Surfaces should be free of pig manure and other organic matter as these can inactivate disinfectants	
5. Let dry completely (at least 12 hours)	
6. Apply disinfectant on all surfaces and let it dry (at least 48 hours) – see guidance on disinfection	
7. Passageways, floors, walls, equipment, loading areas, hoppers, bowls, all surfaces including undersides should be cleaned and disinfected.	
8. Do not rinse after disinfecting feeders and drinkers but if disinfectant pools in feeders or drinker bowls, mop out before pigs are placed.	
9. If the building is left empty for a long period (more than two weeks), check for recontamination by rodents and other pests. If necessary, repeat disinfection of contaminated areas.	

Think clean – act clean

APPENDIX NI1

Cleaning equipment – between batches

TASKS	Tick when done
1. Use a pressure washer to clean the tractor, scraper blade and other large pieces of equipment e.g. trailers, weighers etc.	
2. Clean all smaller pieces of equipment – e.g. pig boards, brushes, shovels, buckets, stepladders, toolboxes, slap marker, waterproof overalls, aprons – using disinfectant applied by immersion or with a brush	
3. Disinfect all cleaned equipment. For larger pieces of equipment, this may be applied using a pressure washer.	
4. Allow all cleaned and disinfected equipment to dry before use.	
5. Complete the cleaning and disinfection of your equipment before the first pig is delivered to the site.	
6. **Very important** – Clear feed from the previous batch immediately and set up baits in pig areas while unit depopulated	

Other cleaning – between batches

TASKS	Tick when done
1. Clear site of overgrown vegetation, rubbish or unnecessary equipment, especially near pig buildings	
2. Clean and disinfect all areas of the unit which pigs are moved through. This includes loading ramps, races, weigh pens, holding pens, weighers, corridors between pens, barriers, hurdles, and gates etc.	

Cleaning Equipment – during production

TASKS	Tick when done
1. At least once each week, pressure wash the scraper blade and tractor tyres and then disinfect.	
2. Clean shovels, brushes or other equipment used to clean pig pens regularly using disinfectant.	
3. If any equipment (e.g. tractor and trailer) is moved off the farm, then it should be cleaned and disinfected when returned to the pig unit.	
4. If any equipment (e.g. bucket loaders, scoops, trailers etc) that is used to handle pig feed is used for any other purpose, it should be thoroughly cleaned and disinfected before it is in contact with feed again.	

Rodent Control

Rats, and especially mice, can leave millions of Salmonella *bacteria in each dropping. One highly infected dropping in a feeder or drinker can undo the whole of the control programme so please take rodent control seriously.*

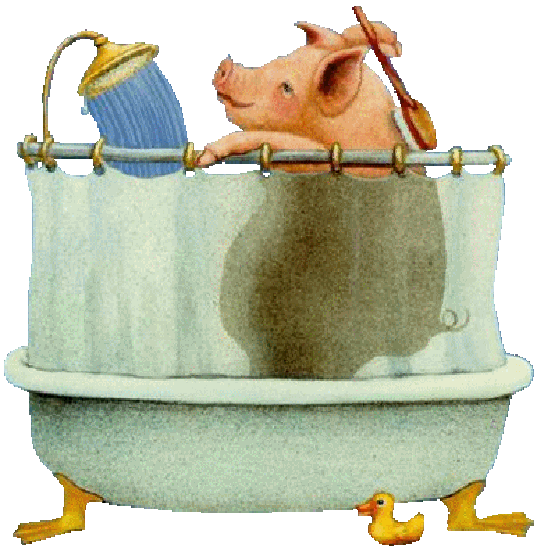
TASKS	Tick when done
1. Check thoroughly for any evidence of rodent activity (sightings especially at night, droppings, chewing damage, footprints in dust, urine pillars and grease marks on ledges, disturbed bait), and review your rodent control program adding new bait points. Include bait points around the outside of houses and the perimeter of the unit. If there is a large rodent population use traps and rodenticide tubes, as well as bait and consider additional water bait when site is empty.	
2. Bait should be checked and replaced at least weekly and more often if required. Use a good quality bait of the right kind for the right pest (mice or rats), and keep it free of dust.	
3. If you carry out your own rodent control, then consider what you are doing and look for possible improvements.	
4. Ensure that spilled feed is always promptly cleared up	
5. **Very important** – Clear feed from the previous batch immediately and set up baits in pig areas while unit depopulated	

Biosecurity Measures

These are *in addition* to any which you normally carry out

1. Boot dips

TASKS	Tick when done
1. Provide a boot dip (containing a phenolic disinfectant at its maximum recommended concentration, see page 16 for more details), and a boot brush at the entry to every building and at every entrance to the pig unit. Boot dips should be large enough to hold at least one large boot and should contain enough disinfectant to cover the whole foot to over the ankle when immersed. Make sure that boot dips are in covered areas if this is possible to prevent them from being diluted by heavy rain or replace dip if it has become diluted.	
2. Use the brush and boot dip to remove visible muck from boots every time you enter and leave the site, and every time that you enter and leave a building.	
3. Empty the bootdips and replenish them when visibly soiled, but at least once every week. If the site is muddy it may be worth having separate boot washes to use before dipping boots in disinfectant.	



2. Personal hygiene

TASKS	Tick when done
1. All staff and visitors must wear clean overalls and boots that are kept in a clean changing area on the pig unit.	
2. Do not use any of the farm protective clothing on any other site.	
3. Boil wash all overalls in a washing machine at least once every week.	
4. Any other protective clothing (e.g. aprons/waterproofs) worn onsite should also be thoroughly cleaned weekly.	
5. Please leave a container of the alcohol based hand sanitizer provided* next to the bootdip at each building entrance, and use it every time you enter and leave a building.	
6. Wash your hands thoroughly and use the alcohol based hand sanitizer as necessary during the working day, for example as you: • arrive on the pig unit • complete any task that involves handling pigs • complete any task that has possible contact with pig dung • are going to eat, drink or smoke • leave the unit	
7. If you visit any other livestock unit, take a shower and change all of your outer clothes before you return to the pig unit.	
8. If your farm has more than one livestock enterprise, then you should wear separate protective clothing for the pig enterprise.	

3. Pig movements

TASKS	Tick when done
1. The unit should ideally be stocked with pigs from a single source. If this is not possible, all pigs in each row of pens must come from one source, and overall from as few sources as possible.	
2. Clear any areas which pigs walk through of puddles or muck.	
3. Do not mix pigs (e.g. when the first batch has been sent to slaughter), except if they are moved into a sick pen.	
4. Do not move pigs from one pen to another during the study.	
5. All pigs on the site should be sent to slaughter within one week or within as short a period of time as possible.	
6. If all pigs within one pen are not sent to slaughter at the same time, remainders should not be mixed with other pigs from different pens.	
7. If groups of pigs are ever split they must not be remixed later on.	

4. Sick pens

TASKS	Tick when done
1. Sick pens should, if possible, be in a separate building. If this is not possible, then ensure that sick pens are placed at the end of a row so that dung etc is not pushed into contact with other pigs.	
2. Place boot dips and a hand sanitizer outside the sick pen, and use before and after entering.	
3. The sick pen should be the last pen visited for routine tasks, such as cleaning or feeding.	
4. Pigs that are moved into a sick pen must not be returned to another pen. If they recover, they must remain in the sick pen or a convalescence pen until they are sent to slaughter.	
5. The smell of dead pigs attracts farm pests, so carcasses should be disposed of as quickly as possible and there should be no seepage from holding areas.	
6. Sick pens, and any bins or holding areas used for dead pigs, should be cleaned and disinfected whenever they are emptied and at the start of the between batch cleaning programme.	

5. Visitors – and other animals!

TASKS	Tick when done
1. No unnecessary visitors should visit the unit.	
2. Visitors should not enter any building containing pigs unless it is essential.	
3. Visitors should not enter any pen containing pigs unless it is essential.	
4. Every visitor must wear clean boots and overalls, provided by the unit. VLA can provide disposable boiler suits and overboots if required.	
5. Do not allow any domestic animals (including dogs & cats) to enter pig accommodation or feed or bedding stores.	
6. Ensure that wild birds do not have access to pig housing, or feed or beddings stores.	

6. Feed and water

TASKS	Tick when done
1. All feed stores and feed hoppers should be covered.	
2. All header tanks should have a solid cover.	

DISINFECTANTS

These sheets give advice on a number of disinfectants that we recommend for use in this study. You do not have to use these disinfectants but to help you we have provided guidelines for the correct concentrations and volumes for use at each stage.

The following disinfectants are recommended for use during this study:

Intensive disinfection of pig areas & equipment:

- **Macroline 500** @ 1:103 (Phenolic)
- **Longlife 250S*** @ 1:80 (High boiling point tar acid)
- **Farm Fluid** @ 1:100 (High boiling point tar acid)
- **Sorgene 5** @ 1:75 (Peroxygen)
- **Hyperox** @ 1:100 (Peroxygen)

Small equipment- protective clothing (e.g. aprons and waterproofs) wash off after a minimum of 1h contact, especially the phenolics

Bootdips

- **Longlife 250S*** @ 1:80 (High boiling point tar acid)
- **Farm Fluid S** @ 1:100 (High boiling point tar acid)

Water flush system

- **Hyperox** @ 1:500 (Peroxygen)
- **Sorgene 5** @ 1:400 (Peroxygen)
- **Virkon S** @ 1:200 (Peroxygen)

***Most highly recommended*

Disinfectants should be made up fresh before each job and at the highest recommended concentration. It is important that enough disinfectant is used at each stage. The following sheets give advice on making up the disinfectants and we suggest you pin these up in a suitable place where staff will have access to them.

Veterinary Laboratories Agency

CLEANING OF PIG AREAS AND EQUIPMENT

Disinfectant should be used to saturation point on dry surfaces. **Bowls and nipples should be cleaned with full strength disinfectant but do not leave pools of disinfectant in drinkers or feeders when new pigs are introduced – mop them up if present.** Aim to use approximately 300ml of made up disinfectant solution for every square meter of floor space, including corridors and passageways.

This table gives the volume of water and volume of disinfectant needed to make up the correct recommended concentration for use in this study:

		DISINFECTANT USED				
		Longlife 250S (high boiling point tar acid)	**FarmFluid S (high boiling point tar acid)**	**Sorgene 5 (Peroxygen)**	**Hyperox (Peroxygen)**	**Macroline 500 (Phenolic)**
Recommended dilution:		**1:80**	**1:100**	**1:75**	**1:100**	**1:103**
		Volume of disinfectant required:				
Volume of water:	**1L**	12ml **(1)**	10ml	15ml	10ml	10ml
	5L	62ml	50ml	70ml	50ml	50ml
	20L	250ml	200ml	14L	200ml	200ml
	50L	625ml	500ml	27L	500ml	500ml
	100L	12.5 Litres	1 Litre	67L	1L	1L

(1) Example: In one litre of water, you need to add 12 ml of disinfectant.

Think clean – act clean

BOOT DIPS

Boot dips should be situated at all main farm/site entrances and at the entrance to every house. Ensure they are protected from rain and that all staff use them.

Boot dips should be replaced **at least once a week** or more often if they become soiled.

A good sized boot dip should hold approx 2 buckets or 30 litres of diluted disinfectant. Boot dips should be large enough for a man to stand with one foot submerged above the ankle. Using too little boot dip or not changing it frequently enough will reduce its effectiveness. For best results keep a stiff brush beside each boot dip and remove as much organic matter as possible from the boots (remembering to pay particular attention to the sole) before dipping.

This table gives the volume of water and volume of disinfectant needed to make up the correct recommended concentration for use in this study:

		DISINFECTANT USED	
		Longlife 250S (high boiling point tar acid)	**Farm Fluid S (high boiling point tar acid)**
Recommended dilution:		**1:80**	**1:100**
		Volume of disinfectant required	
Volume of water:	**1L**	12ml	10ml
	10L	125ml	100ml
	15L	188ml	150ml
	30L	375ml	300ml

Average bucket = 3gal, 15L

WATER FLUSH SYSTEM

DRAIN HEADER TANK AND WATER LINES AND REPLACE WATER WITH WELL MIXED DISINFECTANT AS SPECIFIED BELOW. Leave disinfectant for at least 1h and flush through with clean water.

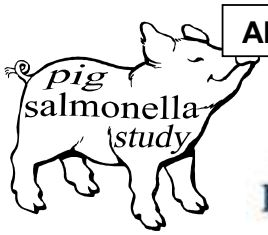
This table gives the volume of water and volume/weight of disinfectant needed to make up the correct recommended concentration for use in this study:

		DISINFECTANT USED		
		Virkon S (Peroxygen)	**Sorgene 5 (Peroxygen)**	**Hyperox (Peroxygen)**
Recommended dilution:		**1:200**	**1:400**	**1:500**
		Amount of disinfectant required:		
Litres of water to be sanitised:	**100L**	500g	250ml	200ml
	250L	1.25Kg	625ml	500ml
	500L	2.5Kg	1250ml	1L
	1000L	5Kg	2.5L	2L

PLEASE WRITE DATE OF REPORT ON FORM

APPENDIX NI5

Weekly Farm Report



Veterinary Laboratories Agency

FARM ID:
Date:

*Please complete this form at the **end** of every week and post back to the VLA using the pre-paid envelopes provided.* ***Please answer for the past week only*****.**

A. General

1) Have any visitors been on the farm? Yes ☐ No ☐

If Yes, did they wear their own protective clothing or did you supply it? Own ☐ Supplied ☐

If Yes, did they enter buildings containing pigs Yes ☐ No ☐

2) Have any farm staff, visited any livestock farms in the past week? If yes, how many times?

	Pig	Cattle	Poultry	Sheep	Other
	☐	☐	☐	☐	☐
How many times?	……..	……..	……..	……..	……..

3) How many pigs have died in the last week? ……

B. Cleaning equipment during production

4) Have you cleaned & disinfected:

a) Scraper Cleaned ☐ Disinfected ☐ Neither ☐

b) Tractor tyres Cleaned ☐ Disinfected ☐ Neither ☐

5) Other larger equipment e.g. tractors, scrapers, trailers, weighers

Cleaned ☐ Disinfected ☐ Neither ☐

6) Cleaned & disinfected smaller equipment e.g. pig boards, brushes, shovels, buckets, stepladders, toolboxes, slap marker? Cleaned ☐ Disinfected ☐ Neither ☐

7) Has any equipment been off the farm in the last week? Yes ☐ No ☐

If yes, did you clean and disinfect it on return? Cleaned ☐ Disinfected ☐ Neither ☐

8) Please estimate how much time was spent cleaning equipment in the last week:

Hours spent cleaning and disinfecting equipment	Was this more or less time than normal? (Circle as appropriate)	If not same, how many hours do they normally spend?
	More Less Same	
	More Less Same	
	More Less Same	

If the cleaning is done by a contractor, what is the hourly rate? _____
(or what was the total cost?)

C. Rodent control

9) Have you checked rodent baits? Yes ☐ No ☐

Have you replenished rodent baits? Yes ☐ No ☐

10) Have you seen any evidence of rodents on your farm? Yes ☐ No ☐

11) Please estimate how much time was spent on rodent control in the last week:

Hours spent controlling rodents	Was this more or less time than normal? (Circle as appropriate)	If not same, how many hours do they normally spend?
	More Less Same	
	More Less Same	
	More Less Same	

D. Biosecurity measures

	ALL	SOME	NONE
12) Have you emptied and changed boot dips?	☐	☐	☐
13) Have you washed staff overalls?	☐	☐	☐
14) Washed/cleaned other protective clothing (aprons, waterproofs):	☐	☐	☐
15) Did staff clean their hands before entering pig buildings?	☐	☐	☐

16) Please estimate how much time was spent on biosecurity measures in the last week:

Hours spent on biosecurity	Was this more or less time than normal? (Circle as appropriate)	If not same, how many hours do they normally spend?
	More Less Same	
	More Less Same	
	More Less Same	

COMMENTS: Please write any events or other information that you think might be important for us.

_____

_____

_____

_____

_____

_____

THANK YOU!